

## MODIFIED ATMOSPHERE FOR THRIP DISINSECTION ON CUT LOTUS FLOWERS

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**Abstract.** In Thailand, lotus flowers are one of exportable products for flower trade industry. Common blossom thrip (*Frankliniella schultzei*) contamination on cut lotus flowers after harvest has been a major problem for export. *F. schultzei* is a quarantine insect pest. Modified atmospheres (MA) without oxygen content are considered an alternative to methyl bromide fumigation to control thrips. MA treatment has been used to control insect pests in agricultural product commodities. Different combinations of carbon dioxide, nitrogen, ozone levels, and treatment times were used to effect mortality of the common blossom thrips and postharvest quality of cut lotus flowers. Exposure to different concentrations of O<sub>3</sub> fumigation for 2 days could kill almost all *F. schultzei* (100% of larvae and  $\geq 96\%$  of adults). However, a concentration of 50-250 ppm of O<sub>3</sub> could not control *F. schultzei* on lotus flowers completely. O<sub>3</sub> fumigation caused color change in lotus flowers. High concentrations of O<sub>3</sub> ( $\geq 150$  ppm) had a negative effect on the visual quality of lotus flowers. The results revealed that thrips mortality increased with increased CO<sub>2</sub> level and storage time. 100% CO<sub>2</sub> caused 100% mortality of both adults and larvae of *F. schultzei* when there was a 6-h exposure. MA were more effective in disinfecting of thrips on cut lotus flowers after 9 h fumigation to  $\geq 50\%$  CO<sub>2</sub> caused complete mortality to *F. schultzei*. There was not much difference in lotus color in response to atmosphere modified by a combination of CO<sub>2</sub> and N<sub>2</sub>. Therefore, CO<sub>2</sub> disinfection treatment has the potential to be developed commercially as an alternative postharvest control for common blossom thrips on lotus flowers.

**Keywords:** CO<sub>2</sub> fumigation, color change of lotus flowers, *Frankliniella schultzei*, post-harvest treatment, visual quality

### Introduction

Sacred lotus (*Nelumbo nucifera* Gaerth) is an interesting plant. All parts of the lotus can be utilized and processed into a variety of products. Thailand has a suitable climate for this plant. Currently, Thailand has plenty of wetlands and often there are flooding problems; therefore it is suitable for lotus planting. Growers can generate an income throughout the year. *N. nucifera* has the potential to develop into a Thai economic crop for export. Major lotus flower markets in the country include Pak Klong Market, Four Corner Thai Market, and flower markets in each province. The major foreign markets include Hong Kong, Singapore, the Netherlands, Japan, and the USA.

Thrips are a major problem for lotus flowers before and after harvest. *Frankliniella schultzei* (Trybom) are found throughout the year and predominantly attack lotus flowers (Bumroongsook, 2018). Therefore, postharvest control is an important process to export cut lotus flower. Lotus flowers for export require consistent quality and phytosanitary certification to meet international standards. However, it is difficult to find effective and sustainable pest management both in pre-harvest and post-harvest. The lotus shape contributes to the habitat for thrips. In order to control the thrip population among lotus flowers, growers regularly spraying insecticides, which is

ineffective because not all of the thrips are killed. Therefore, it is necessary to remove post-harvest thrips in accordance with the standard of agricultural material export to foreign countries. The Thai Department of Agriculture has set the criteria for the removal of insect pests for orchids and ornamentals, with the methyl bromide rate of 20-24 g/m<sup>3</sup> for 90 min or insecticide dips (Thai Department of Agriculture, 2003). However, some shipments of lotus flowers have been rejected over the past few years because of the presence of thrips on lotus flowers.

