Preventing Postproduction Leaf Yellowing in Easter Lily

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ABSTRACT. Postproduction leaf yellowing of Easter lily (*Lilium longiflorum* Thunb.) can be prevented by using growth regulators. Solutions containing benzyladenine (BA) reduced the percentage of yellow leaves in cold-stored plants, but solutions containing gibberellic acid (GA_3) were not effective. Treatment with commercial products containing GA_{4+7} (Provide) or GA_{4+7} and BA (Promalin) nearly completely prevented the development of leaf yellowing. Concentrations as low as $25\text{ mg}\cdot\text{L}^{-1}$ were effective. Leaf yellowing was prevented by growth regulators only on leaves that had been treated, indicating that the growth regulators were not mobilized in the plants. Growth regulator solutions halted further development of leaf yellowing when applied to plants that already had some chlorotic basal leaves. This result suggests that growth regulators need not be applied preventively. Treatment can be delayed until chlorotic lower leaves are first seen on plants. The striking effects of growth regulators in preventing leaf yellowing did not affect the development and opening of flower buds.

Foliar chlorosis is one of the factors that shorten postproduction life of potted Easter lilies (*Lilium longiflorum* Thunb.) (Staby and Erwin, 1977). Leaf yellowing typically starts in the basal leaves and moves progressively upward. Postproduction leaf yellowing symptoms are worse when plants have been treated with growth retardants (Jiao et al., 1986), are grown in a negative DIF (differences between day and night temperatures) greenhouse environment (Miller et al., 1993), or have been cold stored before marketing (Prince et al., 1987; Staby and Erwin, 1977). Reduced carbohydrate levels have been suggested to be related to the development of leaf yellowing during (Jiao et al., 1986) and following (Miller, 1992) greenhouse production. Neither application of spermidine, which delays chlorophyll breakdown in some plant species, nor treatment with silver thiosulfate, which inhibits the action of ethylene, retards development of foliar chlorosis on dark-stored Easter lilies (Prince et al., 1987; Prince and Cunningham, 1989).

The effects of growth regulators on leaf senescence vary greatly among plant species. Exogenous application of cytokinins or gibberellins has been reported to delay leaf senescence in various plant species. In Easter lily, growth regulators such as GA_3 and BA (benzyladenine) significantly delay leaf yellowing and inhibit respiration rates of excised leaves (Franco and Han, 1997; Han, 1995). Using excised leaves, however, did not provide a system with a source–sink relationship, and the dark chamber in which the studies were conducted may have caused senescence of leaves different from that in the light (Thimann, 1987). For practical application, it is therefore critical that studies be conducted on intact plants and that plants be evaluated in a simulated interior environment.

The objectives of this work were to determine the effectiveness of growth regulators in preventing foliar chlorosis of intact Easter lily leaves and to examine if the treatments affect the development and opening of the flower buds.

Materials and Methods

GENERAL PROCEDURES. Precooled ‘Nellie White’ Easter lily bulbs were planted in 1.4-L (15-cm-diameter) pots containing a peat based mix (Pro-Mix BX; Premier Brands Inc., Stamford, Conn.). The lilies were grown under natural daylength in a glasshouse at the University of Massachusetts, Amherst (lat. $42^{\circ}22.5'N$), at a spacing of 23×23 cm. Temperatures of the greenhouse were set at $21/19^{\circ}C$ (day/night). Air temperatures in the greenhouse were recorded by a datalogger (LI-1000; LI-COR, Lincoln, Nebr.) equipped with a thermistor (LI-1000-16).

Unless otherwise stated, plants at the puffy bud stage (1 to 2 d before anthesis) with no chlorotic leaves were used in the postproduction experiments. Experiments were conducted in a $22.6 \pm 0.2^{\circ}C$ room illuminated $12\text{ h}\cdot\text{d}^{-1}$ with $11.2 \pm 0.1\text{ }\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ cool-white fluorescent lamp, simulating an interior environment. All treatments contained 0.1% surfactant Tween 20, [polyoxyethylene (20) sorbiton monolaurate]. Benzyladenine (BA) and gibberellic acid (GA_3) were purchased from Sigma Chemical Co. (St. Louis) and Promalin (a.i. 1.8% of GA_{4+7} and 1.8% of BA), Provide (a.i. 2.0% of GA_{4+7}), ProGib (a.i. 4.0% of GA_3), and ABG-3182 (a.i. 1.8% of BA) were obtained from Abbott Chemical Co. (North Chicago). Unless otherwise stated, senesced leaves were defined as leaves with $\geq 50\%$ of their area chlorotic or necrotic. Data such as the percentage of senesced leaves on the plant and the longevity of the flower buds were collected every other day.

