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Responses of Whitefly and Poinsettias to Insecticidal Controlled Atmospheres

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ABSTRACT. Eggs, larvae, pupae, and adult stages of greenhouse whitefly (*Trialeurodes vaporarium* Westwood) and silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring) were exposed to insecticidal controlled atmospheres at 20 °C or 30 °C. Mortality data were calculated for each stage and results demonstrated that reduced- O_2 atmospheres (an O_2 level of <2 μ L·L⁻¹ balance in N_2) resulted in faster and higher mortality than elevated- CO_2 atmospheres (25% or 50% CO_2). Responses, from the least to most tolerant stage was adult < larvae < eggs = pupae, regardless of the species of whitefly and treatment temperature. At 20 °C, treatment time required to kill >90% of adults, larvae, and eggs and pupae was 2, 4, and 8 hours, respectively. Increasing the treatment temperature from 20 to 30 °C reduced the treatment time to one-half that of 20 °C. Treatment time required to achieve complete elimination of the insects also caused phytotoxicity symptoms on poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch), thus, limiting use of insecticidal controlled atmospheres as the sole means for managing whitefly.

Infestation of poinsettia (Euphorbia pulcherrima) with greenhouse whitefly (Trialeurodes vaporarium) and silverleaf whitefly (Bemisia argentifolii) can cause major economic losses of this leading floricultural crop. Although there are pesticides that effectively control this insect, restrictions on the amount and type of pesticide used in greenhouses have forced the industry to examine other methods for control of pests.

Controlled atmosphere (CA) is an alternative method for controlling insects. Insecticidal controlled atmospheres are those which are lethal to insects as a result of alteration of surrounding air, usually through application of high CO_2 or low O_2 . Elevated CO_2 (>50%) or reduced O_2 (<1%) treatments have been investigated largely for postharvest insect control in long-term storage areas or for quarantine treatment of some edible crops. CA treatments are effective on various commodities for controlling insects such as the Caribbean fruit fly (Anastrepha suspensa Loew) (Benschoter, 1987), codling moth (Cydia pomonella L.) (Soderstrom and Brandl, 1987), and San Jose scale (Quadras-pidiotus perniciosus Comstock) (Chu, 1992), and the treatments have been suggested as a potential quarantine treatment for imported edible crops (Ke and Kader, 1991). The time required to cause 100% mortality depends on the particular species and developmental stage of the insect as well as the temperature and atmospheric composition during the treatment.

Commodities differ in their tolerance of insecticidal controlled atmospheres (Ke and Kader, 1991). High CO₂ or reduced O₂ may retard ripening and degradation of chlorophyll, and reduce chilling injury. Prolonged exposure of edible crops to CA frequently causes anaerobic respiration and thus the accumulation of ethanol, acetal-dehyde, and other volatile compounds in the tissue which, in turn, may result in an off-flavor of the fruit (Davis and Chace, 1969; Norman and Croft, 1971; Pesis and Avissar, 1989). In contrast to the bulky anatomy of most edible crops, the higher surface to volume ratio of floricultural crops may increase the tolerance of CA (Joyce and Reid, 1985). Vase life of cut anthuriums (*Anthurium andraeanum*

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Lind.) (Akamine and Goo, 1981), carnations (*Dianthus caryophyllus* L.) (Hanan, 1967), daffodils (*Narcissus pseudonarcissus* L.) (Parsons et al., 1967), gladiolus (*Gladiolus ×hortulanus* L.), roses (*Rosa* L. sp.), and snapdragons (*Antirrhinum majus* L.) (Thornton, 1930) has been extended by various combinations of CA treatments. Thus, the objectives of this research were to test the effectiveness of insecticidal controlled atmospheres for control of whitefly on poinsettias and to evaluate the effects of the treatments on poinsettia.