Fumigation has been the primary method of postharvest disinfection for insect pests of cut flowers and agricultural products to meet export requirements (Pupin et al., 2013; Williams and Muhunthan, 1998). At present, methyl bromide is the main method which exporters use for insect disinfestation of cut flowers prior to export. It is smokeless, colorless, odorless, and non-flammable. It is an ozone-depleting substance; therefore, safe alternatives must be used to protect life and the environment. Innovative insect disinfestation techniques should be assessed. Modified atmosphere (MA) is defined as altering the composition of atmosphere in the storage packaging in order to extend the shelf life of agricultural products (Jayas and Jeyamkondan, 2002; Liguori et al., 2015). MA treatments have been used to control insects in agricultural product commodities (Beaudry, 1999, 2000; Liu, 2008, 2013). MA includes ultra low oxygen, ozone, carbon dioxide, and a dynamic concentration of carbon dioxide and oxygen (Kerr et al., 2013; Pinhero et al., 2009; Raffo et al., 2009; Tiwari et al., 2010). Ozone causes mortality throughout all life stages of the bean thrips (Leesch et al., 2007).

Carbon dioxide (CO<sub>2</sub>) has direct and indirect effects on the physiological response and mortality of insects (Grodzinski et al., 1999). It affects the knockdown and recovery of insects (Nilson et al., 2006). In addition, it induces toxicity at the cellular level of living organisms; it can cause damage to the respiratory system of insects and deplete air reserves and prevents the exchange of oxygen through the insect egg chorion (Lagunas-Solar et al., 2006). The rate of insect egg development depends on the CO<sub>2</sub> concentration (Kerr et al., 2013). High levels of CO<sub>2</sub> concentration are applied as an anesthetic for insect knockdown. Badre et al. (2005) showed that CO<sub>2</sub> blocked glutamate receptors. High CO<sub>2</sub> concentrations caused hearts of *D. melanogaster* larvae to stop working and interfered with synaptic transmission at the neuromuscular junction. Carbon dioxide exposure might affect insect longevity, mating success and growth, feeding, development, reproduction, and behavior (Bartholomew et al., 2015). With a 24-h exposure time at 25 °C, a lethal concentration of CO<sub>2</sub> for all bed bugs stages was approximately 30% (Wang et al., 2014). The use of high CO<sub>2</sub> concentrations in gastight large bags is a possible method for preventing the occurrence of post-harvest pests on agricultural commodities, including rice, cocoa, beans, and various dried herbs during storage (Pons et al., 2010).

Ozone (O<sub>3</sub>) is the most common air pollutant. It is a product of nitrogen oxides and volatile organic compounds under sunlight. It has harmful effects on humans, plants, and insects (Kampa and Castanas, 2008; Díaz-de-Quijano et al., 2012; Farré-Armengol, 2016). Surface ozone decreases crop production, disables forest growth, and effects plant species composition. (Ashmore, 2015). At relatively low concentrations, tropospheric ozone is toxic to susceptible plant species and causes chlorotic mottle, pigmented stipple, and necrosis. Foliar injury of cutleaf coneflower growing near the edge of the Clingmans Dome trail was significantly greater than that in the Purchase Knob (70% vs. 40% ozone-injured plants, respectively), due to different ozone exposures (Szantoi et al., 2009). Injury were consistent within species, genetic variation

among plants and diversification of physiological vigor (Brace et al., 1999). Ozone gas fumigation is a quarantine treatment to control stored pests and insect pests on fresh agricultural products (Hollingsworth and Armstrong, 2005). O<sub>3</sub> treatment can be used to control thrips and mealybugs on some selected agricultural products (Liu, 2013).

This research is undertaken to assess MA using dynamic concentrations of CO<sub>2</sub> balanced with N<sub>2</sub> and O<sub>3</sub> for thrip disinfestation and their impacts on the color of lotus flowers and quality.

## Material and methods

### *Sample collection*

Pesticide-free lotus farms that are situated at Ladkrabang District in Bangkok, Thailand were selected for sample collection. Standard buds of Roseum Plenum lotus flowers aged 9 to 10 days were collected from these lotus farms. Both adults and larvae of *F. schultzei* were obtained from blossom lotus flowers.

### *Experimental design*

The experimental design was 6 × 7 factorial in completely randomized design to assess the effects of the combination of CO<sub>2</sub> and N<sub>2</sub> against *F. schultzei* on lotus flowers (30 replicates/group). Factor A is different composition of CO<sub>2</sub> balanced N<sub>2</sub> and factor B is exposure time.