EFFECTS OF GROWTH REGULATORS (EXPT. 1.). Plants were sprayed with deionized water, $500\text{ mg}\cdot\text{L}^{-1}$ of GA_3 , $500\text{ mg}\cdot\text{L}^{-1}$ of BA, $500\text{ mg}\cdot\text{L}^{-1}$ each of BA and GA_3 (BA+ GA_3), $1000\text{ mg}\cdot\text{L}^{-1}$ of Promalin (containing $500\text{ mg}\cdot\text{L}^{-1}$ each of GA_{4+7} and BA), or $500\text{ mg}\cdot\text{L}^{-1}$ of ProGib (containing $500\text{ mg}\cdot\text{L}^{-1}$ of GA_3). Following the application, one-half of the plants from each treatment was sleeved with paper sleeves and placed in a $2 \pm 0.7^{\circ}C$, dark room for 3 weeks. The other group of plants was placed directly in the simulated interior environment for evaluation. In addition to the data described in the general procedures, the longevity of each flower bud (number of days from opening to senescence of the bud) was determined. There were eight replicate plants per treatment.

ROLE OF FLOWER BUDS (EXPT. 2.). To determine if the developing flower buds affect leaf yellowing, plants at the puffy bud stage were placed in the interior environment. For half of the plants, the entire inflorescence (four to five flower buds) was removed whereas the inflorescences on the remaining plants were left intact.

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Table 1. Effects of growth regulators on the development of postproduction leaf yellowing in Easter lilies. Plants at the puffy bud stage were treated with the growth regulator solutions. Half of the plants were then sleeved and stored in a 2 ± 0.7 °C room for 3 weeks before being kept in a 22.6 ± 0.2 °C room illuminated $12 \text{ h} \cdot \text{d}^{-1}$ with $11.2 \pm 0.1 \mu\text{mol} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ cool-white fluorescent lamp. Leaf yellowing was recorded on day 17. There were eight replicate plants per treatment.

Growth regulator treatment	Leaves with >50% area yellow (%) ²	
	No cold storage	3 weeks cold storage
Control (water)	$39.7 \pm 2.4 \text{ a}^y$	$44.3 \pm 10.4 \text{ a}$
GA ₃ (500 mg·L ⁻¹)	$32.8 \pm 3.1 \text{ a}$	$34.1 \pm 6.7 \text{ ab}$
BA (500 mg·L ⁻¹)	$29.5 \pm 4.0 \text{ a}$	$17.0 \pm 3.6 \text{ bc}$
GA ₃ + BA (500 mg·L ⁻¹ each)	$31.8 \pm 2.7 \text{ a}$	$10.5 \pm 3.6 \text{ c}$
ProGib ^x (500 mg·L ⁻¹ of GA ₃)	$36.5 \pm 3.6 \text{ a}$	$25.6 \pm 6.5 \text{ abc}$
Promalin ^x (500 mg·L ⁻¹ each of GA ₄₊₇ and BA)	$2.7 \pm 1.1 \text{ b}$	0.0 d

²Data are means $\pm$ standard errors of eight replicate plants.

^yLetters indicate significance among treatments within each column, determined by Duncan's multiple range test at $P = 0.05$.

^xGrowth regulator solutions from Abbott Chemical Co.

The percentage of senesced leaves was collected as previously described. There were six replicate plants per treatment.

CONCENTRATIONS AND ACTIVE INGREDIENTS OF PROMALIN (EXPT. 3.). Plants were sprayed with deionized water or with Promalin at 50, 250, or 1000 mg·L⁻¹. Promalin was applied either to all of the leaves on the plant or only to leaves located on the lower half of the plants. Data on the percentage of senesced leaves and the longevity of flower buds were collected as previously described.

