The Residual Effect of 1-Methylcyclopropene on Protecting *Phalaenopsis* Flowers against Ethylene Injury

Jiunn-Yan Hou, Wei-Li Lin, Nean Lee, and Yao-Chien Alex Chang¹

Department of Horticulture and Landscape Architecture, National Taiwan University, 1 Roosevelt Road Sec. 4, Taipei 10617, Taiwan

Additional index words. temperature, flower maturity, flower senescence, ethylene receptor, moth orchid

Abstract. *Phalaenopsis* flowers are prone to wilting under ethylene (C₂H₄) stress. 1-Methylcyclopropene (1-MCP) can protect *Phalaenopsis* flowers against ethylene injury. In this study, we determined the residual effect of 1-MCP and how it is affected by temperature. The efficacy of multiple applications of 1-MCP was also investigated. The residual effect of 1-MCP was determined by pretreating blooming *Phalaenopsis amabilis* plants with 0.8 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for 8 hours on Day 0 followed by 2 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene fumigation for 12 hours on designated days. Without 1-MCP pretreatment, flowers began to wilt within 2 days after exposure to ethylene. Duration of the residual protection of 1-MCP on *P. amabilis* was ≈ 6 to 8 days during summer in Taiwan. Lower temperatures after 1-MCP application prolonged protection times. The full protection times under day/night temperatures of 25/20, 20/15, and 15/13 °C were 4 to 8, 10 to 13, and 13 to 17 days, respectively. Furthermore, multiple applications of 1-MCP extended the duration of 1-MCP protection against ethylene. Three applications increased the residual protection of *P. amabilis* by 1-MCP to at least 24 days.

Phalaenopsis spp. have become popular commodities in the world; their long-lasting flowers are an attractive characteristic for consumers. The postharvest life of potted *Phalaenopsis* can extend to more than 3 months. Like most orchids, however, the *Phalaenopsis* flower is susceptible to C₂H₄ stress (Hew and Yong, 2004). Exposure of *Phalaenopsis* Snow Mist 'Large Sepal' flowers to 0.09 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene for 66 h resulted in wilting (Lee and Lin, 1992). Ethylene can come from many sources such as emissions from internal combustion engines, pollutants released into the atmosphere, normal emissions from plant organs and from fungal metabolism, etc. (Martínez-Romero et al., 2007). Damaging concentrations of ethylene commonly occur during postharvest, transportation, and marketing and can reduce product quality.

1-Methylcyclopropene is an ethylene inhibitor that is widely used to maintain the freshness of ornamental plants and flowers and delay the ripening of fruits. 1-Methylcyclopropene works by tightly binding to ethylene receptors

in plants, thus competing with ethylene for available binding sites. The affinity of 1-MCP for ethylene receptors is 10 times greater than that of ethylene. Therefore, once ethylene receptors are fully occupied by 1-MCP molecules, both endogenous and exogenous ethylene responses are inhibited (Serek et al., 2006).

The residual protection by 1-MCP against ethylene-induced effects is limited (Sisler and Serek, 1999). *Cymbidium* flowers subjected to 1-MCP pretreatment resisted ethylene stress for up to 2 weeks but wilted when ethylene was applied at Week 3 (Philosoph-Hadas et al., 2005). When ethylene receptors in plant tissues are fully blocked by 1-MCP, 7 to 12 d are required for 50% receptor regeneration (half diffusion time; Sisler and Serek, 1999). After that, the inhibitory efficacy on the ethylene response gradually decreases. Based on the abscission percentage after 2 h of ethylene exposure, the half-lives of 1-MCP activities in *Pelargonium peltatum* flowers were ≈ 2 , 3, and 6 d after 1-MCP treatment at 25, 20.7, and 12 °C, respectively (Cameron and Reid, 2001), demonstrating that the residual effect of 1-MCP is affected by temperature.

