Efficacy of New Inhibitors of Ethylene Perception in Improvement of Display Life of Ornamental Crops

Edward C. Sisler[1], Zeddy Kebenei[2], Traud Winkelmann[2] and Margrethe Serek[2,3]
[1]Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh NC 27695, USA
[2]Department of Horticulture, Institute of Floriculture, Tree Nursery Science and Plant Breeding, University of Hanover, Herrenhaeuser Str. 2, 30419 Hanover, Germany
[3]Department of Agricultural Sciences, Horticulture, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, 1871 Frederiksberg C., Denmark

Keywords: 1-HCP, 1-hexylcyclopropene, 1-MCP, 1-methylcyclopropene, 1-OCP, 1-octylcyclopropene, 1-CPs, 1-substituted cyclopropenes, ethylene inhibitors, *Kalanchoë blossfeldiana, Lathyrus odoratus,* postharvest life, receptor

Abstract

Selecting and testing various strained olefines as ethylene antagonists led to the discovery that 1- methylcyclopropene (1-MCP) and other cyclopropenes effectively block ethylene responses at the receptor level. 1-MCP is now commercially available. It is very effective in blocking ethylene responses in a range of ornamental crops.

We have prepared and tested the effectiveness of many other substituted cyclopropenes. Some require a much higher concentration than 1-MCP and inactivate the receptor for from 3 to 12 days. Some recently prepared 1-substituted cyclopropenes (1-CPs) inactivate the receptor up to 35 days at very low concentrations. The study with *Kalanchoë blossfeldiana* and *Lathyrus odoratus* showed that 1-hexylcyclopropene (1-HCP) and 1-octylcyclopropene (1-OCP) are effective ethylene receptor blockers, which efficiently prolong the display life of flowers in both ornamentals.

INTRODUCTION

The effectiveness of 1-MCP in preventing deleterious ethylene effects has been shown in the range of studies (Serek et al., 1994, 1995; Sisler and Serek, 2003). This substance is now commercially available under the names EthylBloc and SmartFresh and distributed by two companies: FloraLife Inc. and SmartFresh, Rohm and Haas. The registration for ornamental crops in the US has been approved already in year 2000. 1-MCP has been also registered for use with several edible products in the USA, some countries in South America, and also in New Zealand and South Africa. The registration in Europe is in progress.

Several other cyclopropenes have been investigated for their effectiveness as ethylene antagonists (Sisler et al., 2001), but very recent studies (Sisler et al., 2003) have demonstrated that an extension of chain length in the 1-position improves the activity of the subsequent cyclopropenes. A spectacular effect of the compounds with the chain length of more than 5 carbon atoms was reported as more effective than 1-MCP treatment in preventing chlorophyll degradation in bananas.

We report here on the effectiveness of newly synthesized 1-substituted cyclopropenes (1-CPs), 1-HCP and 1-OCP, in protecting two ornamental crops, *Kalanchoë blossfeldiana* and *Lathyrus odoratus,* from deleterious effect of ethylene.

MATERIAL AND METHODS

Preparation of Chemicals

1-HCP and 1-OCP were synthesized from 2-bromoalkenes and bromoform using 50% NaOH to produce a carbene and form a 1,1,2-cyclopropane, which was then reacted with methyllithium at dry ice temperature, to form the cyclopropenes (Al Dulayymi et al.,

1996, 1997). The compounds were divided into smaller samples of 0.5 ml and kept at -80 ^{o}C until needed for the experiments. Each sample was then diluted with ether to a volume of 50 ml before used in experiments. 1-MCP was obtained from Rohm and Haas Company, USA, in powdered form as a commercial product named SmartFresh. The structures of the 1-CPs are presented in Table 1.

Plant Material

Two plant species have been used in the experiments: *Kalanchoë blossfeldiana* cv. Alexandra and *Lathyrus odoratus* cv. Winter Elegance. Plant material was obtained from commercial growers (Gartenbau Dippon GbR, Bad Rappenau, Germany and Knud Jepsen A/S, Hinnerup, Denmark) and transported to University of Hanover, where it was used for experiments.