Effects of O<sub>3</sub> against *F. schultzei* on lotus flowers was performed by using 7 × 7 factorial experiments in completely randomized design (30 flowers/group). Factor A is different composition of O<sub>3</sub> and factor B is exposure time.

The experiments were carried out at the entomological laboratory, King Mong's Institute of Technology Ladkrabang.

### *Effects of the combination of CO<sub>2</sub> and N<sub>2</sub> on F. schultzei*

The experiment involved exposing MA to both adults and larvae of *F. schultzei*. *F. schultzei* were transferred to a young lotus petal and placed in a vial. Then, they were placed in a plastic chamber. The air was expelled and a gas mixture of CO<sub>2</sub> and N<sub>2</sub> was introduced into the air-tight plastic chamber. The experiments were conducted for various modified atmosphere packaging: 0, 25, 50, 75, 100% (v/v) CO<sub>2</sub> with remaining N<sub>2</sub>, and stored for 1 to 3 days at 15 °C. After the treatments, insects were considered dead if they did not move. They were scored as alive if they moved. The dead insects were observed at 1, 2, and 3 days of exposure to the MA and storage temperature of 15 °C.

### *Effects of the combination of CO<sub>2</sub> and N<sub>2</sub> on F. schultzei on lotus flowers*

A cut lotus flower aged 9 to 10 days was exposed to different combinations of CO<sub>2</sub> and N<sub>2</sub> and stored for 3-72 h. The ambient air was removed and the CO<sub>2</sub> and N<sub>2</sub> mixture was introduced into the polypropylene plastic package (10 × 15 inches) before being sealed by a gas filling vacuum packing machine (Hualiangthai model DZQ400/500). After the specified treatments, the package was opened to investigate dead or alive thrips after 1, 2, and 3 days of exposure to MA and a storage temperature of 15 °C.

### **Effects of O<sub>3</sub> on *F. schultzei***

*F. schultzei* was placed on a young lotus petal, transferred to a vial, and kept in a gas-tight plastic chamber. The experimental procedure exposed different concentrations of O<sub>3</sub> (0, 50, 75, 100, 125, 150, 250, 500, 750, and 1,500 ppm) using an ozone generator (Enaly model OZX-300 AT). The observation times were 3, 6, 9, 12, 24, 48, and 72 h. The mortality of adult and larva of *F. schultzei* was assessed.

### **Effects of O<sub>3</sub> on *F. schultzei* on lotus flowers**

A cut lotus flower aged 9 to 10 days was placed in a plastic chamber and exposed to different concentrations of O<sub>3</sub> (0, 50, 75, 100, 125, 150, 250 ppm) and stored for 3 to 72 h at 15 °C. The observation times were 3, 6, 9, 12, 24, 48, and 72 h. The mortality of *F. schultzei* was assessed. The color of petals at the outer curvature was measured with a miniscan (EZ4500, Hunter) and displayed color as three numerical values: L \*, a \*, and b \*.

### **Data analysis**

Analysis of variance was used to evaluate these treatment differences by comparing the mean of the control with the mean of each treatment followed by the Duncan multiple range test for mean separation ( $p \leq 0.05$ ).

## **Results**

### **Effects of the combination of CO<sub>2</sub> and N<sub>2</sub> on *F. schultzei***

The experiments evaluated the effects of a CO<sub>2</sub> and N<sub>2</sub> mixture on *F. schultzei*. Total mortality was achieved at  $\geq 6$  h using 100% CO<sub>2</sub> atmospheres (Table 1). At 25% CO<sub>2</sub>, the mortality of thrip larvae during all exposure time was significantly lower ( $p < 0.05$ ) when compared with any other CO<sub>2</sub> concentration. At 100% N<sub>2</sub>, the percentages of dead insects were 63% at an exposure time of 24 h. Complete mortality occurred in 72 h. Within 48 h of exposure time, 100%N<sub>2</sub> was more effective in controlling thrip larvae than 25% CO<sub>2</sub> ( $p < 0.05$ ). At 72 h exposure time, all treatments killed 100% of thrip larvae.

