

# Gibberellic Acid and Benzyladenine Promote Early Flowering and Vegetative Growth of *Miltoniopsis* Orchid Hybrids

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**Abstract.** Flowering of *Miltoniopsis* orchids is influenced by a combination of cool temperatures and short photoperiod. To determine if application of plant growth regulators could promote flowering without the need for costly structural modification to control photoperiod or temperature, we used drenches of gibberellic acid (GA<sub>3</sub>) (2.5 or 5 mM), N<sup>6</sup>-benzyladenine (BA) (25 or 50 mM) alone or in combination. BA (25 or 50 mM) treatments promoted new vegetative shoots and decreased the number of plants with inflorescences compared to the untreated control plants. This reduction of flowering and increased vegetative shoot production was alleviated by the addition of GA<sub>3</sub> in combination with BA. However, the number of plants with inflorescences remained less than the control. GA<sub>3</sub> hastened *Miltoniopsis* inflorescence emergence during the first flowering season by 10.9 to 14.9 days for Bert Field 'Eileen' and by 48.7 days for Rouge 'Akatsuka'. The number of 'Eileen' inflorescences produced per plant increased from 2.2 to 3.0 with 2.5 mM GA<sub>3</sub> treatment. Flower deformities were not observed in the GA<sub>3</sub> treated plants, and flower size and inflorescence length were unaffected by the GA<sub>3</sub> treatment.

In the U.S., orchids rank second in value for potted flowering plants (between poinsettias and chrysanthemums) and were valued at \$128 million in 2004, a 5% increase over 2003 production (USDA–NASS, 2005). Commercial poinsettia and chrysanthemum production rely heavily on standard practices of flower induction to fulfill market demand (Larson, 1980). In contrast, availability of most orchids is based on the genetic diversity of this large family and on flowers produced at locations that vary in climatic conditions.

Potted orchid production in Hawaii ranks third in the U.S. behind California and Florida and is valued at \$17 million (USDA–NASS, 2005). Growth of Hawaii's orchid industry is attributed to increased export sales with two-thirds of Hawaii's orchid production being exported out of the state. Demand for potted orchids is increasing in southern and western states of the U.S., where >90% of potted orchids are sold. Hawaii orchid growers face many challenges in producing orchids to meet the market demand, including foreign competition from The Netherlands and countries in

Southeast Asia. Hawaii orchid farms are usually smaller than their counterparts in California and Florida and often less mechanized (Hawaii Department of Agriculture, 2004). These types of growing conditions make it economically difficult to modify environmental conditions such as the photoperiod and temperature to promote flowering.

Flowering relies on a developmentally competent plant to favorably perceive environmental signals to initiate the transition from vegetative to reproductive growth (Bernier et al., 1993; Goh et al., 1982). Natural flowering seasons of orchids may be dependent upon photoperiod, light intensity, temperature, water relations and plant hormones (Goh, 1985). Native to Costa Rica, Panama, Venezuela, Columbia, and Ecuador, *Miltoniopsis* or pansy orchid, is comprised of six species: *M. bismarckii*, *M. vexillaria*, *M. roezlii*, *M. phalaenopsis*, *M. warscewiczii*, and *M. santanaei*, which bloom during different times of the year (Baker and Baker, 1993; Sweet, 1978). Most cultivated hybrids have *M. vexillaria* and *M. roezlii* as parents and consequently bloom during March to July (Baker and Baker, 1993). Hybrids of *Miltoniopsis* may be mistaken for *Miltonia* because four of the six species were previously classified and registered as *Miltonia* (Baker and Baker, 1993). *Miltoniopsis* plants consist of one-leaved flattened pseudobulbs that are tightly clustered and light grayish blue-green in color compared to *Miltonia* plants that consist of two-leaved pseudobulbs that are yellowish green to honey-colored and separated by a long rhizome (Baker and Baker, 1993; Sweet, 1978).