Treatment with Cyclopropenes

1) For investigation of display life of flowers kalanchoë florets and sweet pea flowers were exposed to desired concentrations of the compounds in the range of 50 to 2000 nl l^{-1} for 6 h at 20 ^{o}C in sealed glass chambers.

2) The optimum concentrations of 1-CPs were used for investigations on effect of treatment time on display life of flowers. Plant material was exposed to 1-CPs at 20 ^{o}C for different exposure times: 0, 0.5, 1, 2, 4, 6 or 12 h.

3) Effect of treatment temperature on display life was investigated by enclosing plant material in four different temperatures: 5, 10, 15, 20 ^{o}C and exposing to optimal concentrations of 1-CPs.

Ethylene Treatment

After 1-CPs treatments and 1-hour ventilation of the flowers enclosed in glass chambers, 1 or 2 µl l^{-1} ethylene was applied daily until termination of the experiment. Ethylene concentrations inside the chambers were monitored using GC.

Experimental Design and Statistics

The experiments were conducted in a completely randomised design using eight spikes (pea), ten florets and eight inflorescences (kalanchoë) per treatment and two replications. Multiple comparisons among means were done using the Honestly Significant Difference (Tukey's). All comparisons were made at P=0.05.

RESULTS AND DISCUSSION

Effects of 1-CPs Concentrations on Display Life

It has been shown by Serek and Reid (2000) that kalanchoë flowers require an exposure to ethylene for several hours to produce the senescence symptoms, which are irreversible in-rolling of flowers leading to premature wilting. In the present experiment kalanchoë flowers wilted after 2 days of exposure to 2 µl l^{-1} of ethylene (Fig. 1). Pre-treatment with 1-MCP, 1-HCP and 1-OCP inhibited the ethylene effects for several days. The results of optimum concentrations of 1-MCP and 1-HCP pre-treatment were equally good, however 1-OCP treatment gave the best results (Fig. 1).

The senescence of sweet pea flowers is characterized by loss of petal turgor, wilting and petal abscission (Mor et al., 1984). In our experiment 3 days exposure of flowers to 1 µl l^{-1} ethylene accelerated wilting and abscission of petals and display life of the whole spikes decreased (Figure 2). Pre-treatment with the optimum concentrations of 1-MCP and 1-OCP increased display life up to 7 days.

Effects of 1-CPs Treatment Exposure Time

Plant material was treated with chemicals at different exposure times (0, 1, 2, 4, 6 or 12 h) with following exposure to ethylene (Table 2). The display life of both species was dependent on exposure time to 1-HCP and 1-OCP, such that shorter exposure times

resulted in less protection that longer exposure times. An exposure time of 2 h was sufficient to increase the display life of kalanchoë flowers from 2 to 7.3 (1-HCP) or to 8 (1-OCP) days. For sweet pea florets the improvement was from 2.2 days up to more than 6 days. No significant improvement was observed for longer than 4 h exposure (Table 2). 1-MCP treatment for 6 h exposure was used as a positive control based on previous investigations (Dupille and Sisler, 1995; Sisler et al. 1996).

Effects of 1-CPs Treatment Temperature

Sweet pea florets were treated with 200 nl l^{-1} 1-OCP for 6 h at different temperatures: 0, 5, 10, 15 or 20 ^{o}C. 1-OCP pre-treatment in all temperatures improved display life from 2 to 5-7 days. The best treatment temperature for 1-OCP was 20 ^{o}C, which was slightly but significantly better than 0, 5, 10 or 15 ^{o}C, but not different from 1-MCP treatment (200 nl l^{-1}, 6 h, 20 ^{o}C).