**Table 1.** Effect of CO<sub>2</sub> and N<sub>2</sub> fumigation on mortality of *F. schultzei* larvae (N = 10)

Treatment	Mortality percentage of thrips after (h) treatment <sup>1</sup>						
	3	6	9	12	24	48	72
Control	1.00c	3.70d	5.50d	10.00e	14.00e	53.00c	100.00a
100% N <sub>2</sub>	4.60bc	16.00bc	34.00b	43.00c	63.00c	95.00a	100.00a
25% CO <sub>2</sub>	1.00c	12.20c	21.00c	30.00d	42.00d	89.00b	100.00a
50% CO <sub>2</sub>	3.70bc	19.00bc	38.00b	61.00b	86.00b	98.00a	100.00a
75% CO <sub>2</sub>	7.40b	22.00b	39.00b	50.00c	85.00b	100.00a	100.00a
100% CO <sub>2</sub>	21.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a

<sup>1</sup>Means followed by different letters indicate significant difference between concentrations

The mortality of *F. schultzei* adults under different MA conditions and storage times are illustrated in Table 2. *F. schultzei* adults showed almost the same response to different levels of CO<sub>2</sub> and N<sub>2</sub> mixtures as did larvae (Tables 1 and 2). Total mortality

of adults occurred at  $\geq 6$  h using 100% CO<sub>2</sub> (Table 2). With the initial time of 48-h exposure, 100% N<sub>2</sub> was more effective in controlling *F. schultzei* adults than 25% CO<sub>2</sub> ( $p < 0.05$ ). The results showed that complete mortality in all concentrations occurred 72 h after treatment.

**Table 2.** Effect of CO<sub>2</sub> and N<sub>2</sub> fumigation on mortality of *F. schultzei* adult (N = 10)

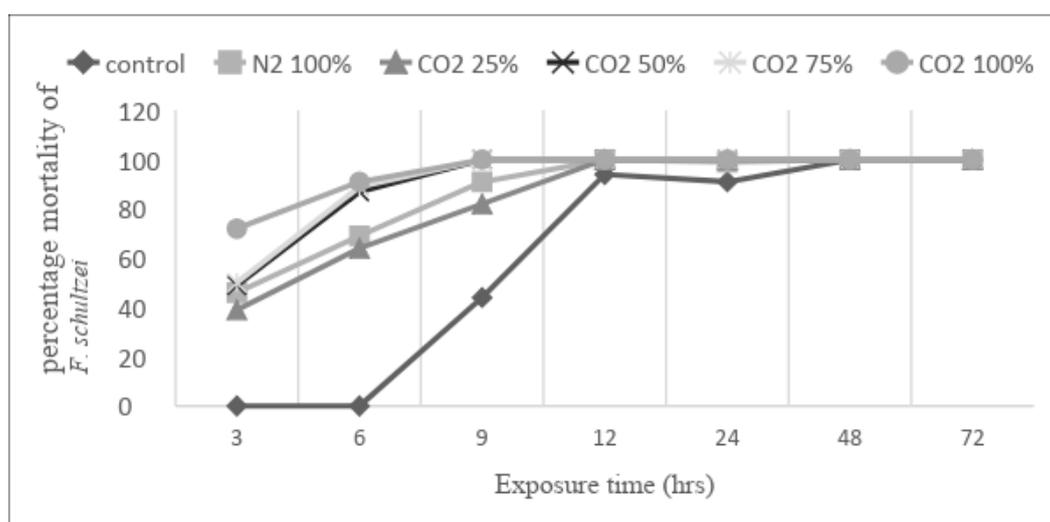
Treatment	Mortality percentage of thrips after (h) treatment <sup>1</sup>						
	3	6	9	12	24	48	72
Control	1.00c	3.70e	4.60e	9.20e	17.10d	60.00c	100.00a
100% N <sub>2</sub>	4.60c	13.00cd	32.00bc	46.00bc	80.00b	96.00a	100.00a
25% CO <sub>2</sub>	1.00c	9.30d	24.00d	40.00c	53.00c	93.00ab	100.00a
50% CO <sub>2</sub>	1.90bc	15.00bc	29.00cd	32.00d	58.00c	86.00b	100.00a
75% CO <sub>2</sub>	6.50b	19.00b	35.00b	51.00b	86.00b	100.00a	100.00a
100% CO <sub>2</sub>	23.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a

