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Growth Regulators Reduce Leaf Yellowing in Easter Lily Caused by Close Spacing and Root Rot

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Abstract. **The development of greenhouse leaf yellowing in Easter lilies (*Lilium longiflorum* Thunb.) was significantly reduced by the application of growth regulator solutions containing gibberellins 4 and 7 (GA_{4+7}) or benzyladenine (BA). Solutions containing BA alone significantly reduced leaf yellowing on plants caused by close spacing but were less effective than GA_{4+7}. Application of BA alone, however, was not effective against root rot-induced leaf yellowing. When plants were treated with GA_{4+7} or BA + GA_{4+7} around the visible bud stage, nearly all of the leaves remained green until the end of the growing season. These growth regulators, however, increased the final height of the plants by 8–10 cm. The developmental rate and size of the flower buds, as well as the length of the pedicels were not affected by the growth regulator treatments. Thus application of these growth regulators greatly improved the quality of the leaves without compromising the quality and timing of the flowers. Chemical name used: *N*-(phenylmethyl)-1*H*-purine-6-amine (benzyladenine, BA).**

The yellowing of lower leaves of Easter lilies in the greenhouse can be caused by many factors (Miller et al., 1993). Recommendations to combat the disorder include a proper nutritional program, adequate spacing of plants on the greenhouse bench, and preventive fungicide drenches. Nevertheless, lower leaf yellowing remains an important issue that often results in a significant economic loss to the growers.

Little is known about the physiological changes occurring in Easter lily leaves during senescence (Miller, 1992). Jiao et al. (1986) showed that leaf senescence was associated with low levels of carbohydrates in the leaves. In that study, treatment with paclobutrazol {(*R**,*R**)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1,-dimethylethyl)-1*H*-1,2,4,-triazole-1-ethanol} and ancymidol [α-cyclopropyl-α-(ρ-methoxyphenyl)-5-pyrimidinemethanol] (to reduce plant height) reduced the concentration of total soluble sugars in all leaves and increased lower leaf senescence. Miller et al. (1993) reported that yellowing of leaves of plants grown under negative difference between day and night temperatures (DIF) temperatures was related to low levels of leaf carbohydrates. Furthermore, in excised leaves, growth regulator treatments (gibberellic acids and BA) that reduce respiration rate significantly delay senescence (Franco and Han, 1997), which is a circumstantial evidence supporting a relationship between carbohydrate status and senescence.

The effects of growth regulators, such as BA and GA_{4+7}, on delaying leaf yellowing after production of Easter lily are well-documented (Franco and Han, 1997; Han, 1995, 1997). Leaf yellowing, however, can begin near the end of the production time while plants are still in the greenhouse (Miller et al., 1993). This results in poor quality plants that may not be marketable. Finding a solution to the disorder has been a top priority of the floriculture industry for many years.

The objective of this study was to investigate if the application of BA and/or GA_{4+7} could prevent or reduce the development of greenhouse leaf yellowing induced by two common causes, close spacing of plants and root rot diseases. In addition, experiments were conducted to determine if the direct application of growth regulators on the developing flower buds affected the quality, developmental rate, and the size of the open flowers, as well as the length of the pedicels.

Materials and Methods

Plant materials. Precooled 'Nellie White' Easter lily bulbs were planted on 5 Dec. 1997 and 7 Dec. 1998 in 1.4-L (15-cm-diameter) pots containing a peat-based mix (Pro-Mix BX; Premier Brands, Stamford, Conn.) and were grown under natural daylength in a glasshouse at the Univ. of Massachusetts, Amherst (lat. 42°22.5′N) until April of 1998 and 1999. Air temperatures were 19 ± 2 °C day/17 ± 2 °C night and were recorded by a datalogger (LI-1000; LI-COR, Lincoln, Nebr.) equipped with a thermistor (LI-1000-16; LI-COR). In accordance with standard practices for growing Easter lily, all plants used in this study were drenched with 0.5 mg of ancymidol per pot 30 d after emergence, when the shoots were ≈10 cm long.