For kalanchoë flowers 1-HCP 1000 nl l^{-1} and 1-OCP 200 nl l^{-1}treatments were used for 4 h at different temperatures: 5, 10, 15 or 20 ^{o}C. 1-HCP treatment gave the same results for all temperatures, and so did also the 1-MCP treatment (200 nl l^{-1}, 6 h, 20 ^{o}C), but the results were significantly different from controls (non-treated). For 1-OCP the display life of flowers treated at temperatures 5 and 10 ^{o}C was equal to the 1-MCP treatment, but significantly shorter than flowers treated at 15 or 20 ^{o}C.

Previous studies (Sisler et al. 1996; Sisler and Serek, 1997) have demonstrated that at lower temperatures, a higher concentration and longer exposure time is required. In this study temperature did not have an effect on the activity of 1-HCP, suggesting that pre-treatment of plant material with this substituted cyclopropene can be done in the temperature interval 5 – 20 ^{o}C, without affecting the activity of the compound. On the contrary, the effectiveness of 1-OCP, similar to 1-MCP, is better after application in higher temperatures.

CONCLUSIONS

In conclusion, our results demonstrate the ability of 1-CPs to function as inhibitors of ethylene binding sites and therefore protecting plant material against deleterious effects of ethylene. However, further work on investigations of interactive effect of 1-CPs concentrations, treatment times and temperature on display life of flowers is needed.

ACKNOWLEDGEMENTS

The study was supported by German Academic Exchange Service (DAAD). The authors would like to thank the growers Knud Jepsen A/S and Dippon Gartenbau for generously supplying the kalanchoë plants and cut sweet pea flowers.

Literature Cited

Al Dulayymi, J.R., Baird, M.S., Simpson, M.J. and Nyman, S. 1996. Structure based interference with insect behaviour-cyclopropenes analogs of pheromones containing z-Alkenes. Tetrahadron 52:12509-12520.

Al Dulayymi, A.R., Al Dulayymi, J.R., Baird, .M.S. and Koza, G. 1997. Simple four and five carbon cyclopropane and cyclopropenes synthetic intermediates. Russian J. Org. Chem. 33:798-816.

Dupille, E. and Sisler, E.C. 1995. Effects of an ethylene receptor antagonist on plant material. In: Ait-Oubahou, A., El-Otmani, M. (eds). Postharvest Physiology, Pathology and Technologies of Horticultural Commodities: Recent Advances. pp 294-301. Institute Agronomique et Veterinaire Hassan II, Agadir. ISBN 9981-9842-2-1.

Mor, Y., Reid, M.S. and Kofranek, A.M. 1984. Pulse Treatments with Silver Thiosulfate and Sucrose Improve the Vase Life of Sweet Peas. J. Amer. Soc. Hort. Sci. 109:866-868.

Serek, M., Sisler, E.C. and Reid, M.S. 1994. Novel gaseous ethylene binding inhibitor prevents ethylene effects in potted flowering plants. J. Amer. Soc. Hort. Sci. 199:1230-1233.

Serek, M., Sisler, E.C. and Reid, M.S. 1995. Effects of 1-MCP on vase life and ethylene response of cut flowers. Plant Growth Reg. 16:93-97.
Serek, M. and Reid, M.S. 2000. Ethylene and postharvest performance of potted kalanchoë. Postharvest Biology and Technology. 18:43-48.
Sisler, E.C., Serek, M. and Dupille, E. 1996. Effect of 1-Methylcyclopropene and Methylenecyclopropane on ethylene binding and ethylene action on cut carnations. Plant Growth Reg. 18:79-86.
Sisler, E.C. and Serek, M. 1997. Inhibition of ethylene responses in plants at the receptor level: recent developments. Physiologia Plant. 100:577-582.
Sisler, E.C., Serek, M., Roh, K.A. and Goren, R. 2001. The effect of chemical structure on the antagonism by Cyclopropenes of ethylene responses in banana. Plant Growth Reg. 33:107-110.
Sisler, E.C., Altwan, T., Goren, R., Serek, M. and Apelbaum, A. 2003. 1-Substituted Cyclopropene: effective blocking agents for ethylene receptor in plants. Plant Growth Reg. 40:223-228.
Sisler, E.C. and Serek, M. 2003. Compounds interacting with the ethylene receptor in plants. Plant Biology (in press)

Tables

Table 1. The chemical structures of 1-MCP, 1-HCP and 1-OCP.