<sup>1</sup>Means followed by different letters indicate significant difference between concentrations

### Effects of the combination of CO<sub>2</sub> and N<sub>2</sub> on *F. schultzei* on lotus flowers

There was a significant effect of CO<sub>2</sub> balanced N<sub>2</sub> and exposure time on thrips mortality ( $F_{5,1218} = 9457.12$ ,  $p < 0.00001$ ;  $F_{6,1218} = 27578.07$ ,  $p < 0.00001$ , respectively). Moreover, the significant interaction between treatments and exposure time on thrip mortality was observed ( $F_{30,1218} = 1864.27$ ,  $p < 0.00001$ ).

High mortality of thrips on lotus flowers packaged in plastic bags was observed under different MA and storage times (Table 3). The exposure time of 3 h and 100% CO<sub>2</sub> had the highest thrip mortality, followed by 100% N<sub>2</sub>, 50% and 75% CO<sub>2</sub> having 46.29, 49.30, and 50.09% mortality, respectively whereby 25% CO<sub>2</sub> having the lowest mortality (39.21%). At 12 h of exposure time, the results showed no significant differences among treatments and no live thrips were observed with 100% N<sub>2</sub> and all concentrations of CO<sub>2</sub>. Higher CO<sub>2</sub> concentrations and longer exposure time could increase mortality of mortality of *F. schultzei* (Fig. 1). All treatments killed 100% of *F. schultzei* kill after 48 h of exposure.



**Figure 1.** Mortality of *F. schultzei* on lotus cut flowers under different concentration of CO<sub>2</sub> fumigation and exposure time

**Table 3.** Effect of CO<sub>2</sub> and N<sub>2</sub> fumigation on mortality of *F. schultzei* on lotus cut flowers (N = 30)

Treatment	Mortality percentage of thrips after (h) treatment <sup>1</sup>						
	3	6	9	12	24	48	72
Control	0.00d	0.00c	44.44b	94.73a	91.00a	100.00a	100.00a
100% N <sub>2</sub>	46.25b	69.34b	91.66a	100.00a	99.00a	100.00a	100.00a
25% CO <sub>2</sub>	39.21c	64.68b	82.77a	100.00a	100.00a	100.00a	100.00a
50% CO <sub>2</sub>	49.30b	86.61a	100.00a	100.00a	100.00a	100.00a	100.00a
75% CO <sub>2</sub>	50.09b	88.88a	100.00a	100.00a	100.00a	100.00a	100.00a
100% CO <sub>2</sub>	72.22a	90.74a	100.00a	100.00a	100.00a	100.00a	100.00a

<sup>1</sup>Means followed by different letters indicate significant difference between concentrations

### Effects of the combination of CO<sub>2</sub> and N<sub>2</sub> on lotus flower color

The color of lotus flower was measured using miniscan (EZ4500, Hunter). The color parameters L\* (lightness), a\* (red–green), and b\* (yellow–blue) of lotus flowers under MA treatments were compared. No differences ( $p > 0.05$ ) in the value of L\* and a\* were observed at 1, 2, and 3 days of exposure (Table 4). The a\* value in the control was less than that of all levels of CO<sub>2</sub> and 100% N<sub>2</sub> on day 1 and 2 after exposure. On day 3, all treatments showed no significant difference in L\*, a\*, and b\* values ( $p > 0.05$ ).