Expt. 1. To assess the role of spacing of the plants in the greenhouse and the effects of growth regulator application on the development of leaf yellowing, plants were spaced either on 15.0-cm (pot-to-pot) or 25.5-cm centers for the duration of the experiment. For each experimental setup, two rows of plants were used as a border and data for them were not recorded. Growth regulator solutions containing 50 mg·L^{-1} of BA, 50 mg·L^{-1} of GA_{4+7}, or 25 mg·L^{-1} each of BA and GA_{4+7} were sprayed on the entire plants (whole plant) either 2 weeks before or at visible flower bud date (40 and 55 d after emergence, respectively). Control plants were sprayed with water. To minimize foliar chlorosis associated with root rot disease, a preventive fungicide drench program was applied to the plants throughout the experimental period. The fungicide treatment consisted of a drench with Cleary's® [dimethyl 4,4′-o-phenylenebis(3-thioallophanate)] and Subdue® {(R)-2-[(2,6,-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester} 3 weeks after planting followed by once a month drenches with Banrot® [5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole and Dimethyl 4,4′-o-phenylenebis(3-thioallophanate)] for the next 3 months. Plant height, flower bud number, days to opening, length of the first flower, and leaf yellowing were monitored. Yellow leaves were defined as those with 10% or more of their area chlorotic or necrotic. The percentage of yellow leaves was determined by dividing the number of yellow leaves by the total number of leaves on each plant. There were six replicate plants per treatment.

Expt. 2. To determine the role of root rot disease and the effects of the growth regulator solution on the development of greenhouse leaf yellowing, plants were grown in the greenhouse as described previously and spaced on 25.5-cm centers. Half of the plants were drenched with the preventive fungicide program described in Expt. 1 while the other half were not treated. Application of the growth regulator solutions and the collection of data were as described for Expt. 1. Eight replicate plants were used per treatment.

Expt. 3. To determine if application of a growth regulator solution directly to the developing flower buds affected the developmental rate and the size of the flower buds, plants with flower buds at five different stages of development were used. The stages were defined by the length of the largest flower bud on each plant, and were as follows: Stage 1 (4.1 ± 0.2 cm); Stage 2 (5.6 ± 0.2 cm); Stage 3 (7.1 ± 0.1 cm); Stage 4 (9.7 ± 0.3 cm); and Stage 5 (puffy bud stage, 15.6 ± 0.2 cm). For each stage, half of the plants were sprayed with a solution containing 25 mg·L^{-1} each of

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BA and GA_{4+7} and the other half (controls) were sprayed with deionized water. Data were collected on the number of days from planting to anthesis, the length of the first three open flowers, and pedicel length at anthesis of the three largest buds on each plant.

Statistical analysis. A completely randomized design was used in all experiments, and within each spacing used in Expt. 1. Data were analyzed with SAS's General Linear Model procedure (SAS Inst., 1992). An arcsin transformation was used on percentage data prior to analysis. Differences among treatments were further analyzed with either Duncan's multiple range test or paired comparisons.

Results

Expt. 1. At 2 weeks before visible flower bud date, the average plant height was 20 to 25 cm and no yellow leaves were evident, regardless of spacing. However, the limitation of light to the lower leaves, due to close spacing of the plants, induced rapid development of leaf yellowing after the visible bud stage (Fig. 1). At visible bud, average height of the plants was 40 and 28 cm for plants spaced at 15- and 25-cm centers, respectively, and the average percentage of chlorotic leaves was 7.0% and 3.1%. The development of leaf yellowing on plants that were spaced at 15-cm centers progressed very rapidly after the visible bud date, averaging 34% by 92 d after emergence. In contrast, chlorosis in plants that were spaced at 25-cm centers averaged 17%.

Treatment with BA, GA_{4+7}, or BA + GA_{4+7} significantly reduced the percentage of yellow leaves, but BA was less effective than GA_{4+7} (Fig. 1). Furthermore, the timing of the growth regulator treatments did not affect the final percentage of yellow leaves. When treatments were applied 2 weeks before visible bud stage to plants spaced at 15-cm center (Fig. 1A), the percentages of yellow leaves at the puffy bud stage (92 d after emergence) were 34%, 14%, 5%, and 5% for those treated with water, BA, GA_{4+7}, and BA + GA_{4+7}, respectively. When the same treatments were applied at visible flower bud stage (Fig. 1B), the percentages of yellow leaves were 34%, 13%, 8%, and 9% for the same treatments. Regardless of treatment time, application of solutions containing GA_{4+7} or BA +GA_{4+7} completely halted the further development of leaf yellowing (Fig. 1A and B). In comparison, leaf yellowing in plants treated with water or BA continued to increase throughout the growing period. The responses of plants to the growth regulator treatments were similar when plants were spaced farther apart (Fig. 1C and D).

