Efficiency of 1-MCP in Neutralizing Ethylene Effects in Cut Flowers and Potted Plants Following Simultaneous or Sequential Application

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Keywords: silver thiosulfate, temperature application, carnations, *Limonium hybrid* 'Beltlaard', roses, *Cymbidium* orchids, *Ficus* 'Green Island'

Abstract

We studied application of 1-methylcyclopropene (1-MCP) in various ornamentals, with the aim of finding optimum application temperature, dose, and duration. We also studied its competition with ethylene, and its synergistic effect with silver thiosulfate (STS). Ethylene (5 µl l^{-1}) was simultaneously applied with 1-MCP (15 or 150 nl l^{-1}) at 4, 12 or 20ºC to carnation (*Dianthus caryophyllus* cv. 'Yellow Candy'). At these application temperatures ethylene negatively affected flower quality. 1-MCP applied at 15 nl l^{-1} neutralized the adverse ethylene effects when applied at 4ºC, but not at 12 or 20ºC. However, when applied at 150 nl, l^{-1} 1-MCP successfully eliminated ethylene effects at all temperatures. It seems, therefore, that 1-MCP is a better competitor with ethylene at low temperatures.

1-MCP pre-treatment (0.2-1 µl l^{-1}/2-20 h), followed by a subsequent exposure to ethylene (1-5 µl l^{-1}/24 h/20ºC or 0.5 µl l^{-1}/12 days/12ºC), significantly improved the quality of cut flowers (carnations cv. 'Yellow Goldy', *Cymbidium* orchids) and potted plants (*Ficus* 'Green island'), respectively. In *Limonium hybrid* 'Beltlaard' cut flowers the combined use of 1-MCP and STS resulted in highest quality. This combined treatment also enabled storage under sea transport conditions (8 days at 2ºC). When applied alone, 1-MCP (0.4 µl l^{-1}/4 h) was also very effective in improving flower opening and the length of vase life in several rose cultivars.

The results suggest that 1-MCP may be very useful in preserving quality of various ornamentals and that it protects against ethylene effects.

INTRODUCTION

Ethylene is a major hormonal regulator of senescence of most plant organs (Abeles, 1992), including leaves (Philosoph-Hadas et al., 1994) and flowers (Reid and Wu, 1992; Borochov et al., 1997) that are classified as ethylene-sensitive (Woltering and van Doorn, 1988). The inhibition of ethylene perception by various ethylene action inhibitors significantly extended longevity of ethylene-sensitive cut flowers. The new gaseous ethylene action inhibitor, 1-MCP, is a very potent and non-phytotoxic blocker of adverse ethylene effects (Serek et al., 1995; Sisler and Serek, 1997; Blankenship and Dole, 2003). Therefore, 1-MCP may replace in ornamentals the very useful but toxic ethylene inhibitor, STS, or act synergistically with it. Ethylene perception requires the activity of a family of membrane receptors, to which also 1-MCP can effectively bind (Hall et al., 2000). Studies performed in floricultural crops have demonstrated that the response to 1-MCP depends on the gas concentration, duration of exposure, tissue sensitivity and application temperature (Sisler and Serek, 1997; Blankenship and Dole, 2003). Low temperatures substantially reduced 1-MCP activity in carnation petals (Çelikel et al., 1999; Reid and Çelikel, 2003). However, the effect of application temperature on 1-MCP binding in the presence of ethylene was not studied. The present study examined the optimal application of 1-MCP in various cut flowers (ethylene-sensitive and insensitive) and potted plants, with regard to its application temperature in the presence of ethylene, doses, duration of protection, and synergistic effect with STS.

MATERIALS AND METHODS

Experiments were performed with fresh cut flowers or potted plants obtained from local growers and subjected to various gas (detailed below) or solution treatments. Solutions were applied by 24 h-pulsing of cut carnation flowers (cv. 'Yellow Goldy') with 0.225 mM STS (STS-75, Milchan Bros, Israel) or 0.2 mM AVG (ReTain^{TM}, Abbott Laboratory, USA), and spraying of *Ficus* 'Green island' potted plants with 0.5 mM of 1-naphthaleneacetic acid (NAA). Following treatments, flowers were transferred to 20°C at a controlled environment room for evaluation of their quality parameters during vase life.

