

Effect of Temperature and Pseudobulb Maturity on Flowering of the Orchid *Miltoniopsis* Augres 'Trinity'

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Abstract

Commercial production of the pansy orchid, *Miltoniopsis*, requires an understanding of how to regulate the flowering process. From previous research, we determined that flowering of *Miltoniopsis* Augres 'Trinity' was promoted most when plants were exposed to short days and then vernalized at 11 or 14°C for at least 8 weeks. Here, we quantified how constant and fluctuating day/night temperatures and pseudobulb maturity influenced time to visible inflorescence (VI) and time from VI to open flower. The diameters of three pseudobulbs were measured on each of 72 *Miltoniopsis* Augres 'Trinity' plants, then plants were vernalized in a growth chamber with a 12-h day/12-h night temperature setpoint of 16/14°C for 8 weeks. Plants were then forced in glass greenhouses under a 16-h photoperiod with constant temperatures of 14, 17, 20, or 23°C, or day/night temperatures (12h/12h) of 20/14 or 23/17°C. Only 27% of the pseudobulbs with a diameter ≤ 1.5 cm initiated flowers, whereas 90% of pseudobulbs with a diameter ≥ 3.1 cm became reproductive. Average time from the end of vernalization to appearance of the first VI was 89 and 8 days for plants with pseudobulb diameters ≤ 1.5 or ≥ 3.1 cm, respectively, regardless of forcing temperature. Average time from VI to flowering was 122, 97, 78 or 62 days at constant temperatures of 14, 17, 20, or 23°C, and 93 or 76 days at day/night temperatures of 20/14 or 23/17°C. Flower diameter increased from 7.4 to 8.6 cm as forcing temperature decreased from 23 to 14°C, but temperature had no effect on flower number and inflorescence height. We conclude that pseudobulbs of *Miltoniopsis* Augres 'Trinity' must be sufficiently mature (diameter ≥ 3.1 cm) before cooling for rapid flowering and a high flowering percentage. Once plants are induced to flower, subsequent development is a function of the average daily temperature.

INTRODUCTION

Commercial production of potted orchids has increased dramatically throughout the world in the past quarter century. Despite the production and value of orchids, the flowering physiology is not well described and large scale commercial scheduling of most orchid genera is not possible. In recent years, several studies have documented the effects of temperature and photoperiod on growth and flowering of *Miltoniopsis* (Lopez et al., 2005; Lopez and Runkle, 2006), *Odontioda* (Blanchard, 2005), *Phalaenopsis* (Lee and Lin, 1984; Yoneda et al., 1991; Wang, 1995; Robinson, 2002; Blanchard, 2005), and *Zygopetalum* orchids (Lopez et al., 2003; Lopez and Runkle, 2004). This information has improved our understanding of the environmental cues that are required for flower initiation and development. For example, flowering of *Miltoniopsis* Augres 'Trinity' was most complete ($\geq 90\%$ of population) and uniform when plants were exposed to short days (SD) for 4 to 8 weeks before 8 weeks of vernalization at 14°C (Lopez and Runkle, 2006).

In the United States, potted flowering plants are commonly produced, marketed, and purchased for holidays such as Christmas, Valentine's Day, Easter, and Mother's Day. Plants must have flowers to be marketable for these holidays, and the flowers must be at a specific stage of development on a target date to obtain the highest price. This requires an understanding of how plant maturity and temperature influence the flowering process, so that greenhouse growers can schedule crops into flower for these specific dates. According to Hew and Yong (1997), for this to be commercially possible, the methods

used for flower initiation and development must be straightforward, cost-effective, and not adversely affect plant and flower quality.

Similar to many other flowering plants, a juvenile phase exists in orchids, in which plants must reach a certain stage of growth (or maturity) before attaining the capacity to flower. Once plants have attained the capacity to flower, environmental or cultural factors can be provided to induce flowering. Once flowering has been initiated, temperature primarily controls the rate of flower development, assuming that plants are provided with adequate water, fertility, and light.