Materials and Methods

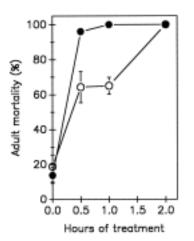
Effects of reduced oxygen controlled atmospheres on whiteflies

ADULT WHITEFLIES. Adult greenhouse or silverleaf whiteflies from a rearing room at the University of Massachusetts, Amherst, were aspirated into 25×150 mm tubes, placed in a 20 °C controlled environment chamber (model 818; Precision Scientific, Inc., Chicago) and connected to a gas stream that delivered air (control) or a reduced O_2 atmosphere (an O_2 level of 100 $\mu L \cdot L^{-1}$ or $<2 \mu L \cdot L^{-1}$ balance in N_2) at a rate of 1 L·h⁻¹. There were three replicate tubes per treatment with ≈ 100 adults per tube. At the end of each treatment time (0, 0.5, 1, or 2 h), tubes were connected immediately to the air stream. At various time intervals after the end of each treatment, live whiteflies (observed to have some movement) were counted. After 24 h, dead insects were removed from the tubes and counted and remaining live whiteflies were gassed with alcohol vapors and counted to determine the total number of whiteflies in each tube at the start of the experiment. Percent mortality was calculated for each tube.

Immature whiteflies. Individual leaves with either the egg, larval, or pupal stage of greenhouse or silverleaf whiteflies were collected from infested poinsettia plants. The particular stage was circled on the leaves. Leaves were then placed in 20 mL vials containing water and treated with a constant flow of air or N_2 (containing an an O_2 level of $<\!2\,\mu L\cdot L^{-1}$ balance in N_2 for larvae and pupae and an O_2 level of $<\!2\,\mu L\cdot L^{-1}$ or $100\,\mu L\cdot L^{-1}$ balance in N_2 for eggs) in the controlled environment chamber as described previously. Following the gas treatments, leaves were maintained in a 20 °C growth chamber with a 16 h photoperiod [17 \pm 3 $\mu mol\cdot m^{-2}\cdot s^{-1}$ measured by a quantum sensor (LI-190SA; LI-COR, Lincoln, Neb.) at leaf level] provided by cool-white fluorescent lamps. Insects at the

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Fig. 1 (**right**). Percent mortality of adult greenhouse whitefly (*Trialeurodes vaporarium*) treated with reduced O_2 levels ($<2\,\mu\text{L-L}^{-1}$) for 0, 0.5, 1 or 2 h. Data were collected between 5 and 24 h after treatment. Data are means \pm se. se bars smaller than the symbols are not shown.



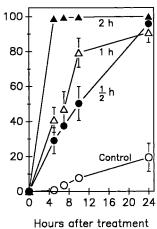


Fig. 2 (**left**). Percent mortality of adult greenhouse whitefly (*Trialeurodes vaporarium*), 24 h after treatment for 0, 0.5, 1 or 2 h with reduced O₂ levels of <2 μL·L⁻¹ (solid circles) or 100 μL·L⁻¹ (open circles). Data are means ± se. se bars smaller than the symbols are not shown

designated stages of development within the circled

areas were counted weekly. The survival rate at each stage was calculated following the passage of sufficient time for the development of insects into the next stage. Eggs were evaluated as percent hatched, larvae as percent killed, and pupae as percent emerged into adults. Data on percent of pupae that emerged successfully into adults were obtained by examination of the pupal cases under a stereoscopic microscope. Pupae that have emerged into adults leave a distinctive opening in the case whereas the cases of those that do not emerge remain intact. In addition, at times, the dead adults were observed in various stages of emergence from the pupal case.

Adult mortality

TEMPERATURE. Silverleaf whiteflies at the egg, larval, and pupal stages were exposed to air or N_2 (containing O_2 at $<2 \,\mu L \cdot L^{-1}$) for 0, 4 or 8 h at 20 °C, or for 0, 2, or 4 h at 30 °C, using the methods described above.

Effects of elevated CO₂ controlled atmospheres on whiteflies

Greenhouse whiteflies were treated with a constant flow of 25% or 50% CO_2 for 0 to 8 h for the adult stage and for 0 to 24 h for eggs and pupae. Percent mortality of adult whiteflies and the survival rate of eggs and pupae were calculated as described previously.

Tolerance of poinsettias to insecticidal controlled atmospheres

ROOTED STEM CUTTINGS. Rooted stem cuttings of nine cultivars of poinsettia ('Celebrate', 'Annette Hegg Red', 'Freedom Red', 'Lilo Red', 'Pink Peppermint', 'Red Sails', 'Supjibi Red', 'V-14 Glory', and 'V-17 Angelica') were obtained from Paul Ecke Ranch, Encinitas, Calif. Six cuttings of each cultivar were placed in 30-L glass tanks housed in a 20 °C dark chamber and vented with a constant flow (45 L·h⁻¹) of air or N₂ (containing O₂ at <2 µL·L⁻¹) for 8h. Cuttings were then transplanted into 10-cm (450-mL) pots filled with a peat based medium (ProMix BX; Premier Brands Inc., Stamford, Conn.) and grown in a glasshouse at the University of Massachusetts, Amherst (lat. 42° 22.5'N) at day/nights of 19/17 °C