Plant growth regulators have been used suc-

cessfully to modify flowering of many orchid species. Auxin application to *Phalaenopsis schillerana* or exogenous auxin supply to decapitated *Aranda* plants reduced flowering while application of anti-auxin and growth retardants stimulated *Aranda* flowering, suggesting that apical dominance and auxin suppress flowering (Goh, 1985). The cytokinin N<sup>6</sup>-benzyladenine (BA) induced flowering of *Aranthera*, *Aranda*, *Holttumara*, *Mokara*, *Dendrobium Lousiae* 'Dark', *Phalaenopsis*, *Dendrobium nodoka*, and *Oncidium*; however, this response is highly depended on orchid variety (Goh, 1985; Hew and Clifford, 1993). Gibberellins transformed the normal vegetative shoot apex to a terminal inflorescence of *Aranda* Deborah and induced flowering of *Bletilla striata*, *Cymbidium*, and *Cattleya* hybrids (Goh, 1985; Goh et al., 1982). Addition of GA<sub>3</sub> with cytokinins enhanced the flowering effect and reduced flower deformity of *Phalaenopsis* and *Dendrobium* (Chen et al., 1997; Sakai et al., 2000). Injection of 100 mM of BA and 10 mM GA<sub>3</sub> into *Dendrobium* Jaquelyn Thomas 'Uniwi Princess' pseudobulbs promoted off season flowering and increased the flower size and number of inflorescences per stem in Hawaii (Sakai et al., 2000).

Market demand for *Miltoniopsis* is highest during December to March in Hawaii, the typical flowering season is in Mar. to June; however, flowering dates may vary slightly with climatic conditions and cultivars. Previous reports suggest that flowering in *Miltoniopsis* Augres 'Trinity' is controlled by a combination of cool temperature (14 °C) and short photoperiod of 9 h (Lopez et al., 2005) or cool temperature (15 °C) alone (Matsui and Yoneda, 1997). The additional expense to provide cooler temperatures for floral induction may be prohibitive for *Miltoniopsis* production. While BA and GA<sub>3</sub> applications have successfully promoted flowering in other orchid species, this is the first study to use plant growth regulators to modify flowering in *Miltoniopsis*. Since the effect of plant growth regulators on *Miltoniopsis* is not known, we used two *Miltoniopsis* grexes with different flowering seasons. Bert Field 'Eileen' typically flowers in Hawaii in February to May and Rouge 'Akatsuka' flowers in March to June. Soil drenches were more effective as a method of plant growth regulator application compared to foliar sprays (Karaguzel, et al., 2004). Therefore, we investigated the effect of BA and GA<sub>3</sub> soil drenches alone or combined to promote earlier flowering of 'Eileen' and 'Akatsuka'.

## Materials and Methods

**Plant materials.** 'Eileen' (*Miltoniopsis* Mulletto Queen × *Miltoniopsis* Woodlands) (Royal Horticultural Society, 2005a) and 'Akatsuka' (*Miltoniopsis* Edmonds × *Miltoniopsis* Hamburg) (Royal Horticultural Society, 2005b) plants were grown by a commercial nursery under covered greenhouses in Kurtistown, Hawaii, with maximum photosynthetic photon flux (PPF) of about 335 μmol·m<sup>-2</sup>·s<sup>-1</sup>. Plants were grown under natural photoperiods (19 °N lat) where the longest and shortest days

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Table 1. The effect of BA and GA treatments on growth, vegetative shoot production, and flowering (number of plants with visible inflorescences) on Bert Field 'Eileen' and Rouge 'Akatsuka' hybrid *Miltoniopsis* orchids.

