Forcing Characteristics of Easter Lily Bulbs Exposed to Elevated-ethylene and -carbon Dioxide and Low-oxygen Atmospheres

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Abstract. Exposure of bulbs of Easter Lily (*Lilium longiflorum* Thunb.) to a maximum of 2 μ l ethylene/liter during vernalization delayed flowering by 5 to 7 days and decreased the number of flower buds. Ethylene exposure for 5 days at 21C after vernalization accelerated shoot emergence and flowering by up to 3 days. No floral or plant abnormalities were observed after bulb exposure to ethylene. Exposure to atmospheres with 0%, 0.5%, or 1% O₂ at 21C for up to 2 weeks before or 10 days after vernalization did not "significantly impair subsequent bulb forcing. Storage in 1% O₂ at 21C for 1 week before vernalization resulted in nearly one additional secondary bud initiated per plant. Exposure to up to 15% CO₂ at 21C for up to 2 weeks before or 10 days after vernalization did not significantly impair subsequent forcing.

We previously reported that lining of cases used for handling and vernalization of bulbs of Easter lily with polyethylene (PE) accelerates floral shoot emergence, floral initiation, and flowering compared to bulbs from nonlined cases (Prince and Cunningham, 1990). One of the reasons for these responses is that PE lining greatly reduces bulb moisture loss during handling, shipping, and vernalization. Because PE liners are a barrier to gas exchange, their use could elevate CO₂ or ethylene or reduce O₂ concentrations in the cases during handling.

Floral abortion and reduction of rooting have been cited as serious symptoms caused by ethylene accumulated in bulbs (Kamerbeek and De Munk, 1976). In tulip bulbs, ethylene production was increased as cold programming progressed (Prince et al., 1982). Ethylene production by lily bulbs, in addition to any microbial source, could result in accumulation of ethylene in the cases during vernalization or during handling periods between vernalization and planting.

A few researchers have exposed lily bulbs to altered O₂ and CO₂ atmospheres. Thornton (1939) found that exposure to 5% O₂ delayed shoot emergence with noncooled bulbs. Later studies (Thornton and Imle, 1941) found that O₂ concentration as low as 2% during storage of bulbs for 28 days at 3.5C had no effect, but that 40% CO₂ delayed shoot emergence, growth, and flowering. Stuart et al. (1970) reported that lily bulbs tolerated anaerobic conditions (100% N₂) for up to 12 weeks at 10, 5, or 0C without apparent damage. Concentrations of 0.25% O₂ during cold storage did delay flowering.

Oxygen depletion and CO₂ accumulation in cases would be most likely to occur before vernalization and during a holding period after vernalization. Temperatures are likely to be highest during these two handling periods, resulting in high respiration rates. Little is known about the possible effects of these atmospheric gases on the vernalization and subsequent forcing re-

sponse of the lily bulb. Our studies examined the forcing response of the bulbs to these gases at these times and the effects of ethylene during and after vernalization.

Materials and Methods

Studies of bulb exposure to reduced O₂ and elevated ethylene were conducted in 1987-88 and those of exposure to CO₂ in 1988-89. Bulbs of 'Nellie White' (23 to 25 cm in circumference) were grown in Oregon, harvested by accepted commercial practice, and packed in cases on 24 Sept. 1987 and 27 Sept. 1988. Bulbs were held in warehouse storage at 14 to 17C until 9 Oct. 1987 and 6 Oct. 1988, when they were shipped to Columbus, Ohio, in refrigerated trucks. Transit required 6 days at 2 to 5C in 1987 and 4 days at 8 to 13C in 1988. After arrival, bulbs were held at 21C until experiments began. All bulbs were vernalized for 6 weeks at 4.5C before, during, or after treatment with various gas mixtures to be described. Gases were humidified to >95% RH (General Eastern Humidity Monitor Model 800B, Watertown, Mass.) and were passed over bulbs held in the dark.

Bulbs were planted in 1.4-liter pots containing Metro-Mix 350 (W.R. Grace, Cambridge, Mass). Bulbs were forced according to standard commercial practice (Tayama et al., 1988). The night minimum was maintained at 15.5C with a maximum growing medium temperature of 18.5C during rooting and floral initiation. Greenhouse ventilation began at 21C after floral initiation.