Simultaneous Application of 1-MCP and Ethylene at Various Temperatures

Carnation (*Dianthus caryophyllus* cv. 'Yellow Candy') cut flowers held in water jars were placed in 30-l PVC containers at 4, 12 or 20ºC for 24 h to allow equilibration. After sealing, 1-MCP (EthylBloc^{TM}, Rohm and Haas, USA; 15 or 150 nl l^{-1}) and ethylene (5 µl l^{-1}) were simultaneously injected into the containers, which were incubated at the three temperatures for additional 24 h. Quality parameters monitored included flower longevity, fresh and dry weight (FW, DW), diameter and ethylene production rates.

Sequential Application of 1-MCP and Ethylene

Cut flowers (carnations cv. 'Yellow Goldy' and pink *Cymbidium* orchids) were exposed first to 1-MCP and subsequently to ethylene by incubating them at 20°C in darkness in sealed 250-l chambers. Quality parameters monitored during vase life included flower diameter (carnations) and labellum redness index (*Cymbidium*). Ficus 'Green island' potted plants were exposed to 0.2 µl l^{-1} 1-MCP for 2 h and then to 0.5 µl l^{-1} ethylene for 12 days at 12°C in boxes, to simulate sea transport. The plants were then transferred to a net-house (80% shade) to monitor leaf abscission and chlorophyll fluorescence in the non-abscised leaves as previously described (Michaeli et al., 2001).

Separate Application of 1-MCP and Ethylene

Various cultivars of rose flowers were exposed to 0.4 µl l^{-1} 1-MCP for 4 h. 'Golden Gate' roses were separately exposed to various ethylene concentrations (1, 5 or 10 µl l^{-1}) for 24 h in darkness to examine the sensitivity to ethylene. Quality parameters assayed during vase life included flower longevity and diameter.

Multiple Application of 1-MCP

Limonium hybrid 'Beltlaard' cut flowers, pulsed for 24 h with 8-hydroxyquinoline citrate and 10% sucrose with or without 0.225 mM STS, were exposed at the beginning of pulsing to 0.2 µl l^{-1} 1-MCP for 2 h. The flowers were then placed in sealed carton boxes, into which another dose of 1 µl l^{-1} 1-MCP was injected. The boxes were incubated for 8 days at 2°C to simulate sea transport. Flower opening was monitored during vase life.

RESULTS AND DISCUSSION

The effects of temperature during simultaneous application of 1-MCP and ethylene were studied in cut carnation (cv. 'Yellow Candy') flowers. Ethylene applied alone decreased flower longevity compared to control at all application temperatures, indicating its binding (Fig. 1). Exposure to 15 nl l^{-1} 1-MCP in the presence of ethylene partially alleviated ethylene effects at 4°C, but not at 12 or 20°C (Fig. 1). Only 1-MCP application at 150 nl l^{-1} neutralized ethylene effects and resulted in increased vase life relative to controls at all application temperatures (Fig. 1). A similar trend was obtained when examining the relative effects of the three treatments (control, 5 µl l^{-1} ethylene without or with 150 nl l^{-1} 1-MCP) applied at different temperatures on other quality parameters of carnation flowers during vase life (Fig. 2). The results show that ethylene binding to the receptor increased with increasing application temperature, and 1-MCP applied at 150 nl l^{-1} in the presence of ethylene was also most effective when applied at 4°C, probably since ethylene binding at this application temperature was less effective (Fig. 2). It seems, therefore, that ethylene (5 µl l^{-1}) can successfully bind to its receptor at

all temperatures. 1-MCP competes better with ethylene at a low temperature for binding to its receptor, and at 150 nl l^{-1} it even has an additional improving effect relative to control. It was hypothesized that lower temperatures might lower the affinity of the binding site for 1-MCP, since 1-MCP was not effective at 2-10°C while showing high efficiency at 20-25°C in carnation petals (Çelikel et al., 1999; Reid and Çelikel, 2003) and other floricultural crops (Blankenship and Dole, 2003). However, the lack of 1-MCP activity at low temperatures was obtained in carnation petals after relatively short exposures of either 30 min to 100 nl l^{-1} (Çelikel et al., 1999) or 6 h to 1 nl l^{-1} (Reid and Çelikel, 2003). Our results show that the low efficiency of 1-MCP at low temperatures could be overcome by longer exposures (24 h) and/or higher doses (150 nl l^{-1}) of the gas (Figs. 1, 2). This conclusion is reinforced by the data of carnation petals showing that 1-MCP, which was ineffective when applied with ethylene at 20°C for 2 h, became inhibitory when applied in the presence of ethylene for 6 h (Reid and Çelikel, 2003). Our results may therefore further indicate a gradual release of ethylene from the binding site and preferential binding of 1-MCP as suggested recently (Reid and Çelikel, 2003), and this process seems to be facilitated at 4°C. This also further confirms previous reports showing that the affinity of 1-MCP for the receptor is much greater than that of ethylene (Sisler et al., 1996; Sisler and Serek, 1997; Hall et al., 2000), even at lower temperatures.