**Table 4.** Color of Lotus flowers after fumigation treatment (N = 30)

Treatment	Day after treatment <sup>1</sup>								
	1			2			3		
	L	a	b	L	a	b	L	a	b
Control	59.90 <sup>ns</sup>	1.37b	18.79 <sup>ns</sup>	57.32 <sup>ns</sup>	2.91ab	18.98 <sup>ns</sup>	58.32 <sup>ns</sup>	4.77 <sup>ns</sup>	16.56 <sup>ns</sup>
100% N <sub>2</sub>	56.89	5.80a	17.70	59.43	5.37a	23.35	61.67	5.47	15.12
25% CO <sub>2</sub>	58.71	4.81a	17.62	59.29	4.88a	17.88	58.21	5.44	16.47
50% CO <sub>2</sub>	57.15	4.57a	16.82	58.51	4.60a	16.75	58.50	5.76	17.14
75% CO <sub>2</sub>	57.41	4.24a	18.35	57.61	2.17b	18.92	59.72	4.66	18.91
100% CO <sub>2</sub>	57.86	4.98a	19.27	58.48	4.31a	17.12	58.62	3.64	18.26

<sup>1</sup>Mean followed by different letters indicate significant difference between concentrations

### Effect of O<sub>3</sub> on *F. schultzei*

The lethal concentration of  $\geq 50$  ppm O<sub>3</sub> to *F. schultzei* larvae in a plastic chamber was 100% thrip mortality at 48 and 72 h of exposure time (Table 5). After 12 h of exposure to ozone fumigation,  $\geq 500$  ppm O<sub>3</sub> caused 100% thrip mortality. The results observed indicated that 1,500 ppm O<sub>3</sub> was the highest effective concentration as compared with the other treatments, and it caused 100% thrip mortality at 3 h of exposure.

Effects of O<sub>3</sub> on *F. schultzei* adults had similar patterns of response to O<sub>3</sub> fumigation as in the larvae (Table 6). At 12 h of O<sub>3</sub> fumigation exposure,  $\geq 500$  ppm O<sub>3</sub> caused 100% mortality of *F. schultzei* adults. The results revealed that 1,500 ppm O<sub>3</sub> at 3 h of exposure caused 100% mortality of adults.

### Effects of O<sub>3</sub> on *F. schultzei* on lotus flowers

The experiment was conducted to evaluate the different levels of O<sub>3</sub> fumigation (50–250 ppm) for disinfestation of *F. schultzei* on lotus flowers. Significant effect of O<sub>3</sub>

concentration and exposure time on thrips mortality was detected ( $F_{6,1421} = 2762.65$ ,  $p < 0.00001$ ;  $F_{6,1421} = 9,318.07$ ,  $p < 0.00001$ , respectively). Moreover, there was a significant interaction between different  $O_3$  concentration and exposure time ( $F_{36,1421} = 34.88$ ,  $p < 0.00001$ ). Whereas, the results were displayed in *Table 7*, indicated that all these concentrations of  $O_3$  could not eliminate the entire thrip infestation on lotus flowers. *F. schultzei* mortality increased, with increased  $O_3$  levels and exposure time.

**Table 5.** Toxicity of  $O_3$  on mortality of *F. schultzei* larvae ( $N = 10$ )

Ozone conc (ppm)	Mortality percentage of thrips after (h) treatment						
	3	6	9	12	24	48	72
Control	0.00e	0.00 f	0.00 e	19.73 c	39.17 bc	54.25 c	74.22 c
50	10.00 d	14.00 e	18.00 d	20.00 c	32.00 c	100.00a	100.00a
75	12.00 d	17.00 e	20.00 d	21.00 c	32.00 c	100.00a	100.00a
100	12.00 d	16.00 e	19.00 d	23.00 c	34.00 c	100.00a	100.00a
125	12.00 d	13.00 e	20.00 d	23.00 c	36.00 c	100.00a	100.00a
150	12.00 d	16.00 e	22.00 d	24.00 c	47.00b	100.00a	100.00a
250	22.00 c	28.00 d	30.00 c	38.00 b	46.00 b	100.00a	100.00a
500	66.00 b	79.00 c	94.00 b	100.00 a	100.00 a	100.00a	100.00a
750	67.00 b	86.00 b	95.00 b	100.00 a	100.00a	100.00a	100.00a
1500	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00a	100.00a