Ethylene exposure. Bulbs were exposed to ethylene during and after vernalization in two separate experiments. On 27 Oct. 1987, bulbs were sprayed to run-off ($\approx$ 5 ml/bulb) with a solution of silver thiosulfate (STS) at 2 mM Ag⁺ or with water and were allowed to dry for several hours. Humidified air containing 0.1-0.2 or 1.0-2.0 μ l ethylene/liter (Salveit, 1978) or ethylene-free air was passed (8 liter-hr⁻¹) through 40-liter cans used for treating the bulbs during vernalization. Ethylene levels exiting the cans were verified periodically by gas chromatography. Three replicate cans for each ethylene level (nine total cans) were placed randomly in the vernalization room. Two mesh bags containing 16 STS-treated or 16 water-treated bulbs were placed randomly in each can in a split-plot design. Following treatment, bulbs were planted and placed on the greenhouse bench for forcing. At 2 and 4 weeks after planting, three STS-treated and three water-treated bulbs from each can were

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Table 1. Forcing characteristics of 'Nellie White' lily bulbs after silver thiosulfate (STS) pretreatment and ethylene exposure during 6 weeks of vernalization at 4.5C.

Pretreatment	Ethylene concn ($\mu\text{l}\cdot\text{liter}^{-1}$)	Root elongation ^a (rating)		Basal leaf elongation ^b (rating)	Leaf no.	Days from planting until			No. floral buds ^c
		2 wk	4 wk			Shoot emergence	Floral buds visible	First bud open	
Water	0	2.4	2.5	2.6	89	26.1	84.2	114	6.4
	0.1–0.2	1.8	3.5	2.8	96	26.0	88.7	119	6.3
	1.0–2.0	1.7	3.8	2.9	98	26.8	90.8	121	5.9
STS	0	1.9	3.2	2.3	92	27.5	86.2	116	6.3
	0.1–0.2	1.5	3.0	2.6	98	29.0	91.1	121	6.0
	1.0–2.0	1.3	3.6	2.7	99	27.4	91.8	121	5.8
SEM ^w		0.2	0.2	0.1	2	1.6	1.5	1.5	0.2
F tests									
STS		*	NS	*	NS	NS	NS	NS	NS
Ethylene vs. no ethylene		NS	NS	NS	**	NS	NS	**	*
STS $\times$ (ethylene vs. no ethylene)		NS	*	NS	NS	NS	NS	NS	NS

^aBased on 1 (poor) to 7 (excellent) scale (see text).

^bBased on a 3-point scale with 1 = short to 3 = long leaves.

^cIncludes primary and secondary buds.

^wStandard error of a mean.

*, **, NS Significant at $\alpha = 0.05$ or 0.01 or nonsignificant, respectively. All low vs. high ethylene concentration effects and STS $\times$ ethylene concentration interactions were nonsignificant.

unpotted and rated for root growth on a 7-point scale, with 0 indicating no root growth and 7 indicating excellent growth, as described by Prince and Cunningham (1990). The remaining 20 bulbs (half STS-treated) in each can were forced to flowering. Days from planting until 'shoot emergence above the medium, number of visible buds, and date of flowering (opening of first flower bud) were recorded. At flowering, plant height, number of leaves and floral buds, and degree of elongation of the lower leaves were determined. A basal leaf rating was assigned to each plant as follows: 1 = short stubby leaves, 2 = medium-length leaves, or 3 = long leaves (extending over pot rim). A photograph of representative plants of each rating was used for standardization of the rating scale. (Prince and Cunningham, 1990).

For postvernalization ethylene exposure, bulbs were pretreated with STS as described above and then vernalized in ventilated PE bags of moist peat for 6 weeks, beginning 30 Oct. 1987. They then were exposed to ethylene during holding at 21C for 5 days using the same experimental design as described above.

Low-oxygen exposure. Bulbs were continuously exposed to atmospheres of 0%, 0.5% $\pm$ 0.05%, or 1% $\pm$ 0.05% O₂ or to a control atmosphere of 19.25% to 19.35% O₂ during holding for 1 or 2 weeks at 21C. Atmospheres were created by mixing controlled flows of humidified N₂ and O₂. Two replicate 12-liter plastic containers, each containing 10 bulbs, were used for each treatment–duration combination in a completely randomized design. Atmospheres were passed through each container at 12 liter·hr⁻¹ and O₂ concentrations exiting the containers were verified periodically using a paramagnetic O₂ analyzer (Model 570A, Servomex, Norwood, Mass.). Treatments began 1 week apart (21 Oct. and 28 Oct. 1987) for two durations so that all bulbs could begin vernalization on the same date (4 Nov. 1987). After exposure, bulbs were vernalized in ventilated PE bags of moist peat and planted. Evaluation of growth and flowering was as described previously, except that a 3-point root rating scale was used at flowering, with 1 indicating poor rooting and 3 excellent.