The effect of sequential application of 1-MCP and ethylene at 20°C was studied in various systems. When applied without ethylene, all ethylene inhibitors improved vase life of cut carnation (cv. 'Yellow Goldy') flowers, but only STS extended it significantly (Table 1). However, in the presence of ethylene, 1-MCP was the most effective inhibitor. It resulted in a significant increase in flower diameter and length of vase life, relative to the controls (Table 1). These results suggest that 1-MCP is a better inhibitor than STS in neutralizing exogenous ethylene effects, at least in this carnation cultivar. A similar higher vase live after 1-MCP treatment, compared to STS, was also found in *Leonotis leonurus* cut flowers (data not shown).

The duration of 1-MCP protection against ethylene was studied in detached *Cymbidium* florets. 1-MCP was effective in protecting the flowers against ethylene only when ethylene was applied immediately or one week following 1-MCP application (Fig. 3). However, when ethylene was applied two or three weeks after 1-MCP application, 1-MCP was not effective (Fig. 3). The results suggest that 1-MCP protects *Cymbidium* florets from ethylene effects up to two weeks.

1-MCP (applied alone or with NAA) was very effective in reducing leaf abscission of *Ficus* 'Green island' potted plants during 4 days of shelf life following 12 days of storage at 12°C with or without 0.5 µl l^{-1} ethylene (Fig. 4A). This positive effect of 1-MCP and NAA was also manifested in retaining the original Fv/Fm values of the non-abscised leaves during 12 days of shelf life (Fig. 4B). This suggests that 1-MCP may serve as a powerful tool for preserving the quality of potted plants and enabling their prolonged sea shipment. Similar effects of 1-MCP in potted plants following short transport simulations were previously reported (Serek et al., 1995; Michaeli et al., 1999).

Cut roses are usually classified as flowers with very low sensitivity to ethylene, at least when considering the time to wilting (Woltering and van Doorn, 1988). However, STS (Reid et al., 1989) and 1-MCP (Muller et al., 2000) were reported to improve flower longevity in a number of rose cultivars. It was recently reported that 1-MCP prevented the ethylene-induced reduction in flower weight, diameter and vase life of 'Marlyse' cut roses (Ait-Oubahou et al., 2003). Our results also show that both flower diameter (Fig. 5A) and vase life (Fig. 5B) of various cut rose cultivars were significantly increased by 1-MCP, relative to control flowers. However, in at least one cultivar ('Golden Gate) the effect of 1-MCP could apparently not be explained by assuming that it reduced ethylene sensitivity, since exposure to increasing concentrations of ethylene (1-10 µl l^{-1} for 24 h) at the half open bud stage affecter neither its vase life nor its flower quality (data not shown). It may however be possible that sensitivity to ethylene in this rose cultivar is greatly increased during flower opening.

A combined treatment of the two ethylene inhibitors, STS and 1-MCP was

assayed in *Limonium hybrid* 'Beltlaard' cut flowers, using either air or sea transport simulation. Flowers treated with either STS or 1-MCP before transport did not have any or only a few open florets after sea transport simulation (data not shown; Table 2). Treatment with 1-MCP alone (treatments 1, 4, 5) were not effective in improving flower opening following sea or air transport. However, a combined treatment with both inhibitors (treatments 2, 3, 6), significantly improved flower opening following sea or air transport (Table 2). Flowers treated with both STS and 1-MCP before transport had the best quality following air transport (treatment 6). However, flowers had to be treated with both inhibitors before transport and with an additional 1-MCP treatment during transport (treatment 3) in order to reach a similar quality following sea transport (Table 2). The results suggest that the combined treatment of both ethylene action inhibitors is good for *Limonium* cut flowers, and may enable their sea shipment. Similar results were obtained for cut *Gypsophila paniculata* 'Perfecta' and 'New Hope' (data not shown). It seems that the combined treatment somehow produces the advantages of both inhibitors, namely the immediate effect of 1-MCP and the more long-lasting effect of STS.

Our work thus demonstrated that 1-MCP application produced good effects in several cut flowers (carnation, rose, *Cymbidium* and *Limonium*) and in a potted plant (*Ficus*), especially when applied for use in prolonged shipment. The results suggest that 1-MCP, applied alone or in combination with STS, may be useful in neutralizing effects of exogenous ethylene.