Multiple applications of 1-MCP can be a strategy to prolong protection times. For example, apple fruit became soft 40 d after 1-MCP treatment, but multiple 1-MCP applications delayed the occurrence of ripening (Mir et al., 2001). A single application of 1-MCP on green-mature tomatoes delayed fruit coloration by 6 d, but a second application applied at 10 d after the first treatment

delayed coloration for another 8 to 10 d (Mir et al., 2004). 1-Methylcyclopropene was reported to be efficacious in retarding ethylene-induced flower wilting in *Phalaenopsis* (Lin et al., 2003; Seglie et al., 2010; Sun et al., 2009). Pretreating *Phalaenopsis amabilis* with 0.8 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for 4 h fully inhibited the wilting of flowers and flower buds (Lin et al., 2003). The residual effect of 1-MCP in *Phalaenopsis* is of great interest because it has emerged as an economically important potted plant. However, such information is not available in the literature.

In the current study, we determined the residual effect of 1-MCP in preventing ethylene damage to flowers and buds of *P. amabilis*. The effects of post-1-MCP-treatment temperatures and multiple 1-MCP applications on the residual efficacy were also investigated.

Materials and Methods

Plant materials. *Phalaenopsis amabilis* plants purchased from commercial nurseries were used. All plants were grown in 10.5-cm-diameter, clear, soft plastic pots (0.75 L) that were tightly filled with sphagnum moss. Each plant had one (Expts. 1 and 3) or two (Expt. 2) inflorescences with the first flower bud nearly open. The total numbers of flowers and buds in an inflorescence were 13.5, 9.8, and 13.5 in Expts. 1, 2, and 3, respectively. Before the experiments began, plants were placed in a greenhouse until they reached a suitable stage of flowering maturity (approximately five open flowers on each inflorescence).

Application of 1-methylcyclopropene. 1-Methylcyclopropene powder was obtained from Lytone Enterprise (Taipei, Taiwan). In this study, 0.8 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP was used to treat plants for 8 h by the following procedure. In an indoor environment, plants were placed in sealable, polypropylene boxes (0.225 m³) containing a small electric fan. A mixture of 0.1 g of 1-MCP and 2 mL of distilled water in a test tube was placed in each box. The boxes were then immediately sealed. After 8 h, plants were moved out of the boxes for further treatments. The average temperature was 23 ± 2 °C.

Application of ethylene. Plants were treated with 2 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene for 12 h in this study. First, plants were placed in sealed boxes (the same as those used for 1-MCP application). Then appropriate volumes of high-concentration ethylene were injected into the boxes to achieve a concentration of 2 $\mu\text{L}\cdot\text{L}^{-1}$. Concentrations of ethylene in the boxes were readjusted after 15 min by the following procedure. Air samples (1 mL) were taken from the boxes, and the concentration of ethylene was measured by a gas chromatograph (GC 14-A; Shimadzu, Tokyo, Japan) equipped with a Porapak Q column (80/100 mesh) and a flame ionization detector. Additional ethylene was injected if needed. After 8 h, plants were immediately moved out of the boxes. The average experimental temperature was 23 ± 2 °C.

Expt. 1: The residual effect of 1-methylcyclopropene. The experiment began in August.

Received for publication 5 June 2012. Accepted for publication 17 Aug. 2012.

Financial support was provided by the Council of Agriculture, Executive Yuan, Taiwan [96AS-1.2.1-ST-a1(4) and 97AS-1.2.1-ST-a1(3)]. This study is part of the theses submitted by Jiunn-Yan Hou and Wei-Li Lin in partial fulfillment of their Master of Science degree requirements.

¹To whom reprint requests should be addressed; e-mail alexchang@ntu.edu.tw.