(day/night) and under 8 h of natural light with a night interruption from $10\,\mathrm{PM}$ to $2\,\mathrm{AM}$ of $3.6\,\mu\mathrm{mol\cdot m^{-2}\cdot s^{-1}}$ provided by incandescent bulbs. Plants were fertilized with 20N-4.3P-16.6K at $200\,\mathrm{Nmg\cdot L^{-1}}$. Phytotoxicity, based on signs of physiological disorders such as discoloration, wilting, and constricted petioles, was recorded on the day after the termination of the treatment and at weekly intervals for 3 weeks. The degree of phytotoxicity was estimated visually, at 10% increments, as the percentage of total bract area with discoloration. Plant height and dry weight of the stems and leaves were determined 4 weeks after planting, and data were subjected to analysis of variance procedures (SAS Institute Inc., 1990). Paired comparisons were used to test for differences between the nontreated (control) and treated plants.

FLOWERING PLANTS. To explore the possibility of using controlled atmosphere as a quarantine treatment for saleable plants, rooted stem cuttings of six cultivars of poinsettias ('Celebrate II', 'Freedom Red', 'V-14 Glory', 'Annette Hegg Red', 'Pink Peppermint', and 'Supjibi') from Paul Ecke range were potted up and grown until anthesis in the University of Massachusetts glasshouse. Cultural conditions (light, temperature, and fertilization) were the same as those described previously. Flowering plants were treated for 8 h with air or N_2 (containing O_2 at $<2~\mu\text{L-L}^{-1}$) in a dark, 20~°C chamber. Following treatment, plants were transferred back to the greenhouse and the appearance of phytotoxicity was recorded 2 d after treatment.

Results

Effects of reduced oxygen CA on greenhouse whiteflies

EFFECTS ON ADULTS. Exposure of adult greenhouse whiteflies to an O_2 atmosphere of 100 μ L·L⁻¹ for 2 h resulted in 100% mortality within 24 h of treatment, whereas a briefer exposure for 0.5 or 1 h at an O_2 atmosphere <2 μ L·L⁻¹ resulted in >90% mortality (Fig. 1). Percent mortality was reduced to 65% when adults were treated with an O_2 atmosphere of 100 μ L·L⁻¹ for <2 h (Fig. 2).

EFFECTS ON EGGS. An 8 h treatment with O_2 at $100 \,\mu\text{L}\cdot\text{L}^{-1}$ did not consistently prevent eggs from hatching (data not presented), whereas an 8-h treatment with an O_2 level of $<2 \mu L \cdot L^{-1}$ significantly reduced egg hatch (Fig. 3B). Percent hatch collected 2 weeks after an 8-h treatment with low- O_2 (<2 μ L·L⁻¹) or air was 8.2% and 100%, respectively. In subsequent experiments, eggs at different stages of development were treated with low O₂. The stages were defined based on the color change of the eggs from white to dark gray (Sanderson and Ferrentino, 1989). Percent hatch of white (earlier stage) and black (later stage) eggs was collected individually. One week after treatment, percent hatch of eggs treated with air was less for white versus dark colored eggs, reflecting differences in developmental stages of the eggs at the time of treatment (Fig. 4). By the second week, however, all of the white and dark colored eggs treated with air hatched. In contrast, there were no differences in percent hatch of white and dark eggs treated with low O₂. Only 10% of the eggs treated with low O₂ hatched, regardless of the stage of development, indicating that the response of eggs to the reduced O₂ treatment is independent of their developmental stage.

EFFECTS ON LARVAE. All larval stages of greenhouse whitefly (crawler through fourths) were highly susceptible to exposure to low $O_2(<2~\mu L \cdot L^{-1})$ (Fig. 3C). Treatment for 4 or 8 h resulted in dehydration and death of larvae within a few days. Percent mortality of larvae treated with 4 or 8 h of O_2 at $<2~\mu L \cdot L^{-1}$ was >90% 1 week after treatment, and was $\approx 100\%$ a week later.

