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Benzyladenine Promotes Flowering in *Doritaenopsis* and *Phalaenopsis* Orchids

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Abstract Two experiments were performed to determine how application of the cytokinin benzyladenine (BA) influenced flowering in *Doritaenopsis* and *Phalaenopsis* orchid clones. In the first experiment, two vegetative orchid clones growing in 15-cm pots were transferred from a 28°C greenhouse that inhibited flowering to a 23°C greenhouse for flower induction (day 0). A foliar spray (0.2 L m^{-2}) containing BA at 100, 200, or 400 mg L^{-1} or 25, 50, or 100 mg L^{-1} each of BA and gibberellins $A_4 + A_7$ (BA+GA) was applied on days 0, 7, and 14. Plants treated with BA alone at 200 or 400 mg L^{-1} had a visible inflorescence 3–9 days earlier and had a mean of 0.7–3.5 more inflorescences and 3–8 more flowers per plant than non-treated plants. The application of BA+GA had no effect on inflorescence number and total flower number at the rates tested. In the second experiment, three orchid clones received a single foliar spray of BA at 200 mg L^{-1} at six time points relative to time of transfer from 29°C to 23°C (−1, 0, +1, +2, +4, or +6 weeks). A separate group of plants received a BA application at week 0 but was maintained at 29°C. Inflorescence number was greatest in all three orchid clones when plants were treated with BA 1 week after the temperature transfer. Plants that were sprayed with BA and maintained at 29°C did not initiate inflorescences. The promotion of flowering by the application of BA suggests that cytokinins at least partially regulate inflorescence initiation of *Doritaenopsis* and *Phalaenopsis*, but its promotion is conditional and BA application cannot completely substitute for an inductive low temperature.

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Introduction

Cytokinins have been implicated in a wide array of physiologic and biochemical processes, including seed germination (Stirk and others 2005), cell division (Zhang and others 2005), organ formation and regeneration (Al-Ramamneh and others 2006), suppression of apical dominance (Bangerth and others 2000), floral development (Taylor and others 2005), senescence of plant organs (Sergiev and others 2007), and long-distance nitrogen signaling (Cline and others 2006). Studies have also reported a role of cytokinins during phloem loading and unloading and an effect on assimilate movement (Thomas and Blakesley 1987).

Flower induction in plants is controlled by primary and secondary environmental factors such as photoperiod, temperature, irradiance, and water availability (Bernier and Périlleux 2005). These environmental cues can promote the synthesis of a floral stimulus that is transported through vascular tissues to the shoot apical meristem and subsequently induces flower initiation (Bernier and Périlleux 2005). Cytokinins are suggested to act as one of the multifactorial components that function as the floral stimulus (Bernier and others 1993). Concentrations of cytokinins have been reported to increase in the apical meristem during floral transition, flower development, or both, in many species, including *Arabidopsis thaliana* L. (Corbesier and others 2003), *Boronia megastigma* Nees (Day and others 1995), *Chenopodium rubrum* L. (Macháčková and others 1993), *Nicotiana tabacum* L. (Dewitte and others 1999), *Sinapis alba* L. (Jacqmard and others 2002), and *Tillandsia recurvata* (L.) L. (Mercier and Endres 1999).

Studies with cytokinin mutants and transgenic plants have revealed a possible role of cytokinins in flowering. Transgenic *N. tabacum* plants deficient in cytokinins flowered 3 months later than wild-type plants, and there was no difference in leaf number at the onset of flowering (Werner and others 2001). In *A. thaliana*, a mutant with elevated cytokinins flowered 4 days earlier than wild-type plants (Chaudhury and others 1993). In a late-flowering mutant of *Lycopersicon esculentum* Mill., explants grown *in vitro* with benzyladenine (BA) flowered earlier and developed fewer leaves before flowering than nontreated explants (Dielen and others 2001). Some reports suggest that cytokinins influence flower development by regulating genes that control functions in the shoot apical meristem (Lindsay and others 2006). For example, in the short-day plant *S. alba*, exposure to 1 long day or application of cytokinins activated the transcription of the MADS box *SaMADS A* gene in the shoot apical meristem, a gene involved in the transition to flowering (Bonhomme and others 2000).

The application of exogenous cytokinins on plants has been shown to induce or promote earlier flowering in several species, generally under environmental conditions that are just above the threshold for flower induction. For example, the addition of cytokinins to the culture medium of the short-day plant *Lemna paucicostata* Hegelm. promoted flowering in plants grown under long days (Gupta and Maheshwari 1970). In *B. megastigma*, application of BA at the time of transfer to low temperature decreased time from flower initiation to anthesis by 8 weeks (Day and others 1994). However, the promotion of flowering by cytokinin treatments is variable and species-specific.