Plants were divided into eight groups. Six groups were pretreated with 1-MCP on Day 0 and then were respectively treated with ethylene (as previously described) on Day 4, 6, 8, 10, 12, or 14. There were two groups of controls: one group did not receive either 1-MCP or ethylene treatment (but were treated with fresh air), and the other group received ethylene treatment on Day 0 but no 1-MCP pretreatment. After 1-MCP or ethylene treatment, plants were placed indoors with a 12-h photoperiod provided by fluorescent lights ($9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Total combined number of flowers and buds in each inflorescence was counted before 1-MCP treatment. The combined number of wilted flowers and buds was recorded daily after ethylene treatment. The percentage of wilted flowers was calculated as the number of wilted flowers and buds divided by the total number of flowers and buds. We also noted the times necessary to reach percentages of flower wilting of greater than 0%, 50%, 70%, and 100%. The average room temperature was 24.7°C . Each treatment included eight single-plant replications. The experiment was performed twice with similar results. Results of the second experiment are reported here.

Expt. 2: The effect of temperature on the residual effects of 1-methylcyclopropene. All plants were pretreated with 1-MCP, after which they were placed in phytotrons with day/night temperatures of 25/20, 20/15, or 15/13 $^\circ\text{C}$. Plants under each temperature regimen were subjected to ethylene treatment at 0, 4, 8, 10, 13, or 17 d after 1-MCP pretreatment. Ethylene-treated plants were moved to an indoor environment with a 12-h photoperiod provided by fluorescent lights ($8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and an average room temperature of 22.1°C . Plants in the control groups received no ethylene treatment and were left at room temperature. The flower-wilting percentage was determined as described in Expt. 1. There were six single-plant replications for each treatment. Each plant had two inflorescences.

Expt. 3: The efficacy of multiple applications of 1-methylcyclopropene. To determine whether multiple applications of 1-MCP could prolong its residual effects on *Phalaenopsis*, plants were subjected to different repetitions of 1-MCP pretreatments and were treated with ethylene at different times. The experiment began in December. Plants were separated into nine groups. Seven of them were treated with 1-MCP for various times including one application of 1-MCP on Day 0 and fumigation with ethylene on Day 6 or 12; two applications of 1-MCP on Days 0 and 6 and fumigation with ethylene on Day 12 or 18; and three applications of 1-MCP on Days 0, 6, and 12 and fumigation with ethylene on Day 18, 24, or 30. There were two groups of controls: one group did not receive either 1-MCP or ethylene treatment (the plants were only treated with fresh air), and the other group received ethylene treatment with no 1-MCP pretreatment. After 1-MCP treatment, plants were placed indoors under a 12-h photoperiod provided by fluorescent lights

($9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The flower-wilting percentage was determined as described in Expt. 1. The average room temperature was 19.5°C . Each treatment consisted of six single-plant replications. The experiment was performed twice with similar results. Results of the first experiment are reported here.

Statistical analysis. All experiments were arranged in a completely randomized design. Daily flower-wilting percentages were graphed with SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA). The numbers of days necessary to reach flower-wilting percentages of greater than 0%, 50%, 70%, and 100% were determined and analyzed using CoStat 6.2 (CoHort Software, Monterey, CA). Means separation between treatments was determined using the least significance difference test at $P \leq 0.05$.

Results

Expt. 1: The residual effect of 1-methylcyclopropene. With neither 1-MCP nor ethylene

treatment, the daily flower-wilting percentage of control plants gradually increased over time (Fig. 1), and the time to reach 50% flower-wilting (per plant) was 64.9 d (Table 1). In ethylene-treated plants with no 1-MCP pretreatment, the daily flower-wilting percentage increased rapidly from Day 2 after ethylene treatment (Fig. 1). Results showed that flowers of *P. amabilis* were very susceptible to ethylene. Plants protected by 1-MCP and subjected to ethylene treatment on Days 4 and 6 had similar rates of daily flower-wilting percentages to control plants (Fig. 1), and the time necessary to reach 50% flower-wilting (per plant) was 54.5 to 73.6 d (Table 1). Plants protected by 1-MCP and subjected to ethylene treatment on Day 8, 10, 12, or 14, however, showed sharply rising curves of daily flower-wilting percentages shortly after ethylene treatment (Fig. 1). These flowers began to wilt within 2 d after ethylene treatment, and these plants took shorter times to reach 50% flower wilting (12.6 to 35.5 d) compared with the