Close spacing significantly increased plant height (Fig. 2). At 2 weeks before visible bud date (40 d after emergence), plants spaced at 15-cm centers averaged 4 cm taller (significant at $P = 0.05$) than those spaced at 25-cm centers. The difference in height between plants at the two spacings increased with time. Closely spaced plants were 13 cm and 27 cm taller than those widely spaced at visible bud date and anthesis, respectively. Application of BA did not affect the height of the plants, whereas the effect of GA_{4+7} or BA +GA_{4+7} on height was dependent on spacing. At the close spacing, the elongation of the plants due to limited lighting apparently nullified the effects of the growth regulator treatments, resulting in no differences in height at anthesis between the treated and control plants (Fig. 2 A and B).

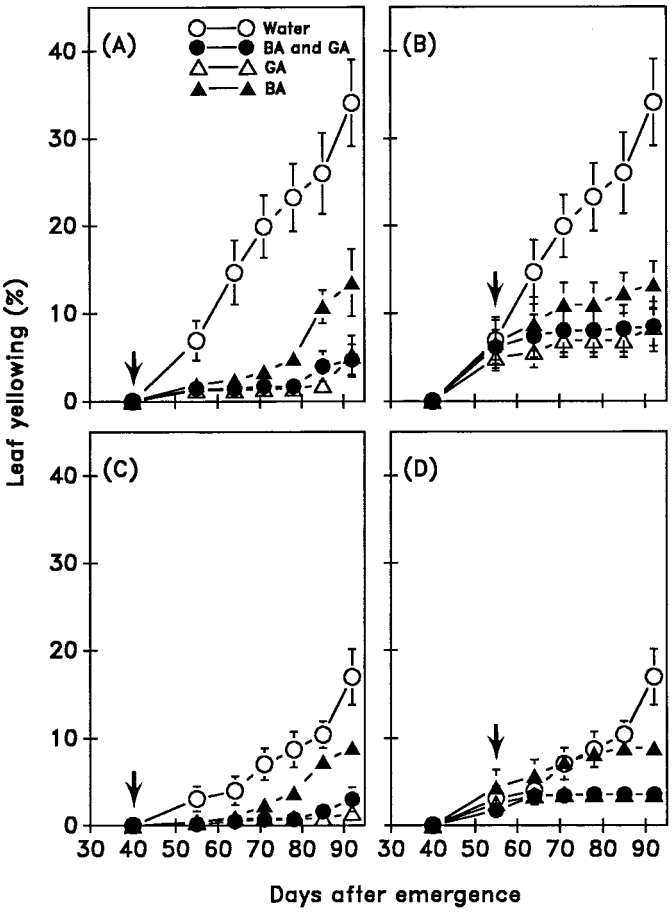

Fig. 1. Effects of spacing and the timing of growth regulator application on the development of greenhouse leaf yellowing in *Lilium longiflorum* Thunb. (Expt. 1). Plants were spaced on 15-cm centers and were treated with 25 mg·L^{-1} each of BA and GA_{4+7} (**A**) 2 weeks before visible bud date or (**B**) on visible bud date, or plants were spaced on 25-cm centers and were treated (**C**) 2 weeks before visible bud date or (**D**) on visible bud date. Data are means ± SE for six replicate plants. Arrows indicate the time of growth regulator treatment.