The genus *Phalaenopsis* Blume consists of approximately 40 species that are native to tropical and subtropical habitats of northern Australia and Asia (Baker and Baker 1991). These orchids are produced commercially in greenhouses as flowering potted plants and are among the most valuable floriculture crops produced in North America and Europe (Nash 2003; US Department of Agriculture 2007; Vereniging van Bloemenveilingen in Nederland 2007). Flower induction in *Phalaenopsis* is primarily regulated by exposure to low temperature. Flowering is inhibited in plants grown at 28°C or above (Sakanishi and others 1980), and inflorescence initiation is promoted in plants after exposure to a day temperature below 26°C (Lee and Lin 1987; Blanchard and Runkle 2006). After inflorescence emergence, there is a linear relationship between increasing temperatures from 14°C to 26°C and the rate of development toward visible bud and open flower (Robinson 2002).

The application of cytokinins has been reported to promote flowering when applied to *Phalaenopsis* and *Doritaenopsis* orchids (Yoneda and Momose 1990; Ichihashi 1997; Kim and others 2000). For example, a foliar spray application of BA or BA+GA_3 on *Phalaenopsis* after transfer to highland (400–500 m above sea level) culture for flowering accelerated inflorescence emergence and decreased time to flower by 13–25 days (Yoneda and Momose 1990). Kim and others (2000) reported that in *Doritaenopsis*, application of BA at the onset of low-temperature exposure (23/18°C day/night) increased the mean number of inflorescences per plant by 0.2 compared to that of nontreated plants.

These investigations indicate that flower induction in *Phalaenopsis* could be regulated by endogenous cytokinins, and the application of exogenous cytokinins during low-temperature exposure may hasten the rate of inflorescence initiation and increase the number of inflorescences per plant. However, the lack of controlled experimental procedures and environments has made it difficult to infer conclusions about the possible role of cytokinins in flower induction in *Phalaenopsis*. For example, it is unknown whether exogenous cytokinins regulate flowering in *Phalaenopsis* when grown at a warm, noninductive temperature. The objective of this study was to determine how plant growth regulators that contain the cytokinin BA, and their application concentration and timing, influence flowering of potted *Phalaenopsis* and *Doritaenopsis* orchids.

Materials and Methods

Effects of Growth Regulators and Application Concentration on Flowering (Experiment 1)

Plant material, year 1 Vegetatively propagated plants of *Phalaenopsis* Brother Apollo '070' and *Phalaenopsis* Golden Treasure '470' were grown in a commercial greenhouse in California, USA, in 11-cm round pots (0.57-L volume) and in a medium containing 75% fine-grade Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) bark, 15% medium-grade perlite, and 10% sphagnum moss (by volume). Plants were grown at 26°C under a natural photoperiod (latitude 37°N) and a maximum photosynthetic photon flux (*PPF*) of 280 μmol m^{-2} s^{-1}. On 22 September 2004, 100 plants of each hybrid were received in East Lansing, Michigan, and were transplanted into 15-cm round pots (1.3-L volume) in a medium consisting of 33% medium-grade Douglas fir bark (Rexius Forest By-Products Inc., Eugene, OR, USA), 45% medium-grade chopped coconut (*Coco nucifera* L.) coir (Millenniumsoils Coir, St. Catharines, Ontario, Canada), 11% long-fiber Canadian sphagnum moss (Mosser Lee Co., Millston, WI, USA), and 11% coarse-grade perlite (OFE Intl. Inc., Miami, FL, USA) (by volume). Plants were grown in a glass-glazed greenhouse at a constant temperature of 28°C to inhibit

flowering. The photoperiod was a constant 16 h (0600 to 2200 HR) consisting of natural photoperiods (latitude 43°N) with day-extension lighting provided by high-pressure sodium lamps delivering a *PPF* of 20–25 μmol m^{-2} s^{-1} at plant height (as measured with a line quantum sensor [Apogee Instruments, Inc., Logan, UT, USA]). Light transmission was reduced using woven shade curtains (OLS 50; Ludvig Svensson Inc., Charlotte, NC, USA) and whitewash applied to the greenhouse glazing so that the maximum *PPF* at plant height was 300 μmol m^{-2} s^{-1}. A vapor-pressure deficit (VPD) of 0.9 kPa was maintained by the injection of water vapor into the air. The mean leaf span of plants was 32–40 cm at the beginning of the experiment. Leaf span was measured by extending the longest opposing leaf on each plant to a horizontal position and then measuring the length from one leaf tip to the opposite leaf tip.

Plant material, year 2 On 27 May 2005, 100 nonflowering plants of *Phalaenopsis* Brother Apollo '070' grown in 15-cm pots (1.5-L volume) in a medium consisting of 90% chunky peat and 10% medium-grade pine (*Pinus* spp.) bark (by volume) were shipped to East Lansing, Michigan, from a commercial greenhouse in Florida, USA. Plants were grown in a glass-glazed greenhouse at a constant temperature of 29°C. The photoperiod, maximum *PPF*, and VPD were maintained as previously described during year 1. The mean leaf span was 46 cm at the beginning of the experiment.