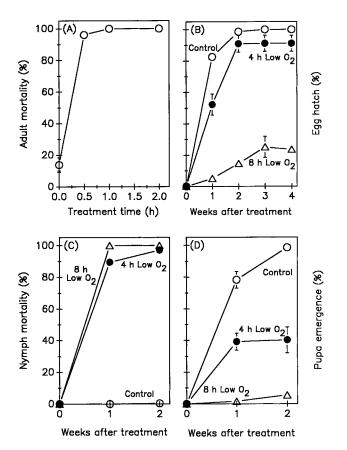
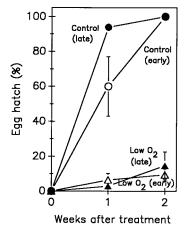


Fig. 3. Effects of low- $O_2(<2\,\mu\text{L}\cdot\text{L}^{-1})$ treatment on (A) percent mortality of adults, 24 h after treatment, (B) hatch of eggs, (C) nymph mortality, and (D) emergence of pupae of greenhouse whitefly (*Trialeurodes vaporarium*). Survival rate of the immature stages of whitefly was calculated following the passage of sufficient time for the development of insects into the next stage. Data are means \pm se. se bars smaller than the symbols are not shown.

Fig. 4. Percent hatch of two stages of greenhouse whitefly (*Trialeurodes vaporarium*) eggs at (A) early (white) and (B) late (black) stage treated with air (circles) or low-O₂ treatment (triangles) (<2 μL·L⁻¹) for 8 h. Data are means ± se. se bars smaller than the symbols are not shown.

EFFECTS ON PUPAE. Exposure of whitefly pupae to O_2 at $<2 \mu L \cdot L^{-1}$ for 4 h decreased percent emergence to $\approx 40\%$, 2 weeks after treatment, in comparison to almost 100% emergence for controls (Fig. 3D). Only 6% of those treated for 8



h emerged within 2 weeks after treatment. The O_2 atmosphere of 100 μ L·L⁻¹ did not reduce emergence of pupae (data not presented).

Effects of insecticidal controlled atmospheres on silverleaf whitefly

RESPONSES OF SILVERLEAF WHITEFLY TO REDUCED OXYGEN AT- MOSPHERES. In general, responses of silverleaf whitefly (Fig. 5) to the CA treatment are similar to those of greenhouse whitefly (Fig. 3). In both species, the adult stage was most sensitive to the CA

treatment with 100% mortality occurring after an exposure time of <2 h at 20 °C. In contrast, eggs were most resistant to the treatment. After a 4 or 8 h treatment with $O_2\!<\!2\,\mu L\!\cdot\! L^{-1},\,10\%$ and 80%, respectively, of the eggs failed to hatch. The larval stage of greenhouse whitefly was killed by a 4-h treatment (Fig. 3C) while sufficient control of silverleaf whitefly required 8 h (Fig. 5C). An 8 h exposure was required to kill >80% of the pupae.

EFFECTS OF TEMPERATURE ON MORTALITY RATE. Increasing treatment temperature, from 20 to 30 °C, significantly decreased the required CA treatment time (Fig. 6). Except for adult whiteflies, treatment times at 30 °C were usually half of those at 20 °C to achieve the same rate of mortality.

Effects of elevated CO₂ atmosphere on whiteflies

An 8-h treatment with 25% or 50% CO_2 was effective at killing adult greenhouse and silverleaf whiteflies but was not effective on immature stages. Increasing the treatment time to 24 h still did not successfully control the pupae: 60% of the pupae emerged after treatment for 24 h with 25% CO_2 compared to 90% of that emerged after the air (control) treatment. Further testing (increased concentrations or duration) of the effects of elevated CO_2 was discontinued because certain cultivars of poinsettia cuttings treated with 24 h of the CO_2 CA developed phytotoxicity, thus, limiting practical application of the treatment. At the termination of CO_2 treatment, there were no signs of damage on the plants.

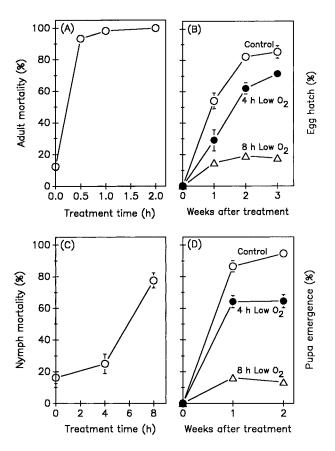


Fig. 5. Effects of low- O_2 treatment (<2 μ L·L⁻¹), applied at 20 °C, on (**A**) percent mortality of adults, (**B**) hatch of eggs, (**C**) nymph mortality, and (**D**) emergence of pupae of silverleaf whitefly (*Bemisia argentifolii*). The mortality rate of adults was collected 24 h after treatment and the survival rate of the immature stages was calculated following the passage of sufficient time for development of insects into the next stage. Data are means \pm se. se bars smaller than the symbols are not shown.