Treatment	'Eileen'					'Akatsuka'				
	Leaves <sup>y</sup>	Ht <sup>z</sup> (cm)	Diam <sup>z</sup> (cm)	Vegetative shoots <sup>x</sup>	Flowering (%)	Leaves	Ht (cm)	Diam (cm)	Vegetative shoots	Flowering (%)
Control	9.0 ab <sup>w</sup>	38.4 ab	19.0 a	0.21 c	96	10.7 bc	45.8 a	25.3 a	0.39 c	88
25 mM BA	9.4 a	39.8 ab	17.5 ab	2.71 a	58	11.5 a	44.0 ab	22.8b	1.89 a	38
50 mM BA	9.0 ab	37.1 ab	16.4 bc	1.86b	33	11.5 a	43.8 abc	22.2b	1.74 a	44
2.5 mM GA	8.6 ab	37.0 ab	17.5ab	0.20 c	84	10.2 cd	41.6bcd	21.7 bc	0.31 c	88
5 mM GA	8.5 b	36.8 b	16.5 bc	0.20 c	96	9.7 d	43.4 abc	21.4bc	0.30 c	89
25 mM BA, 2.5 mM GA	9.0 ab	36.5 b	16.0 bc	0.56 c	74	11.2 ab	41.0 cd	21.1 bc	1.64 ab	73
50 mM BA, 5mM GA	8.7 ab	34.4 c	15.3c	0.52 c	39	10.5 cd	39.2 d	19.8c	0.96 bc	59
MSE <sup>y</sup>	0.81	11.93	4.32	0.73		0.81	11.33	7.22	0.87	

<sup>z</sup>Maximum pseudobulb height and diameter.

<sup>y</sup>Maximum number of leaves.

<sup>x</sup>Number of new shoots.

<sup>w</sup>Mean separation in columns by Tukey's multiple range test at  $P \leq 0.05$ . Any two means within a column not followed by the same letter are significantly different at  $P \leq 0.05$ .

<sup>y</sup>Mean square error.

inclusive of civil twilight are 14.1 and 11.75 h respectively. Civil twilight begins in the morning and ends in the evening when the center of the sun is 6° below the horizon. The monthly temperature mean was 19.0 to 21.9 °C, with minimum temperature ranges from 12.2 to 17.5 °C and maximum temperature ranges from 27.2 to 31.5 °C, with coolest temperatures in February and warmest in July or August.

Plants were clonally propagated in vitro, transplanted from flasks to rooting cubes for 8 months and transplanted to 10-cm pots in February through March 2003 and 2004 for experiments 1 and 2 respectively. Plants were grown in a mixture of 50% fir bark (1 to 1.3 cm particle size), 25% coconut husk (1.3 to 1.9 cm particle size, washed thoroughly in water) and 25% black volcanic cinder (1 to 1.3 cm particle size). Plants were fertilized with 0.8 to 1.6 g of Nutricote 13N–5.7P–10.8K plus micronutrients (Chisso-Asahi Fertilizer Co. Ltd., Tokyo, Japan) 1 month after transplanting and foliar fertilized and irrigated with 85 ppm N; 13.7 ppm P; 74.7 ppm K, twice a week through an overhead boom watering system. Pots were flushed with water once every 2 weeks to prevent salt build up.

Commercially, *Miltoniopsis* plants are shipped in various stages of inflorescence development and flower opening dependent upon customer preference. The first indication of flowering is inflorescence emergence. Thus, flowering in the study was considered to occur when the inflorescence emerged from the leaf axis and was visible without dissection (Lopez et al., 2005). *Miltoniopsis* plants typically flower approximately one year after being transplant to pots from rooting cubes and are approximately two years old since being transplanted from the flask. These inflorescences will be referred to as the first flowering season. Inflorescences from plants left in the pots for an additional year will be referred to as the second flowering season.

**Treatments.** (Expt. 1) On 12 Nov. 2003, 8 months after being transplanted to 10 cm pots (about 1.5 years from in vitro culture), 'Eileen' plants had an average of three pseudobulbs per pot and 'Akatsuka' plants had an average of two pseudobulbs per pot. BA (N<sup>6</sup>-benzyladenine) and GA<sub>3</sub> were dissolved

in 95% ethanol at the concentrations listed for each treatment, and distilled water was added to a final concentration of 10% ethanol. The potting medium of 'Eileen' and 'Akatsuka' plants was drenched with 50 mL of the following treatments; 10% ethanol water solution (control), BA (25 or 50 mM), GA<sub>3</sub> (2.5 or 5 mM) or combination of BA and GA<sub>3</sub> (50 mM BA and 5 mM GA<sub>3</sub> or 25 mM BA and 2.5 mM GA<sub>3</sub>) (Table 1). To ensure complete saturation of the potting medium with the plant growth regulator solution, excess solution that passed through the pot was collected and reapplied to the medium for a total of 3 times. Plants were monitored from 9 May 2003 to 21 June 2004 every 2 weeks for number of leaves, pseudobulb height from the soil line to the leaf tip, pseudobulb diameter perpendicular to the leaf axis and number of vegetative shoots or visible inflorescences. The experiment was a completely randomized design with 25 plants per treatment. The data were analyzed by ANOVA or t-test analysis using SigmaStat (Systat Software, Point Richmond, Calif.).

