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Ethylene and 1-methylcyclopropene action over senescence of nasturtium flowers

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ABSTRACT

This work describes ethylene and 1-methylcyclopropene (1-MCP) action on post-harvest shelf life of four development stages of nasturtium flowers. To reach this goal, we carried out three experiments. In the first and second experiments, we studied five ethylene (0; 0.1; 1; 10; 100 and 1000 μ L/L) and three 1-MCP concentrations (0.25; 0.5 and 0.75 µL/L), respectively. In the third experiment, 1-MCP was followed by combined with ethylene (only 1-MCP; only ethylene; and 24 hours of exposure to $0.75 \mu L/L$ 1-MCP followed by 24 hours of exposure to 100 μ L/L ethylene). All experiments had two control treatments, one keeping non-exposed flowers inside and another outside exposure chambers. Experiments were set in factorial design, in complete blocks at random, with four 10-flower replications each. Flower senescence was determined by a pre-established visual scale and by observing floral bud development. Ethylene dose above 10 μL/L induced flower wilting and premature senescence from the second floral development stage. Furthermore, higher concentrations of exogenous ethylene promoted irregular flower opening and/or morphological abnormalities in opened flowers. 1-MCP effectively extended post-harvest longevity of nasturtium flowers, independent of the concentration and even in the presence of exogenous ethylene.

Keywords: *Tropaeolum majus*, phytohormone, post-harvest, phenological stages.

RESUMO

Ação do etileno e do 1-MCP sobre a senescência de flores de capuchinha

Objetivou-se com este trabalho avaliar a sensibilidade das flores ao etileno e à ação do 1-metilciclopropeno (1-MCP) sobre a conservação pós-colheita de flores de capuchinha em quatro estádios de abertura floral. Para isso, foram realizados três experimentos, sendo que no primeiro, foram estudadas cinco concentrações de etileno (0; $0,1; 1; 10; 100 \text{ e } 1000 \mu\text{L/L}$); no segundo, três de 1-MCP (0,25; 0,5 e 0,75 µL/L), e, no terceiro, combinaram-se 1-MCP e etileno: somente 1-MCP, somente etileno, 1-MCP + etileno em que o 1-MCP (0,75 μL/L) foi aplicado por 24 h e, em seguida, as flores foram expostas ao etileno (100 μL/L) por mais 24 h, e dois controles, dentro e fora das câmaras herméticas. Os experimentos foram arranjados em fatorial, em blocos casualizados, com quatro repetições de 10 flores cada. Avaliou-se a senescência floral, através de escala visual de notas, previamente estabelecida, e o desenvolvimento dos botões. O etileno em dose maior que 10 µL/L provocou o murchamento e a senescência precoce nas flores do segundo, terceiro e quarto estádios de desenvolvimento floral. Nas concentrações maiores, o etileno exógeno afetou o desenvolvimento normal dos botões, promovendo desuniformidade de abertura e/ou da morfologia das flores quando abertas, inibindo a abertura floral. O 1-MCP foi eficiente em prolongar a longevidade pós-colheita de flores de capuchinha, independente da concentração utilizada e até mesmo na presença de etileno exógeno.

Palavras-chave: *Tropaeolum majus*, fitohormônio, pós-colheita, estádios florais.

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Nasturtium (Tropaeolaceae, Tropaeolum majus) has its center of diversity in South America (Friedman et al., 2007). The plant has a highly diversified use: there are reports of its use as a medicinal herb, intercropped crop with other plants, for honey production, as an unconventional vegetable, for ornamental purposes and as a natural dye (Ortiz de Boada & Cogua, 1989). Nasturtium can also be used quite successfully as cover crop, preventing soil degradation.

Currently, nasturtium is also used in fine restaurants, where leaves and flowers are offered as nutritious and attractive salads, adding an exotic touch. Nasturtium fruits, on their turn, once preserved in vinegar, may well replace capers for culinary dishes (Golze, 2008). In Brazil, the state of São Paulo leads the domestic production of edible flowers, with Minas Gerais ranking second, with great expansion potential, reports the Office for Food Safety and Support for Family Agriculture (Susaf) (State

Secretariat Agriculture, Livestock and Supply of Minas Gerais, 2012).

The production of edible flowers as economic activity has become a prosperous business in Brazil. Flowers are sold especially for restaurants, buffets and large supermarket chains. Most of the times, producers and customers set contracts, ensuring the supply and flow of products with very specific characteristics, produced in organic farming systems, therefore with no pesticides. In this way, the

production of edible flowers represents a sustainable alternative source of income for smallholders.