Fig. 1. Effect of 1-methylcyclopropene (1-MCP) against ethylene-induced flower wilting in *Phalaenopsis amabilis*. Plants were fumigated with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP on Day 0 for 8 h followed by ethylene ($2 \mu\text{L}\cdot\text{L}^{-1}$) treatment for 12 h on Day 4, 6, 8, 10, 12, or 14. Control plants were treated with air but did not receive either 1-MCP or ethylene application; $n = 8$.

Table 1. Residual effect of 1-methylcyclopropene (1-MCP) against ethylene-induced flower wilting in *Phalaenopsis amabilis*.

1-MCP treatment ^a	Ethylene treatment ^b	Time to achieve a specific flower wilting percentage (d) ^c			
		Greater than 0%	50%	70%	100%
No ^w	No (control)	23.4 b ^v	64.9 a	68.5 ab	81.6 ab
No	Day 0	1.9 c	14.3 c	29.6 cd	62.5 bc
Yes	Day 4	30.3 b	54.5 ab	60.8 abc	75.6 abc
Yes	Day 6	53.0 a	73.6 a	84.1 a	100.6 a
Yes	Day 8	9.1 bc	33.6 bc	45.5 bcd	66.3 abc
Yes	Day 10	12.0 bc	12.6 c	30.0 bcd	43.6 bcd
Yes	Day 12	12.0 bc	14.5 c	15.1 d	21.1 d
Yes	Day 14	13.6 bc	35.5 bc	37.9 cd	39.0 cd

^aPlants were treated with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP or left untreated for 8 h on Day 0.

^bPlants were fumigated for 12 h with $2 \mu\text{L}\cdot\text{L}^{-1}$ ethylene 4, 6, 8, 10, 12, or 14 d after 1-MCP pretreatment.

^cCalculated from Day 0.

^wControl plants were treated with air but did not receive either 1-MCP or ethylene application.

^vMeans followed by different letters in a column are significantly different at $P \leq 0.05$ by the least significant difference test; $n = 8$.

control plants (64.9 d; Table 1). These results showed that flowers and buds were unaffected by ethylene within 6 d after 1-MCP application, but the protection from ethylene provided by 1-MCP diminished from Day 8 onward. Also, it is worth mentioning that nearly open buds on an inflorescence of plants that received ethylene on Day 10 wilted earlier than younger, unopened buds and older flowers (Fig. 2). This showed that the residual effect of 1-MCP diminished earlier for nearly open buds than other buds and flowers.

These results showed that the residual effect of 1-MCP on protecting flowers against ethylene injury was sustained for 6 to 8 d in the current experiment. The experiment was repeated and similar results were obtained.

Expt. 2: The effect of temperature on the residual effects of 1-methylcyclopropene. All plants in this experiment were treated with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for 8 h. Plants not subjected to any temperature treatments showed similar flower-wilting rates regardless of whether they received any ethylene treatment (Table 2; Fig. 3). At 25/20 °C, plants that received ethylene treatment on Day 4 showed similar rates of daily flower-wilting percentages to control plants (Fig. 3A). Ethylene treatments on Day 8, 10, 13, or 17, however, resulted in rapid increases in the daily flower-wilting percentages (Fig. 3A). Specifically, the times necessary for flower-wilting rates to reach greater than 0%, 50%, and 100% were similar to controls when ethylene was applied on Day 4; however, the times necessary for flower-wilting rates to exceed 0% and 50% decreased when ethylene was applied on Day 8, but the time necessary for flower-wilting rates to reach 100% did not decrease (Table 2). This result indicated that only partial protection was still provided by 1-MCP on Day 8. Ethylene treatments applied on subsequent days (Days 10, 13, or 17) resulted in reductions in the times necessary for flower-wilting rates to reach greater than 0%, 50%, and 100% compared with control plants (Table 2). These results showed that the residual effect of 1-MCP against ethylene-induced wilting at 25/20 °C in *Phalaenopsis* flowers gradually decreased 4 to 8 d after 1-MCP treatment and was totally lost 10 d after 1-MCP treatment.