<sup>1</sup>Mean followed by different letters indicate significant difference between concentrations

**Table 6.** Toxicity of  $O_3$  on mortality of *F. schultzei* adults ( $N = 10$ )

Ozone conc (ppm)	Mortality percentage of thrips after (h) treatment <sup>1</sup>						
	3	6	9	12	24	48	72
Control	0.00f	1.00g	0.00d	12.00d	38.00c	48.00b	60.00b
50	10.00 e	12.00 f	20.00 c	21.00 c	42.00 c	96.00a	100.00a
75	10.00 e	12.00 f	22.00 c	23.00 c	47.00 bc	96.00a	100.00a
100	11.00 e	13.00 ef	23.00 c	24.00 c	48.00 bc	96.00a	100.00a
125	11.00 e	14.00 ef	23.00 c	24.00 c	49.00 bc	96.00a	100.00a
150	13.00 e	20.00 df	23.00 c	25.00 c	52.00 bc	100.00a	100.00a
250	23.00 d	24.00 d	28.00 c	30.00 b	55.00 b	100.00a	100.00a
500	48.00 c	59.00 c	65.00 b	100.00 a	100.00 a	100.00a	100.00a
750	60.00 b	72.00 b	70.00 b	100.00 a	100.00 a	100.00a	100.00a
1500	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00a	100.00a

<sup>1</sup>Mean followed by different letters indicate significant difference between concentrations

**Table 7.** Effect of  $O_3$  fumigation on *F. schultzei* in lotus flowers ( $N = 30$ )

Ozone conc (ppm)	Mortality percentage of thrips after (h) treatment <sup>1</sup>						
	3	6	9	12	24	48	72
Control	8.46 g	12.40 g	22.72 f	29.17 f	44.25 d	50.34d	74.22 c
50	23.81 f	27.03 f	38.88 e	58.73 e	69.47 c	75.22c	85.43 b
75	33.50 e	36.73 e	38.66 e	57.61 e	70.89 c	78.43b	94.90 a
100	36.42 d	38.57 d	44.82 d	61.65 d	85.20 b	90.32a	96.48 a
125	45.33 a	42.68 c	50.06 c	63.99 c	88.46 a	92.46a	98.23 a
150	42.76 c	47.93 b	54.06 b	68.77 a	84.84 b	93.33a	94.12 a
250	42.80 b	51.47 a	55.71 a	68.11 b	85.03 b	95.45a	96.50 a

<sup>1</sup>Mean followed by different letters indicate significant difference between concentrations

### Effects of O<sub>3</sub> on lotus flower color

The L\*a\*b\* value of lotus petals exposed to different levels of O<sub>3</sub> fumigation (50-250 ppm) were different from those of the control (Table 8). The b\* value of lotus petals in 48 and 72 exposure hours were not significantly different among treatments ( $p > 0.05$ ). The color of lotus petals exposed to O<sub>3</sub> fumigation were slightly yellow (negative a\* values), and the petals from control treatments were red (positive a\* values). The color change of lotus flower petals was a response to ozone fumigation. It might not be enough to show much variation in flower color. Ozone fumigation made lotus flower color paler and yellowish. Slight malformation of lotus flowers was detected.

**Table 8.** Effect of ozone fumigation on color of lotus flowers (N = 30)

Ozone conc (ppm)	Day after treatment <sup>1</sup>								
	1			2			3		
	L	a	b	L	a	b	L	a	b
Control	60.90a	1.43a	18.79a	57.32a	2.91a	18.98 <sup>ns</sup>	58.32a	3.97a	16.06 <sup>ns</sup>
50	60.57a	1.33a	18.13a	49.30ab	-0.19b	21.74	51.06ab	-0.71b	16.91
100	51.65b	-0.21b	14.45b	47.62b	-0.81b	21.38	46.35b	-0.84b	14.92
150	51.06b	-0.01b	15.39ab	45.41b	-0.525b	19.91	46.70b	-0.74b	14.74
250	48.38b	-0.55b	13.06b	53.41a	-0.835b	21.43	51.75ab	-0.99b	15.15