**Experiment 2.** On 17 Nov. 2004, the experiment was repeated to confirm the effect of 2.5 mM GA<sub>3</sub> observed in Expt. 1 and to determine if the 2.5 mM GA<sub>3</sub> treatment could promote flowering during the second flowering season. Since foliar damage and slight flower deformities was observed in all treatments containing 10% ethanol and not observed in other plants in the greenhouse, 2.5 mM GA<sub>3</sub> was dissolved in sodium hydroxide (NaOH) instead of ethanol for a final concentration of 0.026 N NaOH. The potting medium of 'Eileen' plants 8 months after transplant to 10 cm pots and 'Akatsuka' plants 1 year and 8 months after transplant to 10 cm pots were drenched with either 2.5 mM GA<sub>3</sub> or water containing 0.026 N NaOH as a control. Inflorescence emergence was monitored weekly, and inflorescence length and flower height and width were measured after all flowers on the inflorescence were fully opened. Days to inflorescence emergence was defined as the number of days until the first inflorescence was visible for each plant. The experiment was completely randomized with 40 plants per treatment. The data were analyzed by *t* test analysis using SigmaStat.

## Results

### Growth of *Miltoniopsis*

**Experiment 1.** *Miltoniopsis* plants are sympodial in growth. At the start of plant growth regulator treatments on 12 Nov. 2003, 'Akatsuka' plants had an average of two pseudobulbs per pot and 'Eileen' had three pseudobulbs per pot. The older pseudobulbs maintained a constant height and diameter although slight reduction was observed in the diameter before growth of new vegetative shoots from the basal buds. The pseudobulbs that produced the inflorescences observed in this study emerged in May through July of 2003. Growth of the immature vegetative shoots proceeded with simultaneous increase in number of leaves, pseudobulb height and diameter. Inflorescences emerged from the top one to four lateral leaf axes after the pseudobulb reached a maximum height and diameter. The pseudobulb height increased until it reached a maximum height, an average of 8 weeks before inflorescence emergence. Pseudobulb diameter continued to expand and reached a maximum diameter, an average of 4 weeks before inflorescence emergence.

### BA and GA<sub>3</sub> effect on plant stature and inflorescence emergence

Since flowering of *Miltoniopsis* is associated with growth of the orchid pseudobulb, we monitored the effect of the plant growth regulator treatments on each pseudobulb to determine the maximum number of leaves, pseudobulb height and diameter, and vegetative shoot emergence of each plant. Although we

Table 2. Number of days to inflorescence emergence for Bert Field 'Eileen' and Rouge 'Akatsuka' *Miltoniopsis* orchid hybrids following soil drenches with GA<sub>3</sub> treatments in 2004.

Treatment	'Eileen'	'Akatsuka'
Control	114.9 a <sup>z</sup>	183.0 a
2.5 mM GA	104.0 b	134.3 c
5 mM GA	107.9 ab	162.1 b
MSE <sup>y</sup>	102.1	494.0

<sup>z</sup>Mean separation in columns by Tukey's multiple range test at  $P \leq 0.05$ . Any two means within a column not followed by the same letter are significantly different at  $P \leq 0.05$ .