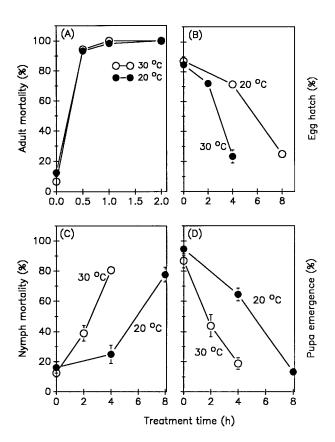


Fig. 6. Effects of low-O₂ treatment (<2 μ L·L⁻¹), at 30 °C or 20 °C, on the mortality of (**A**) percent mortality of adults, (**B**) hatch of eggs, (**C**) nymph mortality, and (**D**) emergence of pupae of silverleaf whitefly (*Bemisia argentifolii*). The mortality rate of adults was collected 24 h after the treatment and the survival rate of the immature stages was calculated following the passage of sufficient time for the development of insects into the next stage. Data are means \pm se. se bars smaller than the symbols are not shown.

However, plants were less turgid (wilted leaves) 12 h after treatment. Within 24 h after treatment, phytotoxicity symptoms such as necrotic areas on the margins of the bracts, upward curling of the margin of the bracts, and collapsed petioles and stems, were observed.

Effects of insecticidal controlled atmospheres on poinsettias

ROOTED CUTTINGS. During vegetative growth, no phytotoxic-

ity was observed on any of the nine poinsettia cultivars after exposure of rooted cuttings to O_2 at $<2 \,\mu L \cdot L^{-1}$ for 8 h. Growth was affected only in the cultivars Supjibi Red and V-17 Angelica where treated plants were shorter and had lower dry weights than the controls (Table 1).

FLOWERING PLANTS. Within 2 d after flowering poinsettias were exposed to O_2 at $<2~\mu L \cdot L^{-1}$ for 8 h, the cultivars Annette Hegg Red and Freedom Red developed phytotoxicity symptoms, evident as areas of discoloration and necrosis, on the bracts (Table 2). Significantly less phytotoxicity occurred on the other cultivars.

Discussion

Insecticidal controlled atmospheres can effectively control whitefly. Reduced O_2 atmospheres, delivered from N_2 cylinders (containing O_2 at $<2~\mu L\cdot L^{-1}$), were more effective in controlling whitefly than were the elevated CO_2 atmospheres (25% or 50%). This is consistent with results of Butler et al. (1986) in which elevated CO_2 levels, up to 200% ambient concentration, did not affect whitefly population in a cotton ($Gossypium\ hirsutum\ L.$) field. The susceptibility of whitefly to the reduced- O_2 ($<2~\mu L\cdot L^{-1}$) atmosphere varies depending on the stage of development. The order from the least to most tolerant was adult < larvae < eggs = pupae. The immature stages (eggs, larvae, and pupae) have been shown to be very resistant to pesticides and their responses to controlled atmospheres are similar.

Raising the treatment temperature significantly increased insecticidal effects of the CA. An increase from 20 to 30 °C significantly reduced the treatment time by one-half, regardless of the developmental stage of the whitefly. The insecticidal effects of controlled atmospheres have also been reported to be dependent on the temperature during treatment of Caribbean fruit fly (Benschoter, 1987), New Zealand thrips (*Thrips obscuratus* Crawford) (Potter et al., 1994), and western flower thrips (Frankliniella occidentalis Pergande) (M.S. Reid, personal communication). In addition, studies have shown that low (0 to 1 °C) (Potter et al., 1994; Seaton and Joyce, 1993) or high [hot water (>45 °C) dips or vapor heat (66 °C)] temperature alone can be lethal to insects (Seaton and Joyce, 1993). However, the similarity in mortality rate of all stages of whitefly treated with air at 20 or 30 °C suggested that the shorter treatment time at higher temperature was due to the increased insecticidal effects of the low-O2 (<2 μL·L⁻¹) atmosphere and not to the temperature.