ACKNOWLEDGEMENT

This research was supported by grant No. 409-0064-01 of The Chief Scientist Fund of the Ministry of Agriculture, Israel. Contribution from the ARO, The Volcani Center, Bet Dagan, Israel, No. 419/03.

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Tables

Table 1. Effect of ethylene inhibitors on flower diameter and vase life of cut carnations (cv. 'Yellow Goldy') in the absence or presence of 5 µl l^{-1} ethylene.

Treatments	Without ethylene			With ethylene		
	Flower diameter (cm)		Vase life	Flower diameter (cm)		Vase life
	Day 0	Day 2	(days)	Day 0	Day 2	(days)
Water	5.9 a	7.6 a	13.8 b	3.5 a	3.5 cd	5.3 c
1-MCP 0.2 µl l^{-1}	5.6 a	7.7 a	17.6 ab	4.5 a	7.2 a	15.7 a
STS 0.225 mM	5.0 a	7.4 a	20.3 a	3.2 a	4.4 b	10.0 b
AVG 0.2 mM	4.7 a	7.4 a	17.8 ab	3.3 a	3.9 bc	5.5 c

Flowers were exposed sequentially to 0.2 µl l^{-1} 1-MCP for 2 h and to 5 µl l^{-1} ethylene for 24 h. A total of 10 flowers per treatment were assayed. Means within each column followed by different letters are significantly different (P = 0.05).

Table 2. Effect of ethylene inhibitors on opening of *Limonium* 'Beltlaard' cut flowers following sea (8 days at 2ºC) or air (2 days at 6ºC) transport simulations.

No	Treatments with ethylene inhibitors	Transport simulation	Flower opening index (0-3)
1.	1-MCP during transport	sea	0.4 ± 0.1
2.	STS + 1-MCP during transport	sea	0.8 ± 0.1
3.	STS + 1-MCP before and during transport	sea	1.4 ± 0.3
4.	1-MCP before and during transport	sea	0.4 ± 0.1
5.	1-MCP before transport	air	0.8 ± 0.1
6.	STS + 1-MCP before transport	air	1.8 ± 0.1

STS (0.225 mM) was applied by pulsing before transport, and 1-MCP was applied either by exposure to 0.2 µl l^{-1} for 2 h before transport, and/or by injection of 1µl l^{-1} into the box during transport. Flower opening index during vase life (0 = closed flowers; 3 = open flowers) was monitored. Results represent means ± SE of 10 measurements.

Figures

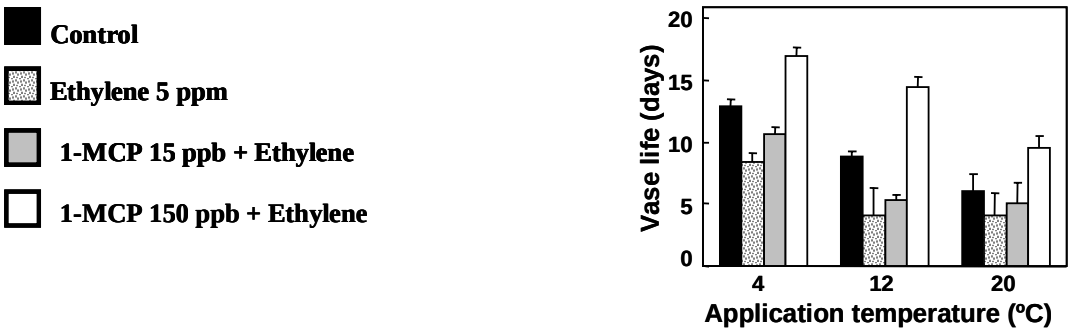

Fig. 1. Effect of gas application temperature on vase life of cut carnation (cv. 'Yellow Candy') flowers following simultaneous application of 1-MCP (15 or 150 nl l^{-1}) and ethylene (5 µl l^{-1}). Control flowers were incubated without 1-MCP and/or ethylene. Results represent means ± SE of 10 replicates.