<sup>y</sup>Mean square error.

detected more leaves on BA treated 'Akatsuka' plants, the BA and GA<sub>3</sub> treatments did not significantly change or decrease the maximal height and diameter of the pseudobulbs in both 'Eileen' and 'Akatsuka' plants (Table 1). Application of BA treatments consistently increased vegetative shoot production in 'Eileen' treated with 25 mM BA (2.71 shoots) and 50 mM BA (1.86 shoots) and 'Akatsuka' plants treated with 25 mM BA (1.89 shoots) and 50 mM BA (1.74 shoots). These vegetative shoots developed normally into pseudobulbs and did not produce inflorescences during the course of the experiment. The pseudobulbs that did produce inflorescences were similar to those in control plants that emerged during May to June of 2003. BA treatments resulted in a decrease in the number of plants with inflorescences and this decrease in flowering was alleviated by the addition of GA<sub>3</sub>. Of 'Eileen' plants treated with 25 mM BA, 58% produced inflorescences and the addition of 2.5 mM GA<sub>3</sub> resulted in 74% plants with inflorescences. Treatment with 25 or 50 mM BA resulted in 38% and 44% of 'Akatsuka' plants with inflorescences. For the same BA treatments supplemented with 2.5 and 5.0 mM GA<sub>3</sub> respectively, the number of plants with inflorescences increased to 73% and 59% (Table 1). However, with the addition of GA<sub>3</sub> the number of plants with inflorescences was still less than the control suggesting that BA inhibited inflorescence emergence and this was alleviated by GA<sub>3</sub>.

#### GA<sub>3</sub> effect on inflorescence emergence

**Experiment 1.** Inflorescences were produced by 96% of 'Eileen' control plants and 5 mM GA<sub>3</sub> treated plants while 84% of the plants treated with 2.5 mM GA<sub>3</sub> (Table 1). Of 'Akatsuka' plants treated with 0 (control) or 2.5 mM GA<sub>3</sub>, 88% to 89% produced inflorescences. The GA<sub>3</sub> treatments were the only treatments that did not significantly alter the total number of plants with inflorescences. Therefore, we determined if GA<sub>3</sub> affected the number of days from treatment to inflorescence emergence (Table 2). The inflorescence of 'Eileen' plants treated with 2.5 mM GA<sub>3</sub> emerged an average of 10.9 d earlier than untreated control plants. The effect of GA<sub>3</sub> on 'Akatsuka' plants was greater, with inflorescences emerging from 2.5 and 5 mM GA<sub>3</sub> treated plants, 48.7 and 20.9 d earlier than control.

Comparing the percentage of plants with inflorescences to the total number of treated plants, we observed that on 17 Feb. 2004 only 4% of the control 'Eileen' plants had inflorescences relative to 56% of plants treated with 2.5 mM GA<sub>3</sub> and 24% of 5mM GA<sub>3</sub> treated plants (Fig. 1). By 29 Mar. 2004 there was no difference between the control and 5 mM GA<sub>3</sub> treatments (96% plants with inflorescences). Inflorescences of 'Akatsuka' plants treated with 2.5 mM GA<sub>3</sub> emerged on 15 Mar. 2004 at 69% while inflorescences of control plants did not emerge until 29 Mar. 2004 at 4% (Fig. 2). By 7 June 2004 the number of flowering plants of control (88%) was similar to those observed in 2.5 mM (88%) and 5 mM (89%) GA<sub>3</sub> treated plants (Fig. 2). These data suggest that GA<sub>3</sub> treatments did not affect the overall

flowering but reduced the time for inflorescence emergence.

**Experiment 2.** To determine if the effect of GA<sub>3</sub> on inflorescence emergence is reproducible, we treated 40 'Eileen' plants approaching the first flowering season with 2.5 mM GA<sub>3</sub>. 'Akatsuka' plants approaching the first flowering season were not available for experimentation. Therefore, we treated 40 plants approaching the second flowering season to determine if 2.5 mM GA<sub>3</sub> would have the same effect on promoting inflorescence emergence on these older plants. Inflorescences of 'Eileen'

emerged on 2 Feb. 2005 with 63% of the GA<sub>3</sub> treated plants with visible inflorescences compared to 37% of the control plants (Fig. 3). 'Akatsuka' inflorescence emergence commenced on 28 Mar. 2005 with 20% of the GA<sub>3</sub> treated plants with inflorescences and none of the control plants. Inflorescence emergence was observed 1 week earlier in 'Akatsuka' plants treated with GA<sub>3</sub>. Inflorescences emerged from 'Akatsuka' control plants an average of 161.7 d after treatment compared 155.1 d for plants treated with 2.5 mM GA<sub>3</sub>. However, this difference was not significant ( $P = 0.13$ ). The