Chemical treatments Plants of each *Phalaenopsis* clone were transferred to a glass-glazed greenhouse that was at a constant temperature of 23°C for flower induction and were randomly assigned to treatments containing ten plants. Plants received a foliar spray (0.2 L m^{-2} [20–25 ml per plant]) of N^6-BA at 100, 200, or 400 mg L^{-1} (BAP-10, Plant-Wise Biostimulant Co., Louisville, KY, USA; BA1) or N^6-BA at 100, 200, or 400 mg L^{-1} (SixPro, Valent de Mexico, Zapopan, Jal., Mexico; BA2) or a solution containing 25, 50, or 100 mg L^{-1} each of N^6-BA and GAs $A_4 + A_7$ (Fascination, Valent USA Corp., Walnut Creek, CA, USA; BA+GA). For each treatment, three spray applications were made at 2 h, 7 days, and 14 days after transfer to 23°C. A surfactant was included in all spray solutions at 1.2 g L^{-1} (Capsil, Scotts-Sierra Crop Protection, Marysville, OH, USA). A group of ten control plants of each clone was transferred to 23°C but received no chemical application. The experiment began on 23 November 2004 (year 1) with both hybrids and was repeated on 23 October 2005 (year 2) with *Phalaenopsis* Brother Apollo '070.'

Effects of BA Application Time on Flowering (Experiment 2)

Plant material On 22 November 2005, vegetatively propagated plants of *Doritaenopsis* 'Alice Girl' (*Phalaenopsis* Rose Girl × *Doritaenopsis* Memoria Alice Ainsworth), *Doritaenopsis* 'Malibu Chablis' (*Phalaenopsis* Ramirito × *Doritaenopsis* Duki), and *Phalaenopsis* 'Pink Twilight' (*Phalaenopsis* Rose Girl × *Phalaenopsis* Lippeglut) were shipped to East Lansing, Michigan, from a commercial greenhouse in California. Plants were grown in 11-cm round pots (0.6-L volume) and in a medium containing 75% fine-grade Douglas fir bark, 15% medium-grade perlite, and 10% sphagnum moss (by volume). Plants were grown in a glass-glazed greenhouse at a constant temperature of 29°C. The photoperiod, maximum *PPF*, and VPD were maintained as previously described for experiment 1. The mean leaf span was 41–47 cm at the beginning of the experiment.

Chemical treatments On 16 December 2005, plants were transferred to a glass-glazed greenhouse at a constant temperature of 23°C for flower induction. A single foliar spray of BA1 at 200 mg L^{-1} and a volume of 0.2 L m^{-2} (20–25 ml per plant) was applied at six time points relative to time of transfer from 29°C to 23°C (−1, 0, +1, +2, +4, or +6 weeks). An additional treatment consisted of the BA application at week 0, but plants were maintained at 29°C for the duration of the experiment. The same surfactant was included in all spray applications, as previously described. Control plants were transferred to 23°C at week 0 but received no BA application. Ten plants of each orchid clone were used in each treatment.

Greenhouse Environment

In both experiments, air temperature was measured by an aspirated thermocouple (0.127-mm type E) every 10 s, and hourly means were recorded by a data logger (CR10, Campbell Scientific, Logan, UT, USA). The actual mean air temperature and daily light integral at plant level per 4-week period were determined (Table 1). Plants were irrigated as necessary with reverse-osmosis water supplemented with a water-soluble fertilizer providing (mg L^{-1}): 125 N, 12 P, 100 K, 65 Ca, 12 Mg, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special, GreenCare Fertilizers, Inc., Kankakee, IL, USA).

Data Collection and Analysis

The date the first inflorescence was visible without dissection (0.1–0.5 cm) and the date that the first flower opened were recorded for each plant. Open flower was defined as when the two lateral petals were completely reflexed. Days to visible inflorescence (VI), days from VI to open flower, and days to open flower from the date of transfer to 23°C were calculated for each treatment. The total number of VI, number of flowers on the first VI, and total number of flowers were recorded for each plant. On

Table 1 Actual Mean Daily Air Temperature and Light Integral at Plant Height per 4-Week Period for Each Experiment

Experiment	Year	Actual mean air temperature (°C)	Daily light integral (mol m^{-2} d^{-1}) per 4-week period				
			1	2	3	4	Mean
1	1[x]	23.3	4.5	4.8	5.4	4.3	4.8
	2[y]	23.3	2.9	3.8	2.9	4.1	3.4
2	2[z]	23.9	2.8	3.7	4.9	4.4	4.0

[x] Period from 23 November 2004 to 14 March 2005

[y] Period from 23 October 2005 to 11 February 2006

[z] Period from 16 December 2005 to 6 April 2006

the date of open flower, the diameter of the first open flower (the distance between the outside edges of opposing petals) and inflorescence length (distance from inflorescence emergence to the first flower plus the distance from the first flower to the inflorescence tip) were measured.