<sup>1</sup>Mean followed by different letters indicated significant difference between concentrations

### Discussion

Arthropods reduce their metabolic rate and increase membrane permeability under high levels of carbondioxide atmosphere (Mitcham et al., 2006; Zhou et al., 2001). Spiracular opening due to low level of CO<sub>2</sub> can cause insect death, whereas at higher CO<sub>2</sub> concentrations, metabolic reduction is an important factor for mortality (Janmaat et al., 2001). Held et al. (2001) observed that exposure over 12 h to >99% N<sub>2</sub> or CO<sub>2</sub> accounted for 100% mortality of aphids, mites, thrips, and whiteflies. CO<sub>2</sub> levels > 20% are toxic to insects and higher concentration cause insect mortality (Carpenter and Potter, 1994; Mitcham et al., 2006; Zhou et al., 2001). Consistent with the previous studies, using different combination of CO<sub>2</sub> and N<sub>2</sub> for the disinsection of *F. schultzei* on lotus flowers in polypropylene plastic packaging could eliminate all thrips on lotus flowers at 12 h of exposure time.

The results obtained in this study agree with Niakousri et al. (2010) whereby lower ozone concentration required longer exposure time to have effective fumigation. Ozone is unstable and has a short half-life and converts to oxygen during ozonation. Our results indicated that under  $\geq 150$  ppm O<sub>3</sub> fumigation caused color change and slight malformation of lotus flowers. This caused the flowers to be unacceptable for the markets. All of the ornamental plants tested were blemished due to O<sub>3</sub> treatments (Hollingsworth and Armstrong, 2005). The studies indicated that  $\geq 500$  ppm of O<sub>3</sub> is needed to have complete control of *F. schultzei*, but the lotus flowers are damaged from this fumigation. The previous studies showed that agricultural products with waxy surfaces could tolerate the high concentrations of ozone fumigation such as waxy leaves, waxed oranges (Hollingsworth and Armstrong, 2005; Leesch et al., 2003). There was more moisture among lotus petals in the bag, which increased the control of thrips. Cut flowers lose water through transpiration (Sankat and Mujaffar, 1994). This might be one factor responsible for thrip mortality.

Gas concentration, fumigation time and temperature are three important key variables for the success of MA (Armstrong and Whitehead, 2005). An increase in concentration of CO<sub>2</sub> or O<sub>3</sub> in fumigation and exposure time would increase *F. schultzei* mortality. Dhouibi et al. (2015) use CO and CO<sub>2</sub> fumigation for *Ephestia kuehniella* and *Ectomyelois ceratoniae* in dates and indicated that insect mortality depended on gas levels and exposure time. Controlled atmosphere and temperature treatment system can control *C. sasakii* found in apples (Son et al., 2012). However, insect pest could become more resistance to MA treatment via a physiological adaptation (Chervin et al., 1996).

## Conclusions

MA treatments are environmentally friendly methods and have no harmful effects to human health. Gas combination treatments and longer storage time along with low temperature during storage are variable factors for effective disinfestation control. MAP of any concentration of CO<sub>2</sub> balance N<sub>2</sub> at 9 h of exposure time had complete mortality of *F. schultzei*. Increased concentrations of CO<sub>2</sub> and longer exposure times increase thrip mortality. Ozone fumigation has the effect as CO<sub>2</sub>, whereas the mortality of thrips increased as the concentration of O<sub>3</sub> increased. O<sub>3</sub> ≥ 500 ppm caused 100% mortality in adult and larvae. However, at this concentration, petals had spot discoloration and slight deformation. Fumigation of lotus flowers with CO<sub>2</sub> balance N<sub>2</sub> could be used to replace methyl bromide for disinfestation. Further research would be necessary to determine how the concentration of CO<sub>2</sub> changes during storage time and the moisture contents from plant transpiration in packaging and interaction among them effect thrip disinfestation.

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