Many studies have been conducted which test either the physiological tolerance of a produce to CA conditions or the CA

Table 1. Effects of low- O_2 ($<2 \,\mu L \cdot L^{-1}$) treatment on plant height and dry weight of leaves and stems of poinsettia. Rooted cuttings were exposed to air or a low- O_2 atmosphere for 8 h at 20 °C and were then grown in a greenhouse for 4 weeks. Data are means \pm SE of six replicate plants.

	Ht (cm)			Dry wt (g)		
		Low-O ₂			Low-O ₂	
Cultivar	Control	$(<2 \mu L \cdot L^{-1})$	Contrast ^z	Control	$(<2 \mu L \cdot L^{-1})$	Contrast
V-17 Angelica	14.1 ± 0.4	11.4 ± 1.0	**	3.7 ± 0.4	2.4 ± 0.3	**
Celebrate II	11.6 ± 0.8	9.9 ± 0.8	NS	0.4 ± 0.3	1.9 ± 0.2	NS
Freedom Red	10.7 ± 0.7	9.9 ± 0.7	NS	2.9 ± 0.3	2.6 ± 0.3	NS
V-14 Glory	11.1 ± 0.5	11.0 ± 0.5	NS	2.5 ± 0.2	2.2 ± 0.2	NS
Annette Hegg Red	10.5 ± 0.5	9.5 ± 0.5	NS	2.0 ± 0.2	1.6 ± 0.1	NS
Lilo Red	10.9 ± 0.4	10.7 ± 0.6	NS	2.5 ± 0.2	2.1 ± 0.1	NS
Pink Peppermint	8.9 ± 0.4	10.0 ± 0.4	NS	2.0 ± 0.2	2.3 ± 0.2	NS
Red Sails	10.7 ± 0.8	11.0 ± 0.8	NS	2.8 ± 0.3	2.3 ± 0.3	NS
Supjibi Red	8.8 ± 0.3	6.5 ± 0.5	*	2.4 ± 0.1	1.5 ± 0.2	*

 $[\]overline{^{NS,*,**}}$ Nonsignificant or significant at P = 0.05 or 0.01, respectively.

Table 2. Susceptibility of bracts of six cultivars of poinsettias to an 8 h, low-O $_2$ (<2 μ L·L $^{-1}$ O $_2$) treatment. The degree of phytotoxicity was estimated visually, at 10% increments, as the percentage of total bract area with discoloration. Data were collected 2 d after treatment and are means \pm SE of six replicate plants.

	Phytotoxicity
Cultivar	(%) ^z
Celebrate II	0.1 c ^y
Freedom Red	7.0 b
V-14 Glory	1.1 c
Annette Hegg Red	31.4 a
Pink Peppermint	0.3 c
Supjibi	4.1 c

^zPercentage of area with symptoms of phytotoxicity.

conditions required for insect disinfestation. Few are a combination of the two and the variation in the individual studies make it difficult to compare conditions that would be tolerated by plants and lethal to insects (Ke and Kader, 1991). However, a study by Seaton and Joyce (1993) evaluated the physiological tolerance of three Australian cut flowers to various combinations of CA (high or low temperature, O₂ and CO₂ concentrations, and treatment time) as well as the CA treatment required for eradication of adult flour beetles (Tribolium confusum Koch.) and Mediterranean fruit fly larvae (Ceratitis capitata Wied.). They concluded that the tolerance level of the cut flowers to the CA treatment varies greatly among species. In addition, the duration of the CA treatment necessary to kill all insects was often longer than that tolerated by the cut flowers. In our study, the tolerance of plants to the CA treatment was dependent on the developmental stages. An 8-h low oxygen treatment ($<2 \mu L \cdot L^{-1}$) at 20 °C, as required to affect the immature stages of whitefly, did not induce phytotoxicity or reduce growth and development of the rooted stem cuuttings of the various poinsettia cultivars that were tested. However, flowering poinsettias were very susceptible to the low O_2 treatment ($<2 \mu L \cdot L^{-1}$) resulting in discoloration of the leaves and petals or collapse of the leaf petiole within 2 d or, in severe cases, death of the plant. The tolerance of flowering poinsettias to low- O_2 treatment (<2 μ L·L⁻¹) was less than that required to achieve complete elimination of whitefly, thus limiting the practical application of low-O₂ treatment as a quarantine treatment for this greenhouse crop.

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^yMean separation by Duncan's multiple range test, P < 0.05.