At 20/15 °C, plants that had received ethylene on Days 4, 8, and 10 showed daily flower-wilting percentages similar to those of control plants, whereas plants that had been treated with ethylene on Days 13 and 17 showed early increases in flower-wilting percentages (Fig. 3B). The times necessary for flower wilting to reach greater than 0%, 50%, and 100% in plants treated at 20/15 °C were similar to those of the control plants when ethylene was applied on Days 4, 8, and 10 (Table 2). When ethylene was applied on Days 13 and 17, however, wilting began earlier than in control plants (Table 2). This result showed that at 20/15 °C, the residual effect of 1-MCP on ethylene-induced wilting in *Phalaenopsis* flowers was partially lost 10 to 13 d after 1-MCP treatment.

At 15/13 °C, plants that had received ethylene on Days 4, 8, 10, and 13 showed similar

Fig. 2. Various degrees of flower wilting of *Phalaenopsis amabilis* pretreated with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-methylcyclopropene (1-MCP) for 8 h on Day 0 followed by $2 \mu\text{L}\cdot\text{L}^{-1}$ ethylene fumigation for 12 h on Day 10. The photograph was taken on Day 23 after 1-MCP treatment. Arrows indicate wilted buds, which were at the nearly open stage before 1-MCP treatment.

Table 2. Effects of temperature on the residual efficacy of 1-methylcyclopropene (1-MCP) against ethylene-induced flower wilting in *Phalaenopsis amabilis*.^z

Ethylene treatment ^y	Temp treatment (day/night, °C) ^x	Time to achieve a specific flower-wilting percentage (d) ^w		
		Greater than 0%	50%	100%
No (control)	—	81.3 a ^u	85.6 a	87.0 a
Day 0 ^v	—	73.8 a	79.0 a	82.3 a
Day 4	25/20	70.9 a	74.2 a	79.2 a
	20/15	71.9 a	81.0 a	84.1 a
	15/13	71.4 a	74.1 a	84.6 a
No (control)	—	81.3 a	85.6 a	87.0 a
Day 0	—	73.8 a	79.0 a	82.3 a
Day 8	25/20	40.4 b	54.0 b	75.0 a
	20/15	77.4 a	80.5 a	87.2 a
	15/13	69.6 a	77.5 a	81.1 a
No (control)	—	81.3 a	85.6 a	87.0 a
Day 0	—	73.8 a	79.0 a	82.3 a
Day 10	25/20	14.4 b	14.6 b	46.1 b
	20/15	81.0 a	82.9 a	87.6 a
	15/13	73.4 a	82.5 a	91.1 a
No (control)	—	81.3 a	85.6 a	87.0 a
Day 0	—	73.8 ab	79.0 a	82.3 a
Day 13	25/20	16.2 c	17.4 b	27.1 b
	20/15	61.6 b	70.6 a	80.6 a
	15/13	74.8 ab	82.6 a	88.2 a
No (control)	—	81.3 a	85.6 a	87.0 a
Day 0	—	73.8 ab	79.0 a	82.3 a
Day 17	25/20	19.9 d	20.6 b	20.9 b
	20/15	49.9 bc	72.7 a	76.7 a
	15/13	58.4 bc	76.7 a	89.9 a

^zAll plants were pretreated with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for 8 h on Day 0.

^yPlants were treated for 12 h with $2 \mu\text{L}\cdot\text{L}^{-1}$ ethylene on the designated days or left untreated and moved to an indoor environment to investigate the flower-wilting percentage.