In a subsequent experiment, plants were sprayed with deionized water or with several growth regulator solutions from Abbott Chemical Co. containing BA (ABG-3182), GA₄₊₇ (Provide), or BA+GA₄₊₇ (Promalin). Concentrations of the growth regulators were 25, 50, or 100 mg·L⁻¹. Plants then were allowed to dry before sleeving and were stored in a 2 ± 0.7 °C cold room for 5 weeks. Following the cold storage, plants were moved to the simulated interior environment for evaluation. The percentage of senesced leaves on each plant was recorded. There were six replicate plants per treatment.

MOBILIZATION STUDIES (EXPT. 4.). Leaves located at different parts of the plants were treated with 100 mg·L⁻¹ of Promalin to determine if the growth regulators mobilize in the plants. Treatments consisted of Promalin applied to leaves on the upper half of the plant, to leaves on the lower half of the plant, to leaves located on only one side of the plant, and to leaves on the entire plant. Control plants were treated with deionized water. The percent of senesced leaves on the treated and untreated leaves of each plants was determined.

DELAYED TREATMENTS (EXPT. 5.). Plants that had developed chlorotic leaves in the greenhouse on the lower 5 cm of the stem were moved to the simulated interior environment and sprayed with 250 mg·L⁻¹ of Promalin. The continued development of chlorotic leaves was monitored in the simulated interior environment.

In a separate experiment, plants with no chlorotic leaves were moved to the simulated interior environment. On the 10th day, chlorosis had developed on some leaves, at which time half of the plants were sprayed with deionized water and the other half were treated with 250 mg·L⁻¹ of Promalin. The percentage of senesced leaves was determined on the 10th day and on the 27th day (17 d after treatment). There were five replicate plants per treatment.

STATISTICAL ANALYSIS. A completely randomized design was used in all experiments. Percent data were transformed before analysis with the SAS's General Linear Model procedure (SAS Institute Inc., Cary, N.C.). Differences among treatments were further analyzed with Duncan's multiple range test.

Results and Discussion

In a previous studies, applying growth regulators such as GA₃ and BA significantly delayed leaf chlorosis of excised Easter lily leaves when kept in the dark (Han, 1995). These growth regulators, however, were not as effective when applied to leaves on intact plants in a simulated interior environment (Table 1). In this study, GA₃ and ProGib (containing GA₃) did not reduce leaf yellowing. BA reduced yellowing on plants that had been cold stored but not on plants that had not been stored. In contrast, Promalin, which contains an equal amount of BA and GA₄₊₇, prevented leaf yellowing on cold-stored and nonstored plants. Flower buds in all treatments, including the controls, opened fully and the longevity of the flowers ranged from 7 to 10 d (data not shown). There were no significant differences between treatments on the longevity of the flower buds despite the marked differences in the development of leaf yellowing. In another experiment, plants with detached

Fig. 1. Postproduction leaf yellowing in Easter lily plants with intact flower buds (○) and detached flower buds (●). Data are the means $\pm$ standard errors of six replicate plants.

Fig. 2. Postproduction leaf yellowing in Easter lily plants treated with deionized water (○), or with Promalin at 50 mg·L⁻¹ (△), 250 mg·L⁻¹ (▲), or 1000 mg·L⁻¹ (□). Data are the means ± standard errors of six replicate plants.

flower buds developed postproduction leaf yellowing at the same rate as those with intact flower buds, also demonstrating that postproduction leaf yellowing in Easter lilies is not related to the development and opening of the flower buds (Fig. 1). These data do not agree with the results of Jiao et al. (1992), in which a positive correlation between the percent of senesced leaves in the greenhouse (determined at the day when the first flower fully opened) and the number of flower buds led the authors to conclude that the strong assimilate requirements of the developing flower buds may have resulted in lower leaf carbohydrates levels and, consequently, an increase in greenhouse leaf yellowing.

To determine the concentrations of Promalin needed to prevent postproduction leaf yellowing, plants were treated with 50 to 1000 mg·L⁻¹ of Promalin. Regardless of concentrations, all plants treated

with Promalin developed <2% senesced leaves 19 d after being evaluated in an interior environment, whereas 22% of the leaves had senesced on plants treated with water (Fig. 2). In a subsequent experiment, various products from Abbott Chemical Co. containing GA₄₊₇, BA, or both were sprayed on the plants to determine which active ingredient most effectively prevented leaf yellowing. Results showed that solutions containing GA₄₊₇ were as effective as those containing GA₄₊₇ and BA and that concentrations as low as 25 mg·L⁻¹ sufficiently prevented leaf yellowing (Table 2). Plants treated with GA₄₊₇ alone or GA₄₊₇+BA developed 1% to 2% senesced leaves 19 d after the treatments. Solutions containing 25 mg·L⁻¹ of BA also significantly reduced leaf yellowing but higher concentrations resulted in necrotic spots on the leaves and were less effective. These data demonstrate that the GA₄₊₇ in the Promalin plays a more important role than BA in preventing leaf yellowing. The solvent used in the commercially prepared products was not the cause for the increase in efficacy of the growth regulators, because no differences in leaf yellowing were observed between plants sprayed with the commercial solution of BA versus that prepared in the laboratory.