A completely randomized design and a completely randomized block design were used for experiments 1 and 2, respectively. Data were analyzed using a SAS (SAS Institute, Inc., Cary, NC, USA) mixed-model procedure (PROC MIXED), and pairwise comparisons between treatments were performed using Tukey's honestly significant difference test at $P \leq 0.05$.

Results

Effects of Growth Regulators and Application Concentration on Flowering (Experiment 1)

The application of BA1 or BA2 increased the number of inflorescences per plant in both *Phalaenopsis* clones (Figure 1). Brother Apollo '072' and Golden Treasure '470' had a mean of 1.0 and 3.0 more inflorescences than nontreated plants, respectively, after application of BA1 or BA2 at 200 or 400 mg L^{-1}. There were no differences in inflorescence number between application concentrations of 200 and 400 mg L^{-1} for either chemical. In both *Phalaenopsis* clones, plants treated with BA+GA at all concentrations had a statistically similar inflorescence number compared with that of control plants.

Application of BA1 or BA2 accelerated time to first VI by 3–9 days compared with that of nontreated plants in both *Phalaenopsis* clones, regardless of concentration (Table 2). In *Phalaenopsis* Brother Apollo '072' there were no significant differences in time to VI among plants treated with BA+GA and control plants. Time from VI to open flower ranged from 72 to 81 days and was not significantly different among treatments for both *Phalaenopsis* clones. Application of BA1 at 200 or 400 mg L^{-1} or BA2 at 400 mg L^{-1} to *Phalaenopsis* Brother Apollo '072' increased total flower number by three to five flowers compared with that of nontreated

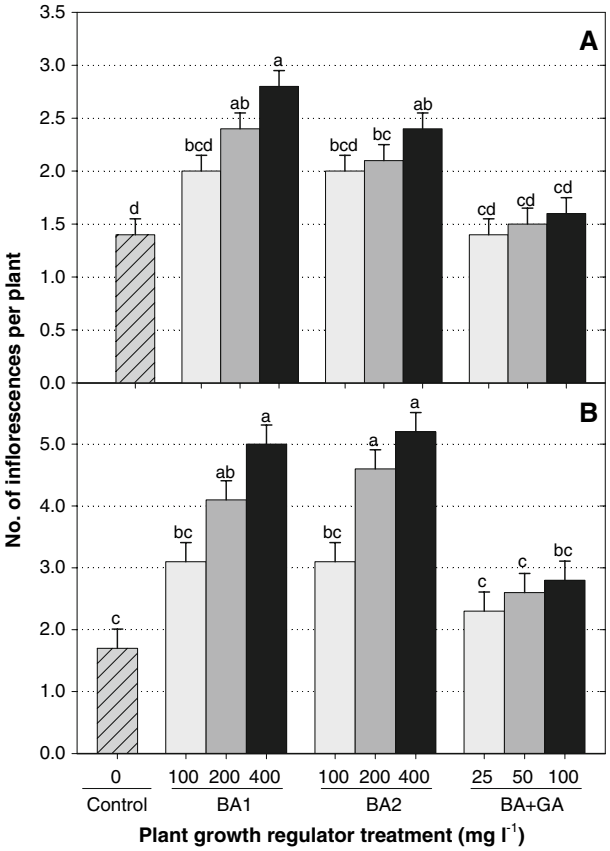

Fig. 1 Effects of plant growth regulator treatments on the number of inflorescences per plant in *Phalaenopsis* Brother Apollo '072' (**A**) and Golden Treasure '470' (**B**). Plants received a foliar spray of commercial products containing benzyladenine at 100, 200, or 400 mg L^{-1} (BA1 or BA2) or a product containing 25, 50, or 100 mg L^{-1} each of benzyladenine and gibberellins $A_4 + A_7$ (BA+GA) at 2 h, 7 days, and 14 days after transfer from 29°C to 23°C. Mean separation by Tukey's honestly significant difference test at $P \leq 0.05$. Vertical bars represent standard errors among the treatment means

plants (Table 3). In *Phalaenopsis* Golden Treasure '470,' plants treated with BA1 or BA2 at 200 or 400 mg L^{-1} had a mean of seven more flowers per plant than control plants. Total flower number was similar among plants treated with BA1 or BA2 at 200 or 400 mg L^{-1} for both *Phalaenopsis* clones. The width of the first open flower in both clones

Table 2 Effects of Plant Growth Regulator (Benzyladenine [BA] and Gibberellins $A_4 + A_7$ [GA_{4+7}]) Foliar Sprays on Time to Visible Inflorescence (VI) and Time from VI to Open Flower in *Phalaenopsis* Brother Apollo '072' and Golden Treasure '470'