Table 2. Forcing characteristics of 'Nellie White' lily bulbs after silver thiosulfate (STS) pretreatment, vernalization, and postvernalization exposure to ethylene for 5 days at 21C.

Pretreatment	Ethylene concn ($\mu\text{l}\cdot\text{liter}^{-1}$)	Days from planting until			No. floral buds ^c
		Shoot emergence	Floral buds visible	First bud open	
Water	0	25.4	91	120	5.5
	0.1–0.2	24.2	90	119	5.0
	1.0–2.0	22.1	88	117	4.7
STS	0	25.9	90	120	5.3
	0.1–0.2	22.7	88	118	5.4
	1.0–2.0	23.5	91	120	4.3
SEM ^y		0.8	0.9	0.9	0.3
F tests					
Ethylene vs. no ethylene		*	NS	NS	NS
Low vs. high ethylene concn		NS	NS	NS	NS
STS $\times$ (low vs. high ethylene)		NS	*	*	NS

^cIncludes primary and secondary buds.

^yStandard error of a mean.

*, **, NS Significant at $\alpha = 0.05$ or 0.01 or nonsignificant, respectively. All STS main effects and STS $\times$ (ethylene vs. no ethylene) interactions were nonsignificant.

For postvernalization exposure, vernalization of bulbs was started in moist peat in shipping cases on 23 Oct. 1987. Treatments were begun on removal, when O₂ levels as described above were applied during holding of bulbs at 21C for 5 or 10 days before planting.

Carbon dioxide exposure. Two studies were conducted identically to the low-O₂ studies, except that atmospheres were 5%, 10%, and 15%, all $\pm$ 1% CO₂, or controls at 0% to 1.5% CO₂. Atmospheres were created by mixing humidified air and CO₂ and were passed through each container at 2.5 liter·hr⁻¹. Carbon dioxide concentrations at the container exits were verified by gas chromatography. For CO₂ exposure before vernalization, treatments began on 18 Oct. 1988 and 29 Nov. 1988 for bulbs treated after vernalization.

Table 3. Forcing characteristics of 'Nellie White' lily bulbs after low-O₂ exposure at 21C for 1 or 2 weeks before vernalization.

Oxygen concn (%)	Duration (wk)	Basal leaf elongation ^z (rating)	Leaf no.	Root elongation ^y (rating)	Days from planting to		No. floral buds	
					Shoot emergence	Floral buds visible	Primary	Secondary
19.3	1	2.6	104	3.0	22.2	88.0	4.9	0.8
1.0		2.4	95	3.0	25.2	84.3	5.0	1.6
0.5		2.4	93	2.9	27.5	87.1	4.8	0.9
0.0		2.4	103	2.7	21.9	85.5	4.8	0.7
19.3		2.7	105	3.0	23.8	89.4	5.0	1.1
1.0	2	2.2	97	2.9	23.9	83.7	4.9	1.5
0.5		2.3	96	2.9	24.3	84.9	4.9	1.0
0.0		1.8	97	3.0	27.9	87.2	5.0	0.8
SEM*		0.2	2	0.07	0.8	1.6	0.1	0.2
F tests								
Oxygen concn								
19.3 vs. others		*	**	NS	*	*	NS	NS
0 vs. low		NS	*	NS	NS	NS	NS	*
0.5 vs. 1.0		NS	NS	NS	NS	NS	NS	*
Oxygen concn × duration								
19.3 vs. others		NS	NS	NS	NS	NS	NS	NS
0 vs. low		NS	*	*	**	NS	NS	NS
0.5 vs. 1		NS	NS	NS	NS	NS	NS	NS

^zBased on a 3-point scale with 1 = short to 3 = long leaves.

^yRating scale of 1 = poor to 3 = excellent.

^xStandard error of a mean.

*, **, NS Significant at $\alpha = 0.05$ or 0.01, or nonsignificant, respectively. All duration main effects were nonsignificant.