Miltoniopsis, or the pansy orchid, produces showy and often fragrant flowers with three to seven flat and large (6 to 8 cm) flowers per inflorescence. It has become the fourth most valuable potted orchid produced commercially in the Netherlands, with 797,000 pots sold in 2001 (Barendse, 2002). We performed experiments to determine how pseudobulb diameter influences time to visible inflorescence (VI) and inflorescence initiation of *Miltoniopsis* Augres 'Trinity'. Another objective of this study was to determine how constant and fluctuating temperatures influence flower development.

MATERIALS AND METHODS

Plant Material and Culture

Young plants of *Miltoniopsis* Augres 'Trinity' were grown by a commercial greenhouse in California, USA at $\approx 25/14^{\circ}\text{C}$ day/night under natural photoperiods (lat. 37°N) with an average photosynthetic photon flux (*PPF*) of 160 to $240\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. They were planted in a bark and perlite-based media in May 2002 (10-cm pots) and were received in East Lansing, Mich. on 22 July 2002. Plants were transplanted into 10-cm pots containing 66% medium-grade fir bark, 17% long-fiber Canadian sphagnum peat, and 17% medium-grade perlite (by volume) in October 2003 and were subsequently maintained at $\approx 20^{\circ}\text{C}$ in a glass-glazed greenhouse until experiments began. The photoperiod was a constant 16h, consisting of natural photoperiods (lat. 42°N) with day-extension lighting from high-pressure sodium (HPS) lamps, which delivered a supplemental *PPF* of $\approx 50\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at plant height. Light transmission through the greenhouses was reduced using a permanent woven shade curtain that reduced light by $\approx 55\%$ (OLS 50; Ludvig Svensson, Charlotte, NC) and by applying whitewash to the glass as needed so that the maximum *PPF* was $\approx 300\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Plants were irrigated as necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following ($\text{mg}\cdot\text{L}^{-1}$): 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).

Vernalization and Forcing

The vernalization treatment consisted of placing 72 plants in a walk-in controlled-environment chamber on 24 Sept. 2004 with a day/night temperature (12h/12h) setpoint of $16/14^{\circ}\text{C}$. Plants remained in the chamber for 8 weeks. The 12-h photoperiod was provided by a combination of cool-white fluorescent (VHOF96T12; Philips, Bloomfield, NJ) and incandescent lamps from 0800 to 2000 HR at $150\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Air temperature and light intensity in the chamber were measured by an aspirated thermocouple and a quantum sensor every 10 s and hourly averages were recorded by a CR-10 datalogger (Campbell Scientific, Logan, UT).

After vernalization treatment, 12 plants were randomly assigned to each of six glass greenhouse sections with constant temperature setpoints of 14, 17, 20, or 23°C , or fluctuating day/night (12h/12h) temperature setpoints of 20/14 or 23/17 $^{\circ}\text{C}$. The photoperiod was a constant 16h, consisting of natural photoperiods with day-extension lighting from high-pressure sodium (HPS) lamps.

Greenhouse Temperature and Irradiance Control

Temperature in each greenhouse section was measured by a thermocouple in an aspirated chamber every 10 s, and hourly averages were recorded by a CR-10 datalogger.

Air temperatures were determined for each pseudobulb from the first VI to flowering. The actual average daily air temperatures (and temperature setpoints) were 14.4 (14), 17.1 (17), 19.7 (20), 22.7 (23) and 16.2 (20/14) and 19.5 (23/17)°C. Light transmission through the greenhouse was reduced as previously described. A vapor pressure deficit (VPD) of 0.9 kPa was maintained at each temperature treatment by the injection of steam.

Data Collection and Analysis

Upon removal from vernalization, pseudobulb diameter was determined for three pseudobulbs with diameters ≥ 1 cm on each of 72 plants (216 pseudobulbs total) by measuring the widest point of the pseudobulb using a digital caliper. The three pseudobulbs chosen for study were randomly selected within each pot but did not include pseudobulbs from which an inflorescence had previously emerged or that were shriveled in appearance. The date of the first VI without dissection (< 0.5 cm long), number of flowers per inflorescence and the number of VI were recorded for each of the three pseudobulbs per plant. Days to VI, days from VI to flower, and VI percentages were calculated. A flower was considered to be open when the sepals and petals were fully reflexed. On the date of flowering, flower diameter at the widest point of the flower and total inflorescence length from the point of emergence to the tip of the inflorescence were measured. Days to flower and air temperature during that period were calculated for each pseudobulb. A completely randomized design was used and data were analyzed using SAS (SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) and linear models procedure (PROC REG).