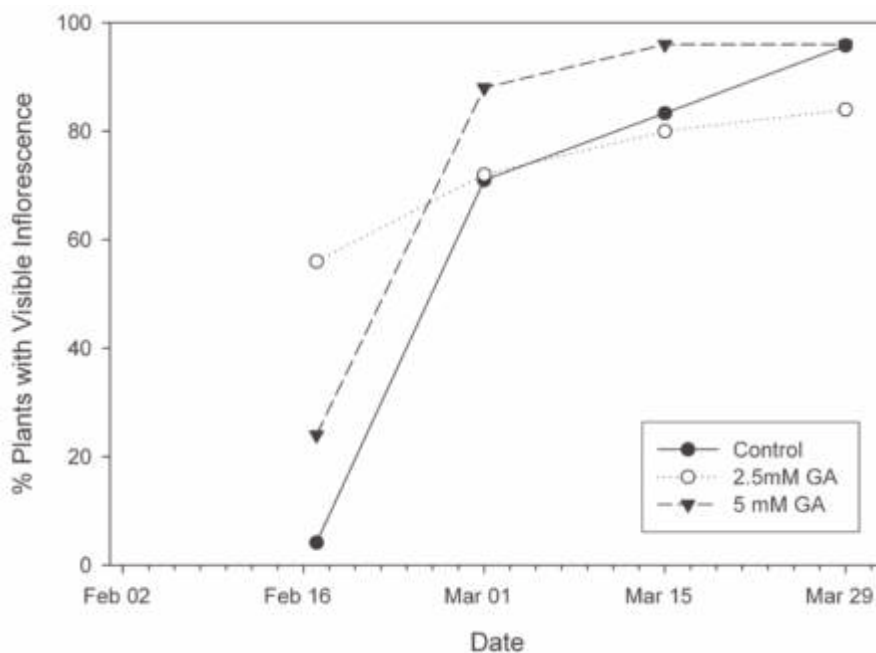


Fig. 1. Percentage of Bert Field 'Eileen' plants with visible inflorescences following treatment with 0 (control), 2.5, or 5 mM GA<sub>3</sub> soil drenches on 12 Nov. 2003. Plants were monitored every 2 weeks from 17 Feb. 2004 to 29 Mar. 2004.