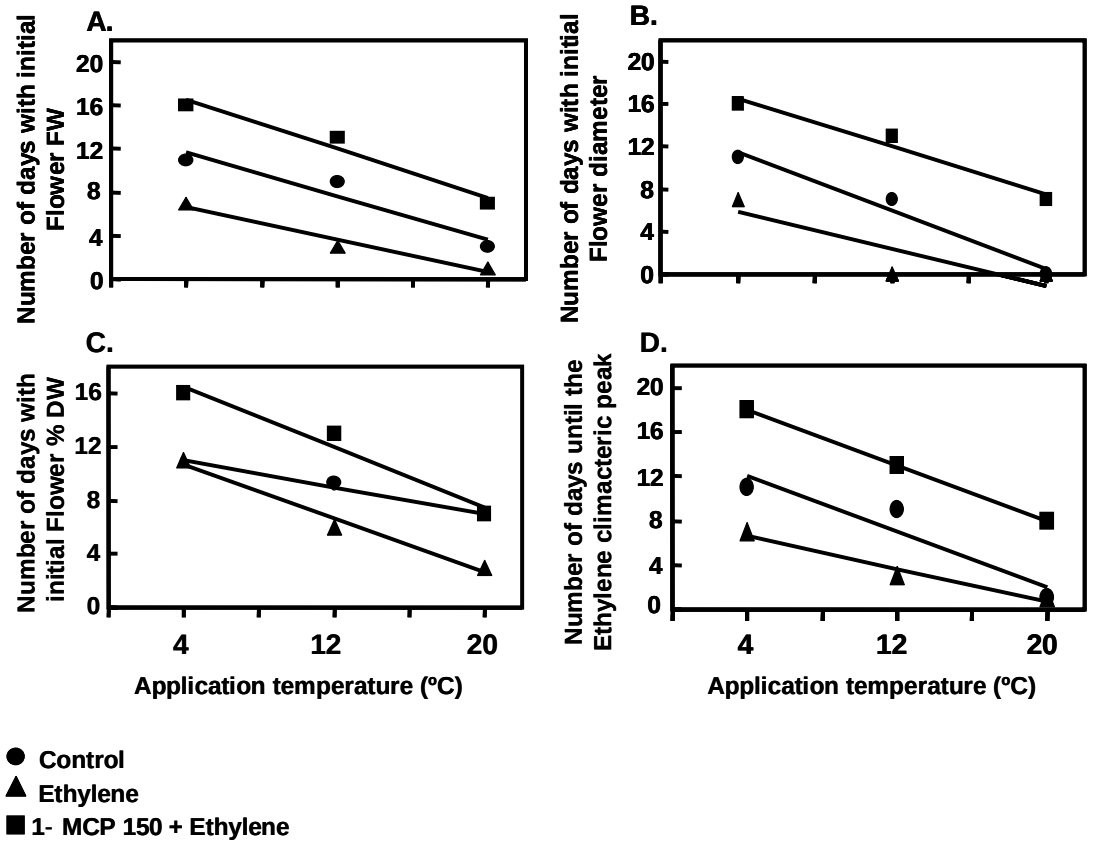

Fig 2. Effect of gas application temperature on quality parameters of cut carnation (cv. 'Yellow Candy') flowers following simultaneous application of 1-MCP (150 nl l^{-1}) and ethylene (5 µl l^{-1}). The parameters included: number of days during which flowers retained their original FW (A), original diameter (B), original DW (C) and number of days until the ethylene climacteric peak was reached in petals (D).

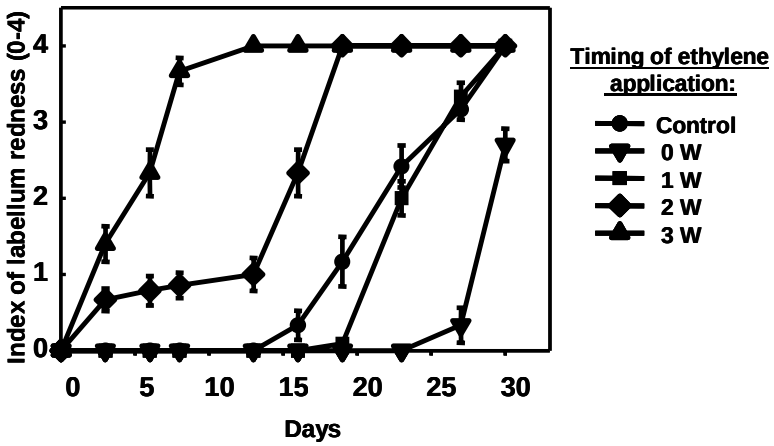

Fig. 3. Effect of timing of ethylene application to 1-MCP-treated *Cymbidium* florets on their sensitivity to ethylene, expressed as labellum redness index (0 = white; 4 = red). Detached florets were exposed first to 0.2 µl l^{-1} 1-MCP for 2 h and then to 1 µl l^{-1} ethylene for 24 h, immediately and 1, 2 or 3 weeks (W) following 1-MCP application. Results represent means ± SE of 10 florets.