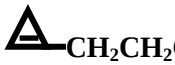
Compound	Chemical structure
1-methylcyclopropene	
1-hexylcyclopropene	
1-octylcyclopropene	

Table 2. Mean display life of sweet pea and Kalanchoë flowers pre-treated with 1-MCP 200 nl l^{-1}, 1-HCP 1000 nl l^{-1} or 1-OCP 200 nl l^{-1} at the temperature of 20 ^{o}C and exposure time of 0, 1, 2, 4, 6 or 12 h. After 1-CPs treatment the flowers were exposed to ethylene (pea 1 µl l^{-1}, kalanchoë 2 µl l^{-1}).

Treatments	Exposure time	Mean Display life (Days) Pea flowers	Kalanchoë flowers	
Control	0	2.2e	2.0d	2.0d
1-MCP	6h	6.5ab	7.0ab	7.2ab
1-HCP	1h		6.1b	
1-HCP	2h		7.3ab	
1-HCP	4h		7.6a	
1-HCP	6h		8.0a	
1-HCP	12h		7.8a	
1-OCP	1h	5.1c		6.9b
1-OCP	2h	6.0b		8.0ab
1-OCP	4h	6.2ab		8.5a
1-OCP	6h	6.4ab		8.9a
1-OCP	12h	6.8a		8.4a

Means marked with the same letter within columns are not statistically different at $P< 0.05$. Means were separated by Tukey's HSD.

Figures

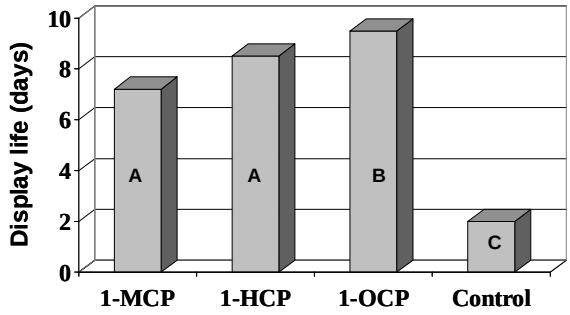


Fig. 1. Mean display life of kalanchoë flowers pre-treated with 1-MCP 200 nl l^{-1}, 1-HCP 1000 nl l^{-1}, 1-OCP 200 nl l^{-1} or control non-treated. The time of 1-CPs treatments was 6 hours and the temperature 20 ^{o}C, then exposure to 2 µl l^{-1} ethylene. Bars marked with the same letter are not statistically different at P< 0.05. Means were separated by Tukey's HSD.

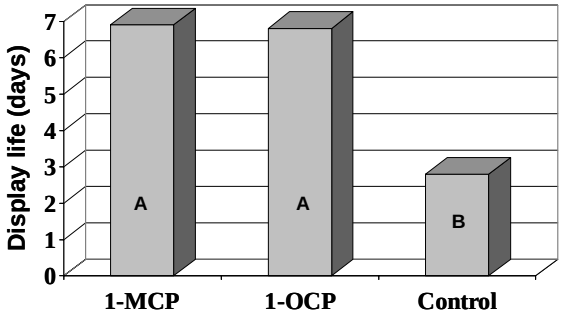


Fig. 2. Mean display life of sweet pea flowers pre-treated with 1-MCP 200 nl l^{-1}, 1-OCP 200 nl l^{-1} or control non-treated. The time of 1-CPs treatments was 6 hours and the temperature 20 ^{o}C, then exposure to 1 µl l^{-1} ethylene. Bars marked with the same letter are not statistically different at P< 0.05. Means were separated by Tukey's HSD.