Other experiments were conducted to determine if the growth regulators are mobile in the plants after application. In a preliminary experiment, six pairs of opposite leaves were chosen on each plant. One leaf of each pair was treated with 100 mg·L⁻¹ of Promalin and the other leaf was treated with water. All of the growth-regulator treated leaves were green 3 weeks after treatment, whereas all of the water-treated leaves were chlorotic. Results of the study suggested that the growth regulators were not mobile in the plants. This was tested further by treating leaves at different locations on of the plants with Promalin. In this study, 32% of the leaves senesced on plants treated with water, whereas almost no leaves senesced on plants when all leaves were treated with Promalin (Table 3). Except for plants treated with Promalin only on the lower leaves, the percentage of leaves that had senesced on the untreated part was the same as that of the control, whereas the percentage of senesced leaves on the treated part was the same as that of plants treated with growth regulators on all leaves. This again suggested that the growth regulator treatment prevented leaf yellowing only on leaves that had been treated and that growth regulators were not mobile in the plants. In the treatment where only the lower leaves of the plants were treated, only about one-third as many untreated leaves senesced as in the treatment where all leaves were treated with water only. This probably was due to

Table 2. The development of postproduction leaf yellowing in Easter lily plants treated with various commercially available growth regulator solutions. Plants at the puffy bud stage were sprayed, allowed to dry, and then sleeved and stored in a 2 ± 0.7 °C cold room for 5 weeks. Plants were then evaluated after 19 d in a 22.6 ± 0.2 °C room illuminated 12 h·d⁻¹ with 11.2 ± 0.1 μmol·s⁻¹·m⁻² cool-white fluorescent lamp. Data are reported as means ± standard errors of six replications.

Growth regulator	Concn (mg·L ⁻¹)	Leaves with >50% area yellow (%)
Control (water)		56.1 ± 6.6 a ²
Promalin ³	25	1.6 ± 0.7 d
	50	2.2 ± 0.6 cd
	100	0.9 ± 0.4 d
ABG-3182	25	9.1 ± 2.2 c
	50	22.2 ± 5.7 b
	100	31.1 ± 5.9 b
Provide	25	1.8 ± 0.9 d
	50	2.0 ± 0.8 d
	100	1.6 ± 0.8 d

²Letters indicate significance among treatments determined by Duncan's multiple range test at *P* = 0.05.

³Growth regulator solutions from Abbott Chemical, Co. Promalin contains 1.8% of BA and 1.8% of GA₄₊₇, ABG-3182 contains 1.8% of BA, and Provide contains 2.0% of GA₄₊₇.

Table 3. The development of postproduction leaf yellowing in Easter lily plants with leaves at various location treated with 100 mg·L⁻¹ of Promalin. Plants at the puffy bud stage were treated and then evaluated in a 22.6 ± 0.2 °C room illuminated 12 h·d⁻¹ with 11.2 ± 0.1 μmol·s⁻¹·m⁻² cool-white fluorescent lamp.

Leaves treated with Promalin	Data from leaves that were	Leaves with >50% area yellow (%) ^c
Control (water)	All leaves	32.4 ± 6.2 a ^b
All leaves	All leaves	1.4 ± 0.6 c
Upper leaves	Treated (upper leaves)	5.5 ± 3.3 bc
	Not treated (lower leaves)	31.4 ± 10.3 a
Lower leaves	Treated (lower leaves)	1.7 ± 0.6 bc
	Not treated (upper leaves)	10.9 ± 3.7 b
One side of the plants	Treated	7.5 ± 1.6 bc
	Not treated	25.3 ± 4.1 a

^aData are means ± standard errors of eight replicate plants.