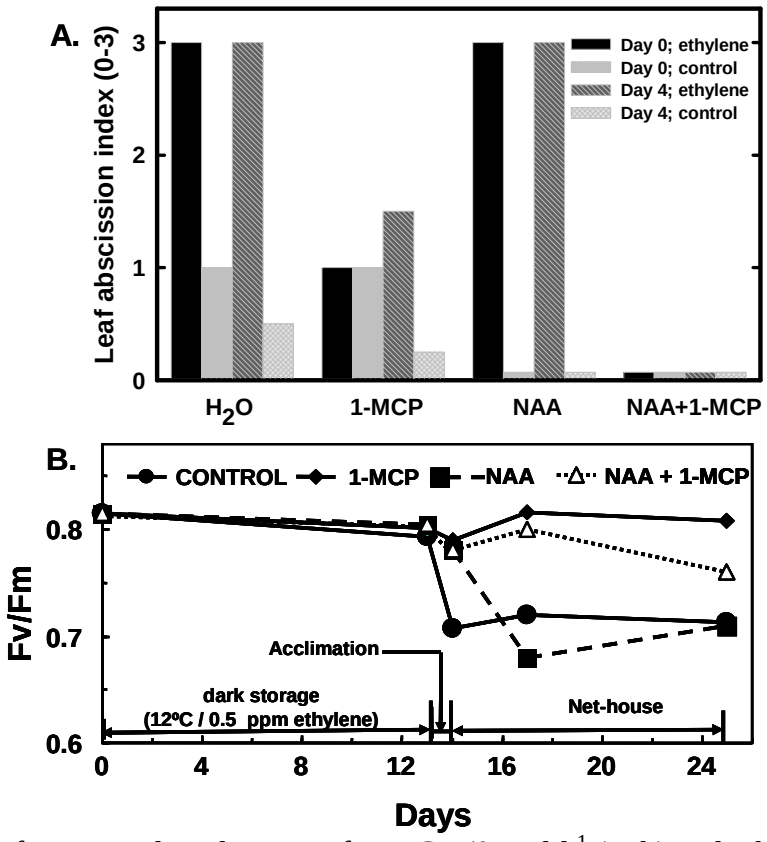

Fig. 4. Effect of sequential application of 1-MCP (0.2 µl l^{-1} / 2 h) and ethylene (0.5 µl l^{-1} for 12 days) on leaf abscission (A) and chlorophyll fluorescence of non-abscised leaves (B) of *Ficus* 'Green island' potted plants during shelf life, following 12-day storage at 12°C. Plants were also sprayed with 0.5 mM NAA prior to the sea transport simulation. Leaf abscission index ranged from 0 = no abscission to 3 = full abscission.

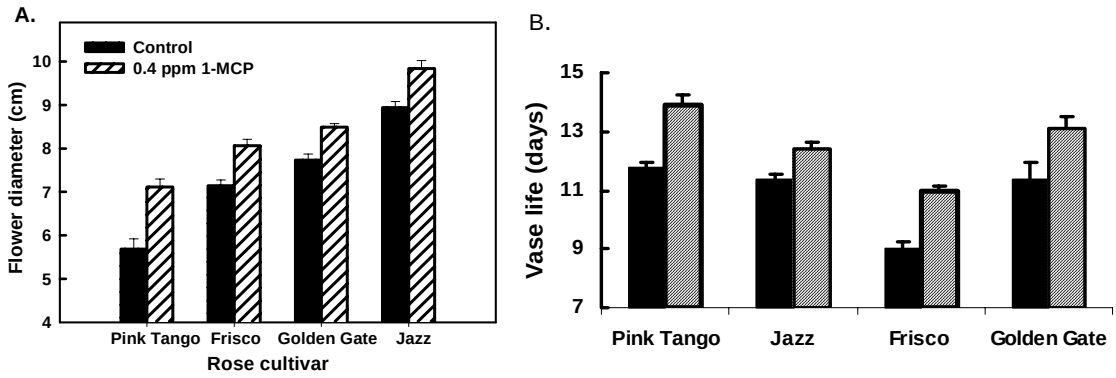

Fig. 5. Effect of 1-MCP (0.4 µl l^{-1} for 4 h) on flower diameter (A) and vase life (B) of various cut rose cultivars. Results represent means ± SE of 10 replicates.