Flowers must be commercialized and used as soon as possible after harvesting, because deterioration starts fast. According to Dukovski *et al.* (2006), flower post-harvest senescence is controlled by many factors of both endogenous and exogenous nature that might act synergistically. However, in many flower species it is possible to extend the postharvest life with inhibitors of ethylene synthesis or action.

The 1-methylcyclopropene (1-MCP) is one of the most frequently employed cyclopropenes for vegetable preservation, since it is stable at room temperature and active at low concentrations, without being toxic (Kebenei *et al.*, 2003). The 1-MCP has been an alternative growth regulator in the conservation of plant products, but its effect is dependent on the genotype, application interval, concentration and maturity stage at time of application (Blankenship & Dole, 2003).

This paper aimed to analyze the sensitivity of nasturtium flowers to ethylene and evaluating the efficiency of 1-methylcyclopropene (1-MCP) on the postharvest senescence of these flowers.

MATERIAL AND METHODS

Nasturtium seeds, hybrid Alta Dobrada, were sown in polystyrene 72-cell trays containing commercial substrate Plantmax®. Seedlings were transplanted to 1x10 m beds, with 20 cm spacing between plants and rows, twenty days after sowing. Flowers were harvested four months later at four pre-established stages (Figure 1): 1= totally enclosed bud, with visible petal tips; 2= expanded buds, with visible although closed petals; 3= newly open buds; 4= fully open flowers. This research consisted of three experiments, namely: ethylene application, 1-methylcyclopropene (1-MCP) application, and 1-MCP application in combination with ethylene. The experiments were carried out separately, using the four floral

stages in all of them.

Ethylene application - Flowers harvested in four floral stages of development were placed in flasks with distilled water and transferred to 110-liter chambers, hermetically sealed. Ethylene was applied at 0.1, 1, 10, 100, and 1000 µL/L. We used two control treatments, one kept inside and the other outside the chamber. Treatments were set up as a factorial 4x7 (four floral stages and 5 concentrations + two controls). The experiment was carried out in complete blocks at random, with four 10-flower replications, and kept at 24±2°C, 10 μmol/m²/s of light intensity, and 41% relative humidity. Flowers were transferred from the chambers to benches after 24 h of exposure to ethylene, and kept in pots filled with distilled water under the same temperature, light and relative humidity conditions.

Application of 1-MCP - Flowers were treated with 1-MCP (Ethylbloc®, 0.14% of active content of 1-methylcyclopropene) at 0.25, 0.5, and 0.75 μ L/L. As in the previous experiment, we used two control treatments, one kept inside and, the other, outside the chamber. The experiment was designed as a factorial 4x5 (four floral stages and three treatments + two controls) in complete blocks at random, with

four 10-flower replications. Flowers remained in a 110-liter chamber, hermetically sealed, for 24 hours, at $20\pm2^{\circ}\text{C}$, $10\,\mu\text{mol/m}^2/\text{s}$ of constant light, 44% relative humidity. Then, flowers were transferred to benches and kept in pots filled with distilled water, under the same temperature, light and relative humidity conditions.

1-MCP application combined with ethylene - The following treatments were applied to flowers in the four floral stages: ethylene (100 µL/L); 1-MCP (0.75 µL/L), and 1-MCP combined with ethylene, with 1-MCP (0.75 μ L/L) applied for 24 h followed by exposure to ethylene (100 µL/L) for another 24 h. We used two control treatments (with no ethylene, nor 1-MCP), one keeping flowers outside the chamber and, the other, with flowers inside the chamber. The experiment was set up in a factorial design 4x5 (four floral stages x three treatments + two controls), in complete blocks at random and four 10-flower replications. The ethylene dose of 100 µL/L shows the maximum flower response, i.e, it was saturating. Flowers were placed in flasks with distilled water and transferred to 110-liter chambers, hermetically sealed, being kept at a 21±2°C, 10 μmol/m²/s constant light, and 50% relative humidity.