RESULTS

Pseudobulb Maturity

Twenty seven percent and 90% of the pseudobulbs with a diameter ≤ 1.5 cm and ≥ 3.1 cm initiated flowers, respectively (Fig. 1). Average time from the end of vernalization to appearance of the first VI decreased from 89 to 8 days as pseudobulb diameter increased from ≤ 1.5 to ≥ 3.1 cm, regardless of forcing temperature. Pseudobulb diameter significantly ($P \leq 0.001$) influenced time to VI of *Miltoniopsis* Augres 'Trinity'. Time from VI to flower, number of flower buds, and inflorescence height and number were not influenced by pseudobulb size (data not presented).

Forcing Temperature

Average time to VI from the beginning of forcing was 31 ± 2 d (\pm standard error) and was not influenced by forcing temperature (Table 1). Time from VI to flower opening was significantly ($P \leq 0.001$) influenced by temperature. Average time from VI to flowering was 122, 97, 78 or 62 days at constant temperatures of 14, 17, 20, or 23°C, and 93 or 76 days at day/night temperatures of 20/14 or 23/17°C. Flower diameter also decreased with increasing temperature, with an average diameter of 8.6 cm at 14°C and 7.4 at 23°C. Rate of progress from VI to flowering was linear within the range of experimental temperatures (data not presented). The number of inflorescences per pseudobulb, the number of flower buds, and inflorescence height were not influenced by temperature.

DISCUSSION

Flower initiation was most rapid, uniform, and complete when pseudobulbs were ≥ 3.1 cm in diameter at the onset of vernalization treatment. Once an inflorescence emerged from a pseudobulb, subsequent time to flowering was dependent upon forcing temperature. We had previously postulated that similar to other flowering plants, a juvenile phase exists in *Miltoniopsis* and plants must reach a certain stage of growth before attaining the full capacity to flower. This was indeed true: if plants with immature pseudobulbs with a diameter ≤ 1.5 cm were placed in an inductive vernalization treatment, only 27% initiated an inflorescence, compared to 90% initiation for pseudobulbs with a diameter ≥ 3.1 cm. Thus, sexual reproduction is delayed until plants reach a size sufficient

to maintain the energetic demands of flowering and seed production. A similar response to pseudobulb diameter has been observed in *Odontioda* hybrids, in which pseudobulbs with a diameter ≥ 5.5 cm developed a VI in 93% of the plants (Blanchard, 2005).

As temperature increased from 14 to 23°C, the rate of development from VI to flower increased. Time from VI to first open flower decreased with increasing temperature, with an average of 122 d at 14°C and 62 d at 23°C. Plants forced at 20/14°C (with an average daily temperature of 17°C) flowered within 4 d of plants forced at a constant 17°C. Similarly, plants at 23/17°C or a constant 20°C flowered in 76 or 78 d. Similar responses to temperature have been observed in *Odontioda*, *Phalaenopsis* and *Zygopetalum* orchid hybrids; as temperature increased from 14 to 23°C, the rate of leaf, bud, and flower development increased linearly (Robinson, 2002; Blanchard, 2005; Lopez and Runkle, 2004). Time to flower from VI for *Odontioda*, *Phalaenopsis*, and *Zygopetalum* was 100 d, 278 d and 64 d at 14°C, respectively, and 64 d, 76 d and 34 d at 23°C, respectively. Similar to *Odontioda*, there were no advantageous effects of a diurnal temperature fluctuation on inflorescence initiation and in fact, flower size and color of *Miltoniopsis* Augres 'Trinity' were negatively affected.