^xPlants were treated with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for 8 h on Day 0 and transferred to phytotrons with day/night temperatures of 25/20, 20/15, or 15/13 °C.

^wCalculated from Day 0.

^vAfter 1-MCP treatment, plants were immediately subjected to ethylene treatment and placed in an indoor environment with no temperature treatment.

^uWithin each group of treatments (each group contained five rows of treatments), means followed by different letters in a column are significantly different at $P \leq 0.05$ by the least significant difference test; $n = 6$.

daily flower-wilting percentages to control plants, but the rate of wilting rose earlier when ethylene was applied on Day 17 (Fig. 3C). In other words, there were no differences in the times necessary for flower wilting to reach greater than 0%, 50%, and 100% in plants treated with ethylene on Days 4, 8, 10, and 13, but wilting began earlier when ethylene was applied on Day 17 compared with controls (Table 2). These results indicated that at 15/13 °C, the residual effect of 1-MCP against ethylene-induced wilting in *Phalaenopsis* flowers was partially lost 13 to 17 d after 1-MCP treatment.

These results showed that the efficacy of 1-MCP against ethylene-induced wilting in *P. amabilis* flowers was gradually lost over time but was sustained for various durations at different temperatures. Higher temperatures resulted in shorter protection times. In this study, full 1-MCP protection was sustained for 4 to 8 d at 25/20 °C, 10 to 13 d at 20/15 °C, and 13 to 17 d at 15/13 °C.

Expt. 3: The efficacy of multiple applications of 1-methylcyclopropene. As shown in Expts. 1 and 2, the efficacy of 1-MCP against ethylene-induced flower wilting in *P. amabilis*

Fig. 3. Influences of temperature on the residual effects of 1-methylcyclopropene (1-MCP) against ethylene-induced flower wilting in *Phalaenopsis amabilis*. Plants were fumigated with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP on Day 0 for 8 h and placed in phytotrons at day/night temperatures of 25/20 (A), 20/15 (B), or 15/13 °C (C). Ethylene treatment ($2 \mu\text{L}\cdot\text{L}^{-1}$ for 12 h) was applied on Day 0, 4, 8, 10, 13, or 17 for 12 h. The control group consisted of plants that did not receive either ethylene (treated with only fresh air) or temperature treatment; $n = 6$.

was transient and was only sustained for 4 to 8 d at 25/20 °C and 10 to 17 d at the lower temperature of 15/13 °C. Therefore, we used multiple 1-MCP applications (up to three times) to determine if the efficacy of 1-MCP protection could be prolonged.

Without 1-MCP protection, flower-wilting percentage curve rose sharply up to 30% soon after ethylene treatment (Fig. 4). After a single application of 1-MCP followed by ethylene treatments on Days 6 and 12, the flower-wilting percentage curves remained similar to those of control plants (Fig. 4), while the time taken to achieve specific flower wilting percentages were also similar to control (Table 3). This showed that the effect of a one-time 1-MCP application was sustained for more than 12 d.

In plants pretreated twice with 1-MCP, flower-wilting percentage curve of plants treated with ethylene on Day 12 (6 d after the last 1-MCP application) was similar to that of control; we observed a small increase in the flower-wilting percentage, however, after ethylene treatment on Day 18 (12 d after the last 1-MCP application; Fig. 4). This result indicated that the efficacy of 1-MCP applied twice, on Days 6 and 12, began to diminish on Day 18.

In plants treated three times with 1-MCP, no differences in flower-wilting percentages were found between control plants and plants treated with ethylene on Days 18 and 24 (6 and 12 d after the last 1-MCP application), but the flower-wilting percentage increased rapidly when ethylene was applied on Day 30 (Fig. 4). This result showed that three applications indeed extended the efficacy of 1-MCP from 18 to more than 24 d compared with only two applications of 1-MCP.