Plant growth regulator treatment			Days to VI		Days from VI to flower	
Product	BA (mg L^{-1})	GA_{4+7} (mg L^{-1})	Brother Apollo	Golden Treasure	Brother Apollo	Golden Treasure
Control	0	0	20 a[z]	22 a	72	78
BA1	100	0	16 bcd	15 b	72	77
	200	0	17 bcd	14 b	72	79
	400	0	14 d	14 b	74	81
BA2	100	0	15 bcd	15 b	73	79
	200	0	14 cd	13 b	73	81
	400	0	14 d	15 b	73	81
BA+GA	25	25	18 abc	17 b	73	80
	50	50	18 ab	15 b	74	79
	100	100	18 ab	14 b	73	78
Significance			***	***	ns	ns

ns = nonsignificant; *** significant at $P \leq 0.001$

[z] Mean separation within columns by Tukey's honestly significant difference test at $P \leq 0.05$

was 0.6–0.9 cm smaller when plants were treated with BA1 at 200 or 400 mg L^{-1}. Flower width was similar among nontreated plants and plants treated with BA2 or BA+GA.

Inflorescence length of *Phalaenopsis* Brother Apollo '072' treated with BA1 at 200 or 400 mg L^{-1} or BA2 at all concentrations was a mean of 9.8 cm shorter than that of control plants (Table 3). In *Phalaenopsis* Golden Treasure '470,' plants treated with BA1 or BA2 at 400 mg L^{-1} had inflorescences that were a mean of 9.0 cm shorter compared with that of nontreated plants. There were no differences in inflorescence length at first open flower among plants treated with BA+GA and control plants.

Effects of BA Application Time on Flowering (Experiment 2)

The number of inflorescences was generally greatest when plants were treated with BA1 at 1 week after transfer from 29°C to 23°C (Figure 2). None of the plants that were sprayed with BA1 and maintained at 29°C initiated inflorescences and thus data were not included in the statistical analysis. In *Doritaenopsis* 'Malibu Chablis,' plants treated with BA1 at 1 week before transfer, at transfer, or 1 or 2 weeks after transfer to 23°C reached VI a mean of 10 days earlier than nontreated plants (Table 4), but there was no effect on the other two clones. The mean time from VI to open flower in all three orchid clones ranged from 65 to 80 days, but there were no significant differences between nontreated plants and any BA1 application time.

In *Doritaenopsis* 'Alice Girl,' plants treated with BA1 at 1, 2, 4, or 6 weeks after transfer had a mean of 5.8 more total flowers than nontreated plants (Table 4). In *Doritaenopsis* 'Malibu Chablis,' BA1 applied at 1 week after transfer increased total flower number per plant by 8.1 compared with that of control plants at 23°C. Application time had no effect on the number of flowers per plant in *Phalaenopsis* 'Pink Twilight,' and mean flower number ranged from 6.7 to 8.5. The width of the first open flower was shorter (by 0.6–1.2 cm) in *Doritaenopsis* 'Alice Girl' and 'Malibu Chablis' treated with BA1 at 1 or 4 weeks after transfer compared with that of nontreated plants. Flower width of *Phalaenopsis* 'Pink Twilight' was similar among all treatments.

The effects of BA1 application time on total inflorescence length at open flower varied among orchid hybrids. In *Doritaenopsis* 'Alice Girl,' inflorescences on plants treated at 2, 4, or 6 weeks after transfer were 8.5 cm longer than those of control plants (Table 4). In *Doritaenopsis* 'Pink Twilight,' BA1 applied 6 weeks after transfer increased inflorescence length by 12.1 cm compared with that of control plants. Application time had no effect on inflorescence length in *Phalaenopsis* 'Malibu Chablis.'

Discussion

In both experiments, the application of the cytokinin BA had a strong promotive effect on inflorescence initiation in all of the *Doritaenopsis* and *Phalaenopsis* clones studied. However, the response to BA depended on the plant growth regulator and application concentration (Experiment 1), application time (Experiment 2), and orchid clone. In *Phalaenopsis* Brother Apollo '072' and Golden Treasure '470,' three applications of BA1 or BA2 at 400 mg L^{-1} nearly doubled and tripled inflorescence number compared with that of nontreated plants, respectively. Lower concentrations of BA had a less promotive effect on stimulating inflorescence initiation. The difference in the magnitude of the responses between these clones could be at least partially attributed to their varied genetic backgrounds. The application of cytokinins also increased the number of flower buds or inflorescences in other species

Table 3 Effects of Plant Growth Regulator (Benzyladenine [BA] and Gibberellins $A_4 + A_7$ [GA_{4+7}]) Foliar Sprays on Total Flower Number per Plant, Width of the First Open Flower, and Total Inflorescence Length in *Phalaenopsis* Brother Apollo '072' and Golden Treasure '470'