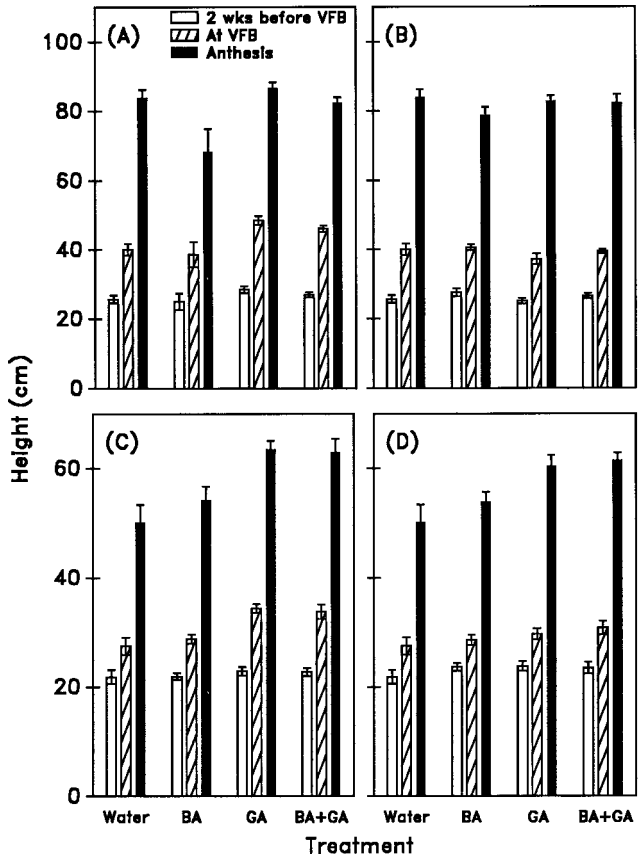

Fig. 2. Effects of close spacing and the timing of growth regulator application (25 mg·L^{-1} each of BA and GA_{4+7}) on the height of *Lilium longiflorum* Thunb. measured 2 weeks before visible bud date, on visible bud date, or at anthesis (Expt. 1). Plants were spaced on 15-cm centers and treated with growth regulators (**A**) 2 weeks before visible bud date or (**B**) on visible bud date, or on 25-cm centers and treated (**C**) 2 weeks before visible bud date or (**D**) on visible bud date. Data are means ± SE for six replicate plants.

However, at the wide spacing, height of the treated plants averaged 8 to 10 cm taller than the controls (Fig. 2 C and D). The developmental rate (determined by the number of days to opening of the first flower), the length of the open flowers, and the number of flower buds on each plant (average number of 6.5 buds) were not affected by the spacing or the type and timing of chemical treatment (data not shown).

Expt. 2. Preventive fungicide applications significantly reduced the development of leaf yellowing (Fig. 3). Without an application of fungicide, 21% of the leaves had became chlorotic by 92 d after emergence vs. 13% of those on plants that were drenched with fungicides. Most of the yellowing began after the visible bud date and progressed rapidly until the end of the production time (Fig. 3). Application of BA did not affect yellowing but GA_{4+7} or BA + GA_{4+7} significantly reduced the percentage of yellow leaves. Treatment with GA_{4+7} or BA + GA_{4+7} was less effective when plants were not treated with fungicides. The combination of fungicide application and treatment with GA_{4+7} or BA + GA_{4+7} greatly reduced the development of leaf yellowing throughout the growing season.

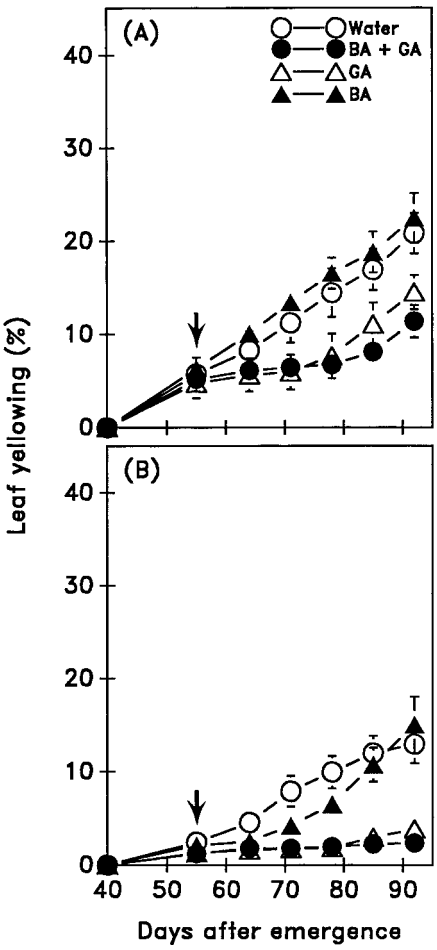

Fig. 3. Effects of treatment with growth regulators (25 mg·L^{-1} each of BA and GA_{4+7}) with and without a fungicide to control root rot on the development of greenhouse leaf yellowing in *Lilium longiflorum* Thunb. Plants were (**A**) not treated or (**B**) treated with a preventive fungicide throughout the growing period (Expt. 2). Growth regulators were applied on the visible bud date. Data are means ± SE for eight replicate plants. Arrows indicate the time of growth regulator treatment.

Plants treated with a preventive fungicide were significantly taller than those not treated (Table 1). The difference in height between the two groups began as early as day 40, the first date of measurement, and thereafter. As in Expt. 1, application of BA did not affect the height of the plants, but treatment with GA_{4+7} or BA + GA_{4+7} significantly increased it. In addition, application of fungicide did not affect the number of flower buds or the rate at which they developed. Flowers on fungicide-treated plants, however, were significantly longer than those on plants not treated.