Results of this experiment showed that multiple applications of 1-MCP after some interval were effective in prolonging the protection by 1-MCP against ethylene-induced flower wilting. This study showed that three applications of 1-MCP could protect flowers of *P. amabilis* for 24 to 30 d (Table 3). The experiment was repeated, and similar results were obtained.

Discussion

Full protection by 1-MCP against ethylene-induced wilting in flowers of *P. amabilis* was sustained for 6 to 8 d during the summer in Taiwan and the protection time declined with time thereafter (Fig. 1). In this study, the responses to ethylene of all plants with the same treatment were very consistent; either all of the flowers remained unaffected because of the protection by 1-MCP or wilting occurred as a result of the loss of protection. The exceptions were plants that received ethylene 10 d after pretreatment with 1-MCP, and these plants exhibited great variations in their responses to ethylene. On some of these plants, all of the flowers and buds wilted; some plants were completely unaffected; whereas flowers in the middle of the inflorescence of other plants wilted (Fig. 2). This phenomenon was also observed in the repeat experiment. These various responses to ethylene indicated that at Day 10, the end of 1-MCP protection was

Fig. 4. Residual effects of multiple applications of 1-methylcyclopropene (1-MCP) against ethylene-induced flower wilting in *Phalaenopsis amabilis*. Plants were fumigated with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP once (Day 0), twice (Days 0 and 6), or three times (Days 0, 6, and 12) followed by $2 \mu\text{L}\cdot\text{L}^{-1}$ ethylene treatment on Day 6 or 12 for one 1-MCP application, Day 12 or 18 for two 1-MCP applications, and Day 18, 24, or 30 for three 1-MCP applications. Control plants were treated with air but did not receive either 1-MCP or ethylene application; $n = 6$.

Table 3. Effects of multiple applications of 1-methylcyclopropene (1-MCP) on its residual protection against ethylene-induced flower wilting in *Phalaenopsis amabilis*.

1-MCP treatment ^z	Ethylene treatment ^y	Time to achieve a specific flower wilting percentage (d) ^x			
		Greater than 0%	50%	70%	100%
No ^w	No (control)	60.0 ab ^y	80.3 a	85.8 ab	93.3 ab
No	Day 0	14.7 c	52.5 b	75.0 bc	99.2 ab
Day 0	Day 6	59.6 ab	80.2 a	83.2 ab	91.8 ab
Day 0	Day 12	50.8 ab	80.7 a	89.3 ab	96.3 ab
Days 0, 6	Day 12	54.5 ab	81.0 a	87.8 ab	98.0 ab
Days 0, 6	Day 18	41.3 b	76.7 a	80.8 b	91.2 ab
Days 0, 6, 12	Day 18	52.0 ab	84.0 a	90.5 ab	100.7 a
Days 0, 6, 12	Day 24	63.5 a	92.0 a	105.5 a	108.8 a
Days 0, 6, 12	Day 30	40.7 b	53.0 b	55.7 c	82.3 b

^xPlants were treated for 8 h with $0.8 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP on the designated days or left untreated.

^yPlants were fumigated with $2 \mu\text{L}\cdot\text{L}^{-1}$ ethylene for 12 h on designated days.

^zCalculated from Day 0.

^wControl plants were treated with air but did not receive either 1-MCP or ethylene application.

^vMeans followed by different letters in a column are significantly different at $P \leq 0.05$ by the least significant difference test; $n = 8$.

almost reached. Sisler and Serek (1999) suggested that the reason for such reduced protection may be that 1-MCP molecules detach from the ethylene receptors or new receptors are regenerated (Sisler and Serek, 1999). Recent research on carnations (*Dianthus caryophyllus* 'White Sim'), however, showed that a supersaturated concentration ($1000 \mu\text{L}\cdot\text{L}^{-1}$) of ethylene was unable to overcome the protection afforded by 1-MCP treatment, which suggests that 1-MCP binding is irreversible (Reid and Çelikel, 2009). In this study, the duration of protection of *P. amabilis* flowers afforded by 1-MCP seemed to vary with the age of the flowers. Among all of the flowers