Plant growth regulator treatment			Avg. total flower No.		Flower width (cm)		Total inflorescence length (cm)	
Product	BA (mg L^{-1})	GA_{4+7} (mg L^{-1})	Brother Apollo	Golden Treasure	Brother Apollo	Golden Treasure	Brother Apollo	Golden Treasure
Control	0	0	9.3 c[z]	8.1 c	9.2 ab	7.3 a	56.8 a	41.9 a
BA1	100	0	12.1 abc	11.7 abc	9.1 ab	7.1 ab	52.6 abc	36.4 ab
	200	0	13.0 ab	16.1 a	8.6 bc	6.7 bc	46.2 c	34.9 ab
	400	0	14.4 a	13.7 ab	8.3 c	6.4 c	46.4 c	32.5 b
BA2	100	0	11.8 abc	12.5 abc	8.9 abc	7.1 ab	47.7 bc	38.5 ab
	200	0	12.3 abc	14.7 ab	9.0 abc	7.1 a	47.1 bc	36.5 ab
	400	0	14.0 a	13.9 ab	8.7 abc	7.0 ab	47.8 bc	33.4 b
BA+GA	25	25	9.8 bc	10.9 bc	9.3 a	7.4 a	55.1 ab	41.5 a
	50	50	9.5 c	10.9 bc	9.1 ab	7.3 a	50.0 abc	41.1 a
	100	100	9.8 bc	10.2 bc	9.1 ab	6.9 ab	51.5 abc	37.9 ab
Significance			***	***	***	***	**	**

** Significant at $P \leq 0.01$; *** significant at $P \leq 0.001$

[z] Mean separation within columns by Tukey's honestly significant difference test at $P \leq 0.05$

including *Aranda* (Goh 1977), *Dendrobium* (Sakai and others 2000), *Hatiora gaertneri* [Reg.] Barthlott. (Ho and others 1985), and *Schlumbergera truncata* [Haw.] Moran. (Boyle 1992, 1995).

The increased number of inflorescences in our study was greater than the increase (0.2 inflorescences per plant) reported by Kim and others (2000). The different responses between these studies could be related to the number and frequency of chemical applications. Kim and others (2000) made two applications of BA at 10-day intervals, beginning on the day of transfer to a low-temperature regimen, whereas in our study, plants received three applications of BA at 7-day intervals, beginning on the day of transfer. Alternatively, the plant material used in our study may have been more mature than that used by Kim and others (2000). Plants with a larger leaf span (>25 cm) at flower induction will generally have more inflorescences and flowers per plant than smaller plants (Runkle and others 2005).

During growth and development of *Doritaenopsis* and *Phalaenopsis*, two undifferentiated bud primordia are produced at each node along the stem, of which the upper bud has the potential to elongate and develop into an inflorescence when environmental conditions are favorable (Rotor 1959; Wang 1995b). However, in general an inflorescence emerges only from the leaf axil of the third, fourth, or both nodes below the apical leaf (Sakanishi and others 1980). Yonenda and Momose (1990) observed that among plants sprayed with BA or BA+GA_3, the percentage of inflorescences that emerged from below the fourth node was 18% and 31%, respectively, whereas only 1% of inflorescences emerged below the fourth node in control plants. These results are consistent with our own observations; exogenous BA applications elicited inflorescences from several nodes along the compressed stem, including nodes that were positioned below the media surface.

The stimulation of flowering after the application of BA suggests that endogenous cytokinins at least partially regulate flower induction in *Doritaenopsis* and *Phalaenopsis*. Chou and others (2000) investigated changes in the concentration of endogenous cytokinins in leaves of *Phalaenopsis hybrida* 'Taisuco Snow' grown at high temperature (30/25°C day/night) and low temperature (25/20°C) for 20 days. At high temperature, the concentration of active cytokinins (zeatin, zeatin riboside, and dihydrozeatin) in leaves decreased by 1.5–10-fold compared with that of plants grown at low temperature. A concomitant increase of glucoside cytokinins in leaves of plants grown at high temperature suggested that active cytokinins were converted into inactive forms (Chou and others 2000). In *Boronia megatigma*, concentrations of zeatin riboside and dihydrozeatin increased in roots and stems after transfer of plants from high temperature (25/17°C day/night) to low temperature (17/9°C) for inflorescence initiation (Day and others 1995). These results suggest that in *Phalaenopsis*, flowering in response to low (≤26°C) day temperature (Blanchard and Runkle 2006) could be influenced by changes in the concentration of endogenous active cytokinins. Thus, the application of BA during low-temperature treatment may supplement the natural increase in endogenous active cytokinins and enhance flower induction.