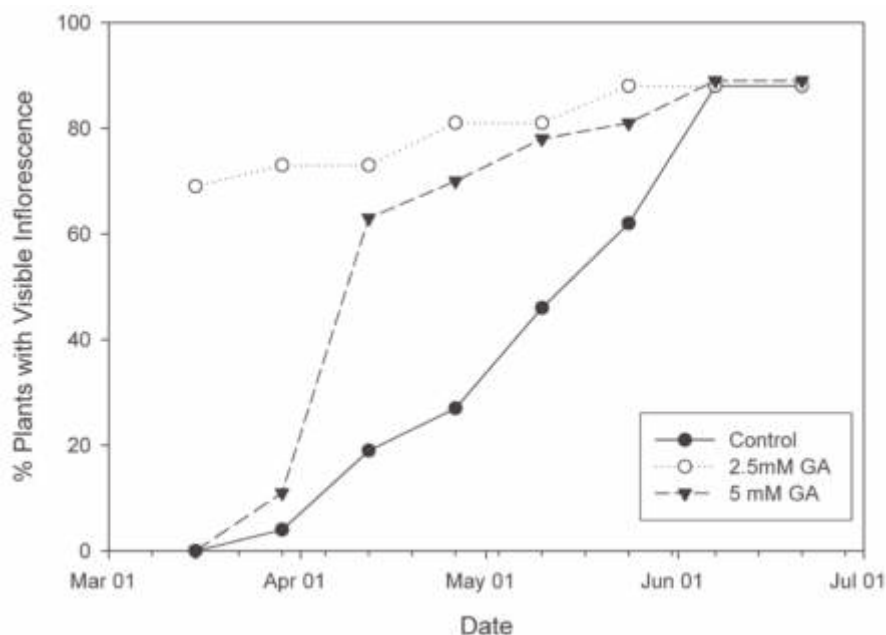


Fig. 2. Percentage of Rouge 'Akatsuka' plants with visible inflorescences following treatment with 0 (control) or 2.5 mM GA<sub>3</sub> soil drenches on 12 Nov. 2003. Plants were monitored every 2 weeks from 1 Mar. 2004 to 12 June 2004.

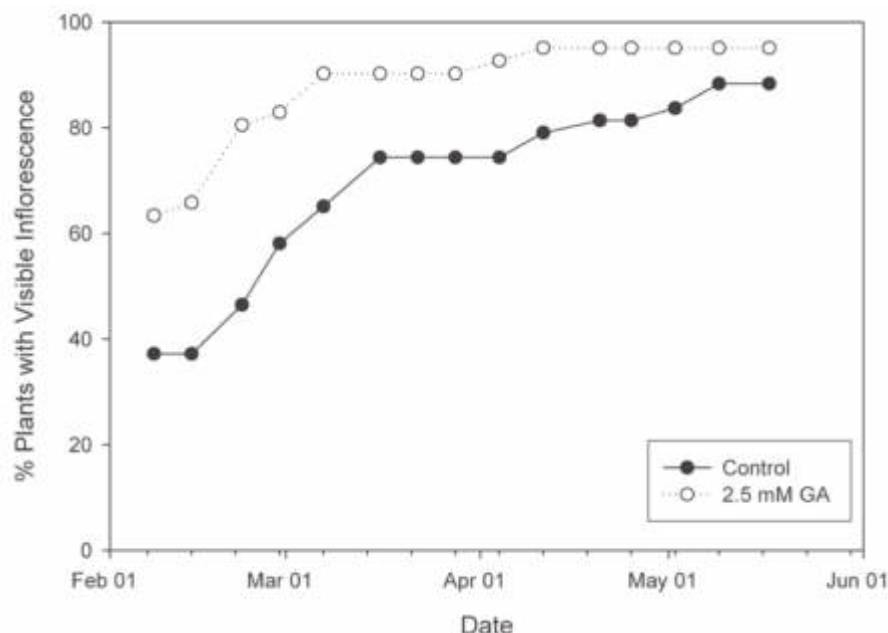


Fig. 3. Percentage of Bert Field 'Eileen' plants with visible inflorescences following treatment with 0 (control) or 2.5 mM GA<sub>3</sub> soil drenches on 17 Nov 2004. Plants were monitored weekly from 8 Feb. 2005 to 17 May 2005.

number of inflorescences per plant was also not significantly different in control plants, with 2.9 inflorescences per plant compared 3 inflorescences per plant for GA<sub>3</sub> treated plants ( $P = 0.78$ ). These data suggest that the 2.5 mM GA<sub>3</sub> does promote inflorescence emergence of *Miltoniopsis* plants approaching the first flowering season.

The mean number of days to inflorescence emergence of 'Eileen' was reduced to 91.2 d in plants treated with 2.5 mM GA<sub>3</sub>, an average of 14.5 d less than control plants which flowered in 105.7 d ( $P = 0.006$ ). The number of inflorescences per plant was increased in 'Eileen' plants treated with GA<sub>3</sub> to an average of 3 inflorescences per plant compared to 2.2 inflorescences per control plant ( $P = 0.02$ ). Flower deformities were not observed in plants treated with GA<sub>3</sub> and no significant differences were observed in the length of the inflorescence or in the width and length of the flower (data not shown).