Statistical analysis. Analysis of variance was conducted for each study. Main effects and interactions of each factorial treatment arrangement were examined. Single-degree-of-freedom contrasts were used to make more-specific comparisons. Where split-plots were used, appropriate error terms were calculated and used for F tests.

Results

Ethylene exposure. Ethylene exposure during vernalization reduced root growth measured at 2 weeks. Ethylene enhanced the root growth measured at 4 weeks on water-treated bulbs, but only the high concentration of ethylene increased root growth at 4 weeks after planting of STS-treated bulbs (Table 1). STS decreased basal leaf elongation. Ethylene exposure delayed flowering by 5 to 7 days and increased leaf numbers at harvest. The delay in flowering occurred between shoot emergence and flowering, since shoot emergence was not delayed by ethylene exposure.^z The number of floral buds was decreased by 0.5 at the high exposure level. STS had no effect on flowering date nor on leaf or bud number.

Ethylene exposure after vernalization (Table 2) accelerated shoot emergence and days to visible bud and flower. STS interacted by reducing these effects somewhat. Bud number was reduced by about one bud per plant, although the ethylene effect was not significant ($\alpha = 0.057$).

Low-oxygen exposure. Low-O₂ exposure of bulbs before vernalization reduced leaf number and basal leaf elongation observed at flowering (Table 3). Oxygen levels of 0.5% and 0% decreased root elongation at flowering compared to control bulbs after 1 week of exposure. Exposure to 0.5% or 1% O₂ for 1 week delayed shoot emergence, but 0% did not delay emergence compared to controls. After 2 weeks of exposure, only 0% O₂ delayed emergence. The low or 0% O₂ treatments for both 1 and 2 weeks reduced the number of days to visible bud com-

Table 4. Forcing characteristics of 'Nellie White' lily bulbs after vernalization and low-O₂ exposure for 5 and 10 days at 21C.

Oxygen concn (%)	Duration (days)	Basal leaf elongation ^z (rating)	Days from planting to shoot emergence
19.3	5	2.4	26.6
1.0		2.0	27.6
0.5		1.6	27.9
0.0		2.1	29.1
19.3	10	2.1	26.8
1.0		1.7	30.2
0.5		1.5	31.0
0.0		1.9	32.7
SEM ^y		0.1	0.8
F tests			
Oxygen concn			
19.3 vs. others		**	**
0 vs. low		NS	*
0.5 vs. 1.0		*	NS
Duration		*	**

^zBased on 3-point scale with 1 = short to 3 = long leaves.

^yStandard error of a mean.

*, **, NS Significant at $\alpha = 0.05$ or 0.01 or nonsignificant, respectively. All interactions were nonsignificant.

pared to controls. Exposure to 1% O₂ for 1 week resulted in the addition of one floral bud per plant. The increased number of buds (Table 3) was due to an additional secondary bud (Wilkins, 1980). All plants forced from low- and 0% O₂-treated bulbs appeared normal. Exposure to low O₂ after vernalization reduced leaf elongation and delayed shoot emergence, but had no other significant effects (Table 4).

Carbon dioxide exposure. Exposing bulbs to 5%, 10%, or 15% CO₂ before vernalization had very few effects on subse-

Table 5. Forcing characteristics of 'Nellie White' lily bulbs after CO₂ exposure at 21C for 1 or 2 weeks before vernalization.

CO ₂ concn ² (%)	Duration (wk)	Basal leaf elongation ³ (rating)	Leaf no.	Days from planting until		
				Shoot emergence	Floral buds visible	First bud open
Control	1	2.3	95	28.9	92.1	124.6
5		2.2	96	30.7	93.0	124.1
10		2.1	91	30.5	93.0	125.3
15		2.4	92	27.1	89.2	121.0
Control	2	2.1	93	27.6	88.4	122.1
5		2.0	91	28.1	88.2	121.6
10		2.1	91	27.7	89.3	122.3
15		2.1	89	26.8	86.8	119.4
SEM*		0.1	1.4	1.1	1.0	1.4
F tests						
CO ₂ concn						
Cont. vs. any		NS	NS	NS	NS	NS
Concn linear		*	NS	NS	*	NS
Duration		*	*	*	**	*

²Air controls had 0% to 1.5% CO₂ at the exit ports.

³Based on 3-point scale with 1 = short to 3 = long leaves.

*Standard error of a mean.