The application of exogenous cytokinins has been reported to induce or increase the synthesis of endogenous

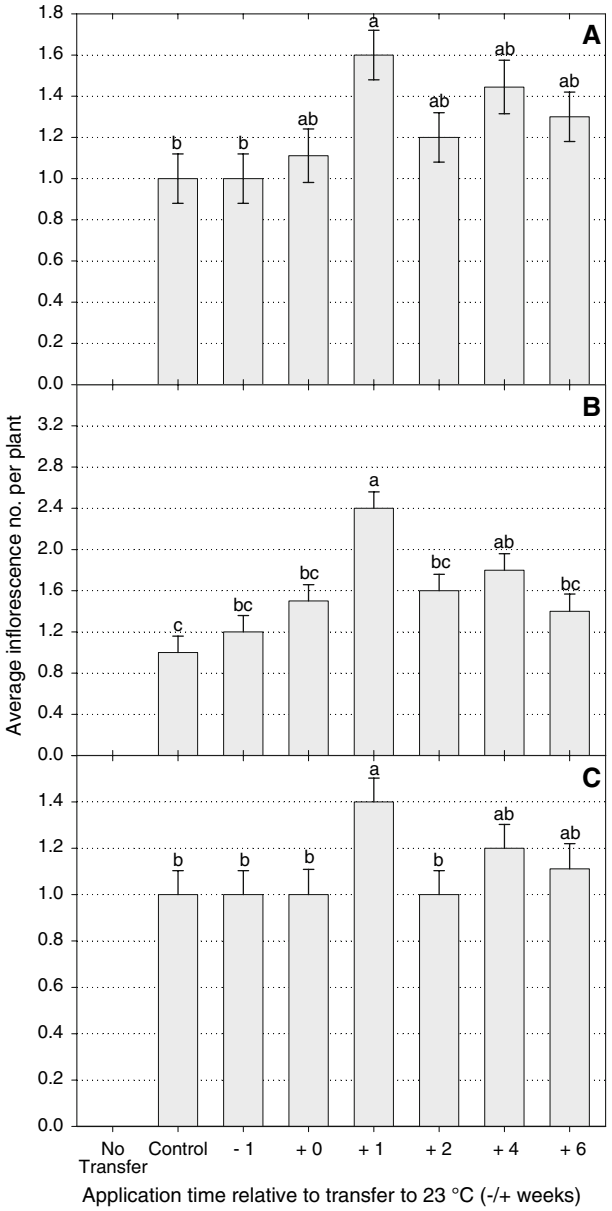

Fig. 2 Effects of benzyladenine (BA) application time on the number of inflorescences per plant in *Doritaenopsis* 'Alice Girl' (**A**), *Doritaenopsis* 'Malibu Chablis' (**B**), and *Phalaenopsis* 'Pink Twilight' (**C**). Plants received a single foliar spray of 200 mg L^{-1} BA at one of six application time points relative to time of transfer from 29°C to 23°C. Control plants did not receive a BA spray. Plants that were sprayed with BA but were maintained at 29°C did not initiate inflorescences and were not included in analysis. Mean separation by Tukey's honestly significant difference test at $P \leq 0.05$. Vertical bars represent standard errors among the treatment means

cytokinins (Letham 1994). For example, in excised cotyledons of *Citrullus vulgaris* Schrad., exogenous BA caused a 36-fold and 10-fold increase in dihydrozeatin riboside and transzeatin riboside cytokinins, respectively, at 1 day after treatment (Rossi and others 1991). Research also indicates that cytokinins can increase the flow of assimilates to growing cells (for example, shoot apical meristem) by upregulating enzymes that control apoplastic phloem unloading (Roitsch and Ehneß 2000). The application of BA on *Pharbitis nil* (L.) Chois. cotyledons caused a significant increase in the import of ^{14}C-labeled assimilate into the shoot apex (Ogawa and King 1979). Chen and others (1994) reported that levels of sucrose, glucose, and fructose in *Phalaenopsis* inflorescences increased in plants exposed to 25/20°C compared to plants held at 30/25°C. The authors postulated that the inhibition of *Phalaenopsis* flowering at a warm temperature could be caused by the reduced translocation of assimilates to the floral meristem. These results collectively suggest that the application of exogenous cytokinins could stimulate flower induction by increasing assimilate movement.

In this study, the application of BA at 200 mg L^{-1} on plants held at a 29°C did not initiate inflorescences. In *Phalaenopsis* Brother Goldsmith '720,' multiple foliar spray applications or higher spray concentrations of BA to plants grown at 29°C promoted inflorescence emergence, but development ceased when inflorescences were less than 1.5 cm in length and plants never flowered (unpublished data). This suggests that exogenous BA cannot completely substitute for an inductive low temperature for flowering in *Doritaenopsis* and *Phalaenopsis*, that other hormones could be required for flowering, or that BA is converted to inactive forms at high temperature. Alternatively, BA may at least partially regulate inflorescence initiation but favorable temperatures could be required for continued inflorescence development. Additional research is warranted to further elucidate the specific effects of BA and high temperature on inflorescence initiation and development of these orchids.