From this and previous studies (Lopez et al., 2005; Lopez and Runkle, 2006), it has been determined that mature pseudobulbs of *Miltoniopsis* Augres 'Trinity' can be induced to initiate an inflorescence by providing vernalization at either constant 11 to 14°C under a 9-h photoperiod or a 12-h day/12-h night temperature setpoint of 16/14°C for eight weeks. Time to VI is dependent on pseudobulb maturity and time from VI to flower is a function of average daily temperature. With this information, large-scale commercial scheduling of *Miltoniopsis* Augres 'Trinity' as a potted flowering plant for winter and spring holidays in North America and Europe (Christmas, Valentine's Day, Easter and Mother's Day) is now possible.

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Tables

Table 1. The effect of forcing temperature on days to visible inflorescence (VI), days from VI to flowering, flower bud number, flower diameter, inflorescence count per pseudobulb, and height of *Miltoniopsis* Augres 'Trinity.'

Forcing temperature (°C)	Days to VI	Days from VI to flower	Flower bud number	Flower diameter (cm)	Inflorescence	
					Count	Height (cm)
14	42	122	2.9	8.6	1.4	22.8
17	32	97	2.9	8.4	1.2	22.9
20	27	78	3.4	7.4	1.2	24.8
23	25	62	3.7	7.4	1.2	26.3
20/14 ¹	29	93	3.8	8.2	1.3	26.7
23/17 ¹	28	76	3.5	6.5	1.3	25.4
	NS	***	NS	**	NS	NS

¹12-h day/12-h night.

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

Figures

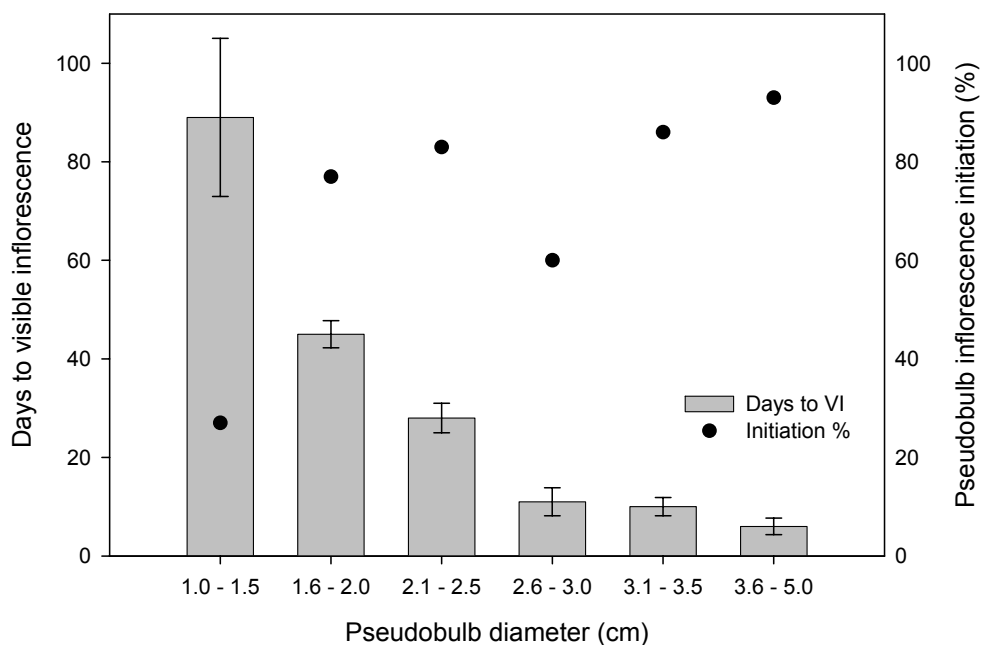


Fig. 1. The effect of pseudobulb diameter on days to visible inflorescence (VI) and the percentage of pseudobulbs that initiated an inflorescence of *Miltoniopsis* Augres 'Trinity'. A 16/14°C (12h/12h) vernalization treatment was provided under a 12-h photoperiod for 8 weeks. Plants were subsequently forced under a 16-h photoperiod with constant temperatures of 14, 17, 20, or 23°C, or day/night temperatures (12h/12h) of 20/14 or 23/17°C. Vertical bars represent standard error of the mean.