^bLetters indicate significance among treatments determined by Duncan's multiple range test at *P* = 0.05.

the fact that yellowing of Easter lily leaves usually occurs on the basal leaves and half of them had been treated with the growth regulator.

Previous studies on excised Easter lily leaves indicated that growth regulators can delay the further development of leaf yellowing when applied to leaves at the initial stage of leaf yellowing (Franco and Han, 1997). Two experiments were conducted to determine if growth regulator treatments can be used to stop the progressive development of leaf yellowing on intact plants after the development of some chlorotic leaves at the base. In the first experiment, an application of 250 mg·L⁻¹ Promalin prevented yellowing on nonchlorotic leaves on plants that had developed chlorotic leaves on the lower 5 cm of the stems during greenhouse production (data not shown). Leaves that were chlorotic at the start of the experiment, however, turned necrotic 1 week later and consequently dehydrated. Therefore, the effects of the growth regulators on yellowing of Easter lily leaves is different from that of cocklebur (*Xanthium pennsylvanicum* L.), in which cytokinins reversed the breakdown of aged chloroplasts and resulted in regreening of the leaves (Leshem, 1986). In a separate experiment, plants with no chlorotic leaves at the start of the experiment were treated with water or 250 mg·L⁻¹ of Promalin when ≈7% of the leaves were chlorotic, 10 d after being placed in the simulated interior environment. Plants treated with water at day 10 continued to develop yellow leaves and, at day 27, 54% of the leaves were chlorotic. In comparison, no further leaf yellowing occurred on plants treated with Promalin. Results of these studies, therefore, suggest that Promalin can be applied to Easter lily plants with basal leaves already yellowing to stop the progressive upward development of chlorosis.

HORTICULTURAL SIGNIFICANCE. Results of this study demonstrated that growth regulator solutions containing GA₄₊₇ or GA₄₊₇ and BA can prevent the development of postproduction leaf yellowing in Easter lily. The effectiveness of the growth regulators did not diminish when used on plants that had been cold stored for

up to 5 weeks. Commercial products such as Promalin and Provide applied in concentrations as low as 25 mg·L⁻¹ nearly prevented leaf yellowing from developing. The growth regulators, however, were not mobile in the plants and must be applied to all leaves, or at least to the lower leaves that are more prone to leaf yellowing. The growth regulators need not be applied as a preventive method since applying the growth regulators to plants with some chlorotic leaves can halt the further development of leaf yellowing.

Literature Cited

- Franco, R.E. and S.S. Han. 1997. Respiratory changes associated with growth-regulator delayed leaf yellowing in Easter lily. *J. Amer. Soc. Hort. Sci.* 122:117–121.
- Han, S. 1995. Application of growth regulators to delay the development of foliar chlorosis on Easter lilies. *J. Amer. Soc. Hort. Sci.* 120:254–258.
- Jiao, J., M.J. Tsujita, and D.P. Murr. 1986. Effects of paclobutrazol and A-Rest on growth, flowering, leaf carbohydrate and leaf senescence in 'Nellie White' Easter lily (*Lilium longiflorum* Thunb.). *Scientia Hort.* 30:135–141.
- Leshem, Y.Y. 1986. Plant senescence, p. 3–18. In: Y.Y. Leshem, A.H. Halevy, and C. Frenkel (eds.). *Developments in crop science*. Elsevier, Amsterdam.
- Miller, W.B. 1992. Easter and hybrid lily production. Timber Press, Portland, Ore.
- Miller, W.B., P.A. Hammer, and T.I. Kirk. 1993. Reversed greenhouse temperatures alter carbohydrate status in *Lilium longiflorum* Thunb. 'Nellie White'. *J. Amer. Soc. Hort. Sci.* 118:736–740.
- Prince, T.A. and M.S. Cunningham. 1989. Production and storage factors influencing quality of potted Easter lilies. *HortScience* 24:992–994.
- Prince, T.A., M.S. Cunningham, and J.S. Peary. 1987. Floral and foliar quality of potted Easter lilies after STS or phenidone application, refrigerated storage, and simulated shipment. *J. Amer. Soc. Hort. Sci.* 112:469–473.
- Staby, G.L. and T.D. Erwin. 1977. The storage of Easter lilies. *Florists Rev.* 161:38.
- Thimann, K.V. 1987. Plant senescence: A proposed integration of the constituent processes. *Proc. X Annu. Symp. Plant Physiol.* 10:1–19.