*, **, NS Significant at $\alpha = 0.05$ or 0.01 or nonsignificant, respectively. All quadratic concentration effects and interactions were nonsignificant.

quent forcing (Table 5). Days to visible bud were reduced 2 to 3 days by the highest CO₂ level, but the resulting reduction in days to flower was not significant. Regardless of the CO₂ content of the gas flowering over the bulbs, 2 weeks of ventilation reduced basal leaf elongation, leaf number, and days to shoot emergence, to visible "bud and to flowering compared to 1 week of ventilation (Table 5).

Exposure to CO₂ during 10 days at 21C after vernalization delayed flowering from 2 to 5 days compared to bulbs held in air for 10 days, but CO₂ caused no other significant effects. Regardless of CO₂ level, ventilation for 10 days reduced days to visible bud and flower by 2 days compared to 5 days of ventilation. Ranges of treatment means were 1.4-1.8 (basal leaf rating), 79-82 (leaf number), 2.8-3.0 (root rating), 15-17 (days to emergence), 72-77 (days to visible bud), 103-108 (days to flower), and 6.2-6.8 (bud number).

Discussion

We have measured ethylene levels ranging from 0.05 to 0.1 $\mu\text{l}\cdot\text{liter}^{-1}$ in internal gas samples vacuum-extracted from healthy bulbs just after removal from 6 weeks of vernalization (unpublished data). We do not know whether this ethylene is from bulb tissue or microorganisms. Our current results indicate that these levels could result in some delay in flowering, and reduced flower number on a commercial scale. However, ethylene does not appear to play a major role in the bulb developmental processes studied. We did not observe floral deformities or bud abortion as we observed on Easter lily plants exposed to ethylene just before bud opening (Prince et al., 1986). Bulbs such as tulips exhibit bulb gummosis, bud abortion, floral deformity, total shoot abortion, and greatly reduced rooting on exposure of bulbs to similar levels of ethylene that we used (Kamerbeek and DeMunk, 1976). The lack of floral and shoot abortion on exposure of lily bulbs to ethylene could be due to the vegetative state of the lily shoot during and immediately after vernalization compared to the reproductive state of the tulip shoot before cold

treatment. The reasons for the lack of bulb gummosis and severe root growth inhibition in the lily bulb are unknown.

Swart (1980) found that bulb immersion in STS just before planting greatly protected cut flowers of 'Enchantment' lily against exogenous ethylene. She did not expose bulbs to ethylene, so a direct comparison to her work is impossible. However, our earlier work (Prince et al., 1986), showing that STS plant sprays reduce bud abortion on Easter lily plants cold stored or exposed to ethylene, suggests that bulb dips might function as observed by Swart (1980). Comparison of our earlier study (Prince et al., 1986) with our present data (Tables 1 and 2) suggests that plants near flowering are more susceptible to ethylene than bulbs themselves. Therefore, STS application to bulbs is not needed to protect bulbs from ethylene exposure, but that some form of STS application can be useful commercially for protecting the flowering plants.

These studies have demonstrated a high degree of tolerance of lily bulbs to low and virtual lack of O₂, and to elevated CO₂ environments at 21C both before and after vernalization. These results extend the observations of Stuart et al. (1970), who found lily bulbs tolerant to anaerobic conditions at low temperatures. Rhizomes of *Iris pseudacorus* L. have also been shown to survive anoxia for 7 days at 22C (Crawford, 1984). Lily bulbs removed from 0% O₂ had a fermentation odor, suggesting anaerobiosis. Regardless, the bulbs survived a period without O₂ that did not impair their subsequent forcing characteristics. The delay in emergence from bulbs exposed to low- or nonoxygenated conditions (Table 4) could have been due to inhibition of respiration or metabolite transport from the bulb scales to the elongating shoot.

The cause of the increased bud count from 1% O₂ storage (Table 3) is unknown. Perhaps an anti-ethylene effect or conservation of respiratory substrates was involved. The prospect that a period of low-O₂ exposure before bulb vernalization could be used to increase bud number deserves further investigation.

These studies have shown that low O₂ and elevated CO₂ concentrations that may develop in PE-lined cases of bulbs before

or after vernalization would not likely be detrimental to bulb forcing. Ethylene accumulations could have negative effects if the bulbs are mishandled, but generally the effects would be minor. Therefore, concerns about the gaseous environment in a PE-lined case should not limit its commercial adoption for handling lily bulbs.

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