Expt. 3. Direct application of BA + GA_{4+7} to developing flower buds at various stages did not affect the rate at which the flower buds developed (based on the number of days from treatment to anthesis) or the length of the pedicels (data not shown). Open flowers were significantly longer (≈1 cm) when 5- to 8-cm-long buds were sprayed with BA + GA_{4+7} (Table 2). No abnormalities or abortion of the buds and open flowers were observed.

Discussion

The dramatic effect of growth regulators (BA and GA_{4+7}) in preventing the development of postproduction leaf yellowing in Easter lilies (Han, 1995, 1997) was also observed to hold true in the prevention of the development of greenhouse leaf yellowing. While BA alone had no effect in preventing postproduction leaf yellowing, it was effective in reducing greenhouse leaf yellowing induced by close spacing (Fig. 1). GA_{4+7} or BA + GA_{4+7} was very effective in preventing greenhouse leaf yellowing, and significantly reduced it under conditions of close spacing or no fungicide application. However, their effectiveness was greatly reduced on plants with root rot (Fig. 3A).

Timing of the growth regulator application, whether 2 weeks before or at visible flower bud date, did not affect final percentage of leaf yellowing. These data are consistent with those of Ranwala and Miller (1999) in which no differences in leaf chlorosis were observed when plants were sprayed at an early stage of development, i.e., 36 d or 55 d after planting. However, when plants were sprayed at 80 d or 90 d after planting, when leaf chlorosis had already begun, the percentage of chlorotic leaves at the puffy bud stage was the same as that of the control (Ranwala and Miller, 1999). Treatment at these later dates, however, prevented further development of leaf yellowing that would otherwise have occurred in the postproduction environment.

Treatment with solutions containing GA_{4+7} can significantly increase plant height. The increase in height from the GA_{4+7} treatment was not detected when plants were spaced close together (Fig. 2A and B), as reported by Heins et al. (1996). However, when properly spaced, height of plants treated with GA_{4+7}, either 2 weeks before or on the visible bud date, was ≈10 cm taller than that of the controls (Fig. 2 C and D). According to Ranwala and Miller (1999), the degree of stem elongation is dependent on the timing of growth regulator application. In their study, plants sprayed with 100 mg·L^{-1} each of BA and GA_{4+7} at 36 and 55 d after planting were 23.5 cm and 10.5 cm taller, respectively, than the controls. However, the developmental stages of the plants at the time of the treatment were not indicated. Thus, comparison with our data is not possible. When

Table 1. Effects of preventive fungicide drench and growth regulator application (25 mg·L^{-1} of BA and/or GA_{4+7}) on plant and flower bud development of *Lilium longiflorum* Thunb. Data are means ± SE for eight replicate plants

Treatment	Time of application[z]	Height (cm)	No. flower buds	No. days to first flower	Length of the first flower (cm)
		No fungicide			
Water (control)	1	51.8 ± 1.3	7.1 ± 0.3	96.0 ± 1.1	16.4 ± 0.2
BA and GA_{4+7}	1	53.6 ± 0.7	6.6 ± 0.2	94.4 ± 0.8	15.4 ± 0.7
	2	49.3 ± 1.7	7.8 ± 0.6	94.6 ± 0.8	15.5 ± 0.5
GA_{4+7}	1	52.8 ± 1.6	7.1 ± 0.4	95.3 ± 1.7	15.0 ± 0.6
	2	49.3 ± 1.4	7.9 ± 0.4	97.0 ± 1.4	15.0 ± 0.3
BA	1	48.0 ± 2.1	7.8 ± 0.9	95.4 ± 1.4	15.4 ± 0.5
	2	49.0 ± 2.5	7.4 ± 0.4	96.5 ± 0.6	15.9 ± 0.7
		Fungicide			
Water (control)		55.6 ± 2.1	7.3 ± 0.5	96.0 ± 1.1	17.9 ± 0.1
BA and GA_{4+7}	1	63.9 ± 2.2	7.5 ± 0.4	102.1 ± 1.2	17.6 ± 0.1
	2	60.4 ± 1.4	6.6 ± 0.4	93.0 ± 0.8	18.5 ± 0.3
GA_{4+7}	1	62.1 ± 1.8	7.5 ± 0.4	97.0 ± 1.0	18.1 ± 0.3
	2	64.6 ± 1.5	7.0 ± 0.4	95.3 ± 0.6	18.6 ± 0.2
BA	1	59.0 ± 2.6	6.9 ± 0.4	95.9 ± 1.3	17.3 ± 0.5
	2	59.4 ± 1.4	7.6 ± 0.4	96.0 ± 1.0	17.6 ± 0.8
		Statistical analysis			
Fungicide vs. no fungicide		*	NS	NS	*
Timing of application		NS	NS	NS	NS
Growth regulator treatment		*	NS	NS	NS

[z]Two weeks before visible bud stage (1) or at visible bud stage (2).
NS, *Nonsignificant or significant at $0.01 < P \leq 0.05$ in paired comparisons.