After the ethylene and 1-MCP



Figure 1. Development stages of nasturtium flowers: 1= totally enclosed bud, with visible petal tips; 2= expanded buds, with completely visible although closed petals; 3= newly open buds; 4= fully open flowers (estádios de desenvolvimento de flores de capuchinha: 1= botões totalmente fechados, apresentando projeção apenas das pontas das pétalas; 2= botões bastante expandidos, apresentando projeção completa das pétalas, ainda que fechadas; 3= botões recém-abertos; 4= flores totalmente abertas). Viçosa, UFV, 2013.

application, we replaced the distilled water in the flasks daily. Flower senescence was assessed according to the pre-established visual scale: 1= totally enclosed bud, with visible petal tips, turgid; 2= expanded buds, with visible although closed petals, turgid; 3= newly open buds, turgid; 4= fully open flowers. Turgid; -1= wilted flowers; -2= start of necrosis at the petal edge; -3= necrosis up to half of the petals; -4= completely dried petals. We considered longevity to be finished when 100% of the buds had grade -4.

The results were submitted to analysis of variance and means were compared by the Tukey test, at 5% probability. Results shown in the tables correspond to 48 h after finishing the treatments. To avoid CO₂ accumulation, NaOH pellets were placed in the chambers.

RESULTS AND DISCUSSION

Ethylene exogenous application induced wilt without causing premature petal fall in flower buds at stages 3 and

4 (Table 1). However, in buds at floral stages 1 and 2, ethylene application in concentrations equal or above 10 μL/L for 24 h, in addition to wilting, prevented buds from opening. There were significant differences in the degree of senescence as function of ethylene concentration and floral stages (Table 1). At first floral stage, all flowers wilted independent of ethylene concentration. At second, third, and fourth floral stages, the higher ethylene concentration, the greater degree of wilting occurred, except for the third and fourth stages, in which the ethylene at dose 1 µL/L showed almost the same effect as the higher doses (Table 1). Floral stage 4 showed the highest wilting level in response to ethylene, with scores ranging from -2 to -3.6, while other stages had grades ranging from -1 to -2.6. Therefore, ethylene had more significant effects on fully opened flowers.

Flowers from different species have different reactions to ethylene, i.e., there are distinct sensitivity degrees (van Doorn & Woltering, 2008). Three types

are flowers that wilt and dry, such as carnations, orchids and petunias; there are flowers in which the petals fall, although with no visible sign of wilting, which is possibly due to ethylene acting at the petal abscission zone, including roses and geraniums, and; there are flowers that are unresponsive to ethylene, such as lilies and iris (Woltering & van Doorn, 1988). In addition, the ethylene concentration needed to sensitize a flower, as well as ethylene effects on flower opening and senescence, are dependent on factors such as exposure time, temperature, stage of development and species or variety (Hoyer, 1996). In nasturtium, it has been observed that ethylene in higher concentrations, 10 to 1000 µL/L, led to early senescence, causing loss of turgor without petal abscission. Thus, according to literature, nasturtium can be classified as moderately sensitive to ethylene (Rudnicki & Nowak, 1990).

of responses have been reported: there

Flowers treated with 1-MCP, independent of the floral stage, were open and turgid, with no wilting, 48 hours after the treatment (Table 2), while flowers from the control treatment, within the same period, had signs of senescence onset. However, increasing 1-MCP dose above 0.25 μ L/L did not prolong flower for a longer period. When 1-MCP was used, there were significant differences among floral stages exclusively for degree of flower opening. Flower buds at stage 1 did not open fully, while flower buds at other stages evolved to fully opened flowers 48 hours after applying the treatment. We concluded that 1-MCP delayed flower opening, as flowers were turgid and developed normally as days passed by. Thus, the use of 1-MCP in nasturtium flowers, at concentrations tested, preserved flower quality during storage for floral stages 2 and 3.

1-MCP has satisfactory results in blocking the negative effects of ethylene in other flower species, as seen in roses, geraniums, *Phalaenopsis* sp. and *Diantus* sp. (Seglie *et al.*, 2010), *Gentiana* sp. (Shimizu-Yumoto & Ichimura, 2012), *Epidendrum* sp. (Finger *et al.*, 2008), and tulip bulbs (Liou & Miller, 2011). 1-MCP has also

Table 1. Scores of four stages of floral opening after ethylene application at different concentrations in nasturtium flowers, 48 hours after removing flowers from the chambers (notas de quatro estádios de abertura floral após a aplicação de etileno em diferentes concentrações em flores de capuchinha, 48 horas após retirá-las das câmaras). Viçosa, UFV, 2013.