## Discussion

A soil drench application of 2.5 mM GA<sub>3</sub> effectively reduced the time of inflorescence emergence in 'Eileen' by 10.9 (Expt. 1) or 14.5 (Expt 2) d compared to the controls and in 'Akatsuka' by 48.7 d (Expt. 1) during the first flowering season. However, this treatment was ineffective in hastening inflorescence emergence in 'Akatsuka' plants during the second flowering season. Since these plants contain a greater number of pseudobulbs per plant compared to plants in the first flowering season, allocation of the GA<sub>3</sub> may result in less GA<sub>3</sub> per pseudobulb and the GA<sub>3</sub> effect on inflorescence emergence may be decreased. Higher concentrations of GA<sub>3</sub> may be required to promote inflorescence emergence in older plants with a greater number of pseudobulbs per pot.

Promotion of inflorescence emergence by GA<sub>3</sub> is more effective in 'Akatsuka' plants than 'Eileen' plants. Differences in the effectiveness of GA on inflorescence emergence of these grexes may be due to the differences in the response to temperature during floral development as a result of the different genetic backgrounds. Previous reports indicate that flowering in *Miltoniopsis* Augres 'Trinity' is promoted by short days and cool temperatures (Lopez et al., 2005). The natural flowering season for 'Akatsuka' (mid Mar. through June) is slightly later than the natural flowering season of 'Eileen' (February to May), and at a time of year where the average daily temperature is increasing. This may be similar to the situation in *Phalaenopsis* where GA applications effectively promoted the development of flowers during normally inhibitory high temperatures. This was due to the promotion of longitudinal growth of the flower primordium by GA<sub>3</sub> application (Chen et al., 1994, 1997). Treatment of *Phalaenopsis* flowering shoots with GA<sub>3</sub> at warmer temperatures increases levels of the active GA<sub>1</sub> required for the promotion of flower development to the same extent as levels found in flowering shoots grown at cool temperatures (Su et al., 2001).

GA treatments of certain *Cypripedium* and *Cattleya* plants resulted in earlier flowering compared to control plants (Smith, 1958). Growth retardants known to inhibit GA synthesis or antagonize GA effects such as, paclobutrazol and uniconazole, progressively delayed flowering of *Phalaenopsis* orchid plants at increasing concentrations of both retardants during the second bloom season (Wang and Hsu, 1994). GA promotes flower development in *Arabidopsis* by inhibiting the DELLA repressor proteins and increasing the expression of floral homeotic genes, APETALA3 (AP3), PISTILLATA (PI) and

AGAMOUS (AG) (Yu et al., 2004). MADS-box genes, which are often associated with the floral transition of the meristem, have been isolated from *Aranda* 'Deborah' using AG as a probe (Lu et al., 1993) and by differential display analysis in *Dendrobium* (Yu and Goh, 2000a, 2000b; reviewed in Mudalige and Kuehnle, 2004) suggesting a similar GA pathway found in *Arabidopsis* may exist in orchids.

Applications of GA<sub>3</sub> (Chen et al., 1997) or BA (Sakai et al., 2000) treatment to increase flowering often results in deformed flowers and usually require the combination of both for normal flower development. However, we observed that low dose (2.5 mM) of GA<sub>3</sub> is effective in promoting flowering without deleterious effects to flower development. Lack of flower deformities may also be attributed to the GA<sub>3</sub> treatment being applied months before flower development. This long period between treatment and response has been previously observed in *Cypripedium* × *Glenarm* and *Cypripedium* × *Maudiae* (Smith, 1958).

Application of BA treatments promoted vegetative growth instead of flowering and this effect was alleviated by the addition of GA<sub>3</sub>. We observed that the majority of flowering pseudobulbs did not produce new vegetative shoots, and in pseudobulbs that did produce new vegetative shoots inflorescence emergence was delayed. In *Phalaenopsis*, flower primordial elongation promoted by GA<sub>3</sub> is fully prevented by BA treatments (Chen et al., 1997).

A working hypothesis of flowering in *Miltoniopsis* may be that GA<sub>3</sub> application can promote uniform inflorescence emergence through the increase in GA in the floral meristem and prevents the development of vegetative shoots however; this will require further investigation. From this study we conclude that GA<sub>3</sub> alone is effective in promoting inflorescence emergence in *Miltoniopsis*.

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