Table 2. Effects of stage of bud development at the time of direct application of growth regulators (25 mg·L^{-1} each of BA and GA_{4+7}) to the developing buds on the length of the open flowers. Data are means ± SE for six replicate plants.

Stage	Length of buds[y] (cm)	Treatment	Length of open flowers (cm)[z] 1st flower	2nd flower	3rd flower
1	4.1 ± 0.2	Water (control)	16.0 ± 0.5	16.4 ± 0.5	15.9 ± 0.3
		BA + GA_{4+7}	16.2 ± 0.6	16.5 ± 0.4	15.9 ± 0.6
2	5.6 ± 0.2	Water	15.3 ± 0.3	15.5 ± 0.2	16.1 ± 0.2
		BA + GA_{4+7}	16.4 ± 0.4	16.8 ± 0.4	17.3 ± 0.3
3	7.1 ± 0.1	Water	15.6 ± 0.3	15.5 ± 0.3	15.2 ± 0.2
		BA + GA_{4+7}	16.7 ± 0.4	16.6 ± 0.5	16.4 ± 0.6
4	9.7 ± 0.3	Water	16.0 ± 0.2	15.9 ± 0.3	15.8 ± 0.2
		BA + GA_{4+7}	16.1 ± 0.4	15.8 ± 0.6	15.7 ± 0.8
5	15.6 ± 0.2	Water	15.8 ± 0.2	15.6 ± 0.4	15.4 ± 0.2
		BA + GA_{4+7}	16.0 ± 0.3	15.8 ± 0.3	16.1 ± 0.4
		Statistical analysis			
Bud length (BL)			NS	NS	NS
Treatment (T)			*	*	*
BL × T			NS	NS	NS

[z]Measured on the day of anthesis.
[y]Measured on the day growth regulators were applied.
[NS, *]Nonsignificant or significant at $0.01 < P \leq 0.05$ in paired comparisons.

plants were treated at later dates, 80 and 90 d (puffy bud stage) after planting, plant height was not affected (Ranwala and Miller, 1999).

The effects of growth regulator treatment on the development of flower buds were also investigated. Heins et al. (1982) reported that the developmental rate of flower buds is a function of temperature and is independent of light intensity. Our results also demonstrated that the spacing of the plants, the lack of fungicide application, and the application of the growth regulator solutions on the leaves and flower buds did not affect the rate at which the flower buds developed or the length of the pedicels. The open flowers, however, were shorter on plants that did not receive a preventive fungicide drench and slightly longer when BA + GA_{4+7} was applied to developing flower buds 5 to 8 cm in length. Wang (1996) also reported an increase in dry weight of flowers when buds 2 to 5 cm in length were sprayed with 500 mg·L^{-1} of PBA [*N*-(phenylmethyl)-9-(tetra-hydro-2*H*-pyran-2-yl)-9*H*-purin-6-amine]. Deformed buds, such as those observed by Heins et al. (1996) when plants were sprayed with 100 mg·L^{-1} each of BA and GA_{4+7}, 30 d after emergence, were not detected in our study. The discrepancy is probably due to the differences in the timing of the application. We treated plants after the completion of bud initiation, whereas Heins et al. (1996) treated them during the initiation of flower buds. Also, deformed buds were also not observed on plants sprayed with 100 mg·L^{-1} each of BA and GA_{4+7} at various time after planting, including those sprayed at 36 d after planting (Ranwala and Miller, 1999). Furthermore, when sprayed at later dates (80 and 90 d after planting), postproduction longevity of flowers was significantly increased.

In conclusion, one application of GA_{4+7} or BA + GA_{4+7} to Easter lily plants prevented the development of greenhouse leaf yellowing induced by limited light (close spacing) and reduced leaf yellowing on plants not treated with preventive fungicides. However, plants grown at close spacing elongated excessively and the final product was not commercially acceptable. These growth regulators, when used on plants grown under proper cultural practices, greatly improved plant quality. When applied at or around visible bud stage, nearly all of the leaves on the plants remained green until the end of the growing season with no effects on the developing flower buds. Application of these growth regulators at the rate used in this study, however, increased plant height by 8–10 cm.

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