Floral stages	EC ¹	IC ²	0.1	1	10	100	1000
			(µL/L)				
1	-1bA	-1aA	-1aA	-1.3aA	-1.3aA	-1.3aA	-2aA
2	4aA	-1aB	-1aBCD	-1.6aB	-1.6abB	-2aB	-2aB
3	4aA	-1aB	-1.6aB	-1.3aBC	-2.6bcCD	-2.3abD	-2.6abD
4	-2bA	-2aA	-2aA	-3.3bB	-3cAB	-3.3bB	-3.6bB

*Means followed by the same capital letter in the line and small letter in the column did not differ significantly from each other, Tukey, p<0.05 (médias seguidas de letras maiuscúlas iguais nas linhas e minúsculas nas colunas não diferem significativamente entre si, Tukey, p<0,05). Flower stages correspond to 1= totally enclosed bud, with visible petal tips; 2= expanded buds, with visible although closed petals; 3= newly open buds; 4= fully open flowers (estádios florais correspondentes a 1= botões totalmente fechados, com projeção da ponta das pétalas; 2= botões bastante expandidos, porém fechados, apresentando projeção de toda a pétala; 3= botões recém-abertos; 4= flores totalmente abertas); Scores correspond to 1= totally enclosed bud, with visible petal tips, turgid; 2= expanded buds, with visible although closed petals, turgid; 3= newly open buds, turgid; 4= fully open flowers, turgid; -1= wilted flowers; -2= start of necrosis at the petal edge; -3= necrosis up to half of the petals; -4= completely dried petals (notas correspondentes a 1= botões totalmente fechados, apresentando projeção da ponta das pétalas, túrgidos; 2= botões bastante expandidos, porém fechados, apresentando projeção de toda a pétala, túrgidos; 3= botões recém-abertos, túrgidos; 4= flores totalmente abertas, túrgidas; -1= flores murchas; -2= início de necrose nas bordas das pétalas; -3= necrose até metade das pétalas; -4= pétalas completamente secas). ¹EC: external control (controle externo); ²IC: internal control (controle interno).

Table 2. Scores of four stages of floral opening after 1-MCP application at different concentrations in nasturtium flowers, 48 hours after removing flowers from the chambers (notas de quatro estádios de abertura floral após a aplicação de 1-MCP em diferentes concentrações em flores de capuchinha, 48 horas após retirá-las das câmaras). Viçosa, UFV, 2013.

Floral stages	EC ¹	IC ² -	0.25	0.5	0.75	
	EC.	IC -	(µL/L)			
1	1.6aB	3.6aA	3aA	3.3aA	3aA	
2	-1bB	-1bB	4aA	4aA	4aA	
3	-1bB	-1bB	4aA	4aA	4aA	
4	-1.3bB	-1bB	4aA	4aA	4aA	

*Means followed by the same capital letter in the line and small letter in the column did not differ significantly from each other, Tukey, p<0.05 (médias seguidas de letras maiuscúlas iguais nas linhas e minúsculas nas colunas não diferem significativamente entre si, Tukey, p<0,05). Flower stages correspond to 1= totally enclosed bud, with visible petal tips; 2= expanded buds, with visible although closed petals; 3= newly open buds; 4= fully open flowers (estádios florais correspondentes a 1= botões totalmente fechados, com projeção da ponta das pétalas; 2= botões bastante expandidos, porém fechados, apresentando projeção de toda a pétala; 3= botões recém-abertos; 4= flores totalmente abertas); Scores correspond to 1= totally enclosed bud, with visible petal tips, turgid; 2= expanded buds, with visible although closed petals, turgid; 3= newly open buds, turgid; 4= fully open flowers. turgid; -1= wilted flowers; -2= start of necrosis at the petal edge; -3= necrosis up to half of the petals; -4= completely dried petals (notas correspondentes a 1= botões totalmente fechados, apresentando projeção da ponta das pétalas, túrgidos; 2= botões bastante expandidos, porém fechados, apresentando projeção de toda a pétala, túrgidos; 3= botões recém-abertos, túrgidos; 4= flores totalmente abertas, túrgidas; -1= flores murchas; -2= início de necrose nas bordas das pétalas; -3= necrose até metade das pétalas; -4= pétalas completamente secas). ¹EC: external control (controle externo); 2IC: internal control (controle interno).

Table 3. Scores of four stages of floral opening after applying 1-MCP in combination with ethylene in nasturtium flowers, 48 hours after removing flowers from the chambers (notas de quatro estádios de abertura floral após a aplicação de 1-MCP em combinação com etileno em flores de capuchinha, 48 horas após retirá-las das câmaras). Viçosa, UFV, 2013.