and buds in inflorescences, nearly opened buds were more susceptible to wilting if treated with ethylene 10 d after 1-MCP pretreatment (Fig. 2). This observation may be explained in terms of the availability of functional ethylene receptors and the innate sensitivity to ethylene in buds and flowers. Nearly open buds may be in a developmental stage when ethylene receptors become functional or when new receptors are being actively regenerated. Hence, nearly open buds became more sensitive to ethylene in the time between the 1-MCP pretreatment and subsequent ethylene treatment. Opened flowers of *P. amabilis* were more sensitive to ethylene than flower buds; moreover, the

sensitivity increased with age, both among flowers and among flower buds (Lin, 2006; Lin et al., 2003). In this study, however, we observed that protection against ethylene in opened flowers by 1-MCP was sustained for a longer time than in nearly opened buds, suggesting the possibility of a lower rate of receptor regeneration in open flowers. Younger buds were less susceptible to wilting after ethylene treatment on Day 10, because younger buds have an innately lower sensitivity to ethylene (Lin, 2006; Lin et al., 2003). This lower sensitivity to ethylene may be the result of fewer functional ethylene receptors in younger buds.

Low temperature (i.e., from 25 to 20.7 and 12 °C) prolonged 1-MCP protection against ethylene in *Pelargonium peltatum* (Cameron and Reid, 2001). Low temperatures, however, are also efficient in delaying the response to ethylene after the binding of ethylene receptors and ethylene (Serek et al., 2006). In this study, responses of plants to ethylene treatment were observed for several days. Therefore, to avoid effects of temperatures on the ethylene response after the binding of ethylene to its receptors, we placed the *Phalaenopsis* plants in the same place at the same temperature after ethylene treatment. Full protection of *P. amabilis* flowers by 1-MCP against ethylene was sustained for 4 to 8 d at day/night temperatures of 25/20 °C, 10 to 13 d at 20/15 °C, and 13 to 17 d at 15/13 °C (Fig. 3). From 15 to 25 °C, the temperature coefficient (Q_{10}) of flower respiration in *Phalaenopsis*

Snow Mist 'Large Sepal' was 1.8 (Lee and Lin, 1992). Therefore, higher metabolism may accelerate the loss of efficacy of 1-MCP. In this study, the effect of temperature on 1-MCP efficacy was also shown when experiments were done in different seasons. After one-time treatment with 1-MCP, *P. amabilis* resisted ethylene-induced flower wilting for 6 to 8 d in the summer (Fig. 1) and for at least 12 d in the winter (Fig. 4). We deduced, therefore, that higher temperatures resulted in shorter 1-MCP protection times.

The transient efficacy of 1-MCP is necessary for fruit-ripening control during postharvest treatment (Hoeberichts et al., 2002). The goals of postharvest treatments of ornamental plants, however, are to maintain product quality and extend the shelf life for as long as possible. The efficacy of 1-MCP in delaying fruit softening of 'Redchief Delicious' apples (*Malus sylvestris* var. *domestica*) increased with a greater application frequency (once every week, every 2 weeks, 1 month, or 1 year) when the fruit was stored at 5 to 20 °C (Mir et al., 2001). In this study, multiple applications of 1-MCP were an efficient method to extend protection times against ethylene stress in flowering *P. amabilis* (Fig. 4). Two applications of 1-MCP protected flowers against ethylene for only 12 to 18 d, but three applications prolonged the protection time to 24 to 30 d (Fig. 4).

In conclusion, the efficacy of 1-MCP against ethylene-induced flower wilting in *P. amabilis* is transient, and its protection duration is predominantly affected by temperature. Once the

efficacy diminishes, 1-MCP retreatment can extend the protection time; retreatment with 1-MCP every 6 to 8 d in the warm season and every 10 to 12 d in the cool season can reduce ethylene-induced flower wilting in *Phalaenopsis*.

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