In both *Phalaenopsis* Brother Apollo '072' and Golden Treasure '470,' the application of BA+GA, which includes GAs $A_4 + A_7$, elicited little or no promotion of inflorescence initiation at the concentrations tested. These results are supported by the work of Kim and others (2000) in which there were no differences in the number of inflorescences between nontreated plants and those that received a foliar application of BA+GA_3 (100 or 200 mg L^{-1} of each). Wang (1995a) reported that inflorescence initiation did not occur after four weekly 50-µl injections of GA_{4+7} at 20,000 mg L^{-1} into the leaf axil of vegetative plants grown during warm summer months. These results collectively suggest that the addition of GA to a spray solution does not enhance the flowering response of *Phalaenopsis* to BA. A higher application concentration of BA+GA (200 or 400 mg L^{-1} each of BA and GA_{4+7}) promoted flowering similar to that of BA applied at the same rate without GA (unpublished data).

Inflorescence number increased as application concentration of BA1 or BA2 increased from 100 to 400 mg L^{-1} in *Phalaenopsis* Golden Treasure '470,' but the mean

Table 4 Effects of Benzyladenine (BA) Application Time on Days to Visible Inflorescence (VI), Days from VI to Open Flower, Total Flower Number per Plant, Width of the First Open Flower, and Total Inflorescence Length in *Doritaenopsis* 'Alice Girl,' *Doritaenopsis* 'Malibu Chablis,' and *Phalaenopsis* 'Pink Twilight'

Application time (weeks, −/+ transfer to 23°C)	Days to VI	Days from VI to flower	Avg. total flower No.	Flower width (cm)	Total inflorescence length (cm)
	Doritaenopsis 'Alice Girl'				
Control	45 ab[z]	72	7.7 c	8.3 a	38.9 b
−1	52 a	72	9.3 bc	8.4 a	44.5 ab
0	38 b	79	10.6 abc	8.0 ab	43.2 ab
+1	38 b	76	14.6 a	7.7 b	42.6 ab
+2	39 b	77	12.3 ab	8.0 ab	46.2 a
+4	46 ab	73	13.3 ab	7.9 ab	47.1 a
+6	41 b	70	13.7 ab	8.1 ab	49.0 a
Significance	***	ns	***	**	***
	Doritaenopsis 'Malibu Chablis'				
Control	42 a	68 ab	8.8 b	10.7 a	52.2
−1	33 bc	72 a	9.4 b	10.5 ab	52.2
0	30 c	71 a	11.5 b	10.4 ab	50.4
+1	33 bc	70 a	16.9 a	9.5 c	51.5
+2	34 bc	70 a	12.1 ab	10.4 ab	51.5
+4	38 ab	68 ab	13.3 ab	10.0 bc	51.9
+6	42 a	65 b	10.3 b	10.4 ab	56.1
Significance	***	***	***	***	ns
	Phalaenopsis 'Pink Twilight'				
Control	28 ab	76	6.7	8.1	35.9 b
−1	26 ab	80	6.9	8.1	39.8 ab
0	29 ab	76	7.1	8.0	38.3 b
+1	29 ab	77	8.5	7.9	39.6 ab
+2	32 a	75	6.9	11.1	39.1 ab
+4	33 a	74	7.7	8.0	38.9 ab
+6	30 ab	75	8.2	8.1	48.0 a
Significance	**	ns	ns	ns	**

Plants received a single foliar spray of 200 mg L^{-1} BA at one of six application points relative to time transfer from 29°C to 23°C. Control plants did not receive a BA spray. Plants that were sprayed with BA and were maintained at 29°C remained vegetative, and thus data are not available

ns = nonsignificant; ** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$

[z] Mean separation within columns by Tukey's honest significant difference test at $P \leq 0.05$

number of flowers per inflorescence decreased from 3.9 to 2.7. The decrease in flower number per inflorescence could be caused by competition among developing inflorescences for available assimilates. During the reproductive stage in monopodial orchids, the greatest sinks for assimilates are developing inflorescences and the vegetative apical shoot, and inflorescence growth is primarily source limited (Hew and Yong 1997).

In Experiment 1, a small percentage (≤20%) of plants treated with BA1, BA2, or BA+GA within each treatment had flowers with abnormal floral structures (for example, additional carpels, stamens, petals, or sepals); however, there were no trends observed among treatments. In *Phalaenopsis* 'Alice Girl,' BA1 applied 6 weeks after transfer to 23°C caused several plants to develop "crooked" inflorescences (for example, acute bend at 1 node). Kim and others (2000) noted that 5–13% of *Doritaenopsis* plants treated with 400 mg L^{-1} BA had malformed inflorescences at emergence and 3–7% of plants had blasted flower buds. In our study, application of BA on some orchid clones significantly reduced the width of open flowers by 1.2 cm or less. Chaudhury and others (1993) reported that an *A. thaliana* mutant with six times the natural levels of endogenous cytokinins had siliques with either additional carpels, wrinkled appearances, reduced seed number, or reduced length. Further studies are merited on other *Doritaenopsis* and *Phalaenopsis* clones to determine whether BA causes any additional adverse effects.

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