Floral stages	EC ¹	IC ²	1-MCP (0.75 μL/L)	Ethylene (100 μL/L)	1-MCP+ethylene (0.75 + 100 μL/L)
1	-1aB	-1aB	4aA	-1.3aB	-1cB
2	-1aC	-1aC	4aA	-1aC	2.3bB
3	-1aB	-1.3aB	4aA	- 1aB	4aA
4	-1aB	-1aB	4aA	-1.6aB	-1cB

*Means followed by the same capital letter in the line and small letter in the column did not differ significantly from each other, Tukey, p<0.05 (médias seguidas de letras maiuscúlas iguais nas linhas e minúsculas nas colunas não diferem significativamente entre si, Tukey, p<0,05). Flower stages correspond to 1= totally enclosed bud, with visible petal tips; 2= expanded buds, with visible although closed petals; 3= newly open buds; 4= fully open flowers (estádios florais correspondentes a 1= botões totalmente fechados, com projeção da ponta das pétalas; 2= botões bastante expandidos, porém fechados, apresentando projeção de toda a pétala: 3= botões recém-abertos: 4= flores totalmente abertas): Scores correspond to 1= totally enclosed bud, with visible petal tips, turgid; 2= expanded buds, with visible although closed petals, turgid; 3= newly open buds, turgid; 4= fully open flowers. turgid; -1= wilted flowers; -2= start of necrosis at the petal edge; -3= necrosis up to half of the petals; -4= completely dried petals (notas correspondentes a 1= botões totalmente fechados, apresentando projeção da ponta das pétalas, túrgidos; 2= botões bastante expandidos, porém fechados, apresentando projeção de toda a pétala, túrgidos; 3= botões recém-abertos, túrgidos; 4= flores totalmente abertas, túrgidas; -1= flores murchas; -2= início de necrose nas bordas das pétalas; -3= necrose até metade das pétalas; -4= pétalas completamente secas). ¹EC: external control (controle externo); ²IC: internal control (controle interno).

significantly extended flower longevity in *Phalaenopsis* sp. and roses, cultivar Sparkle, regardless of the presence or absence of ethylene in the atmosphere after the treatment with 1-MCP (Chang *et al.*, 2013; Nergi & Ahmadi, 2014).

In the current work, when 1-MCP was used, flowers started wilting only 96 h after the treatment (data not shown). while nasturtium flowers usually start to wilt 24 hours after harvest. Therefore, we recommend the use of 1-MCP to circumvent ethylene deleterious effects in the commercialization of nasturtium flowers. We suggest the use of 1-MCP as sachets placed inside the PET packing which nasturtium flowers are typically sold in, since 1-MCP is not toxic. This practice can extend flower commercialization period, benefiting the entire supply chain. Nevertheless, it is important to know that each cultivar has specific requirements that must be analyzed and considered individually to obtain high quality products with extended postharvest shelf life (Dias-Tagliacozzo et al., 2005).

In the experiment in which 1-MCP and ethylene were combined, the treatment in which only 1-MCP was applied showed the best results (Table 3): all flowers were turgid 48 h after the treatment. In all other treatments, flowers exhibited characteristics of starting senescence within the same period. Nevertheless, flowers in stages 2 and 3 remained turgid when ethylene was combined with 1-MCP (Table 3). Therefore, even when exogenous ethylene was present, 1-MCP effectively prolonged the postharvest life of flowers in all these stages. In all other treatments, there were no differences related to the floral stages. These results indicate that 1-MCP can indeed prevent or mitigate either limiting factors and processes dependent on ethylene that concurs to the abbreviation of flower pot life (Uthaichay et al., 2007).

At first stage floral development, the buds exposed to ethylene concentrations equal or above $10 \, \mu L/L$ remained closed (Table 4), while those exposed to lower concentrations, as well as those in the control treatments, were open. This indicates that high concentration of exogenous ethylene promotes quick

Table 4. Scores of first stage flower buds of nasturtium flowers after applying either 1-MCP or ethylene, and 1-MCP and ethylene in combination (notas dos botões do primeiro estádio de abertura floral de flores de capuchinha após a aplicação isolada e combinada de etileno e 1-MCP). Viçosa, UFV, 2013.

Experiments		Treatments							
	ECI	ICI	0.1	1	10	100	1000		
Ethylene	EC ¹	IC ¹	(μL/L)						
	3.6A	3.6A	3A	3A	2.6A	2.6A	1.3B		
1 MCD	EC ¹	IC¹	0.25 (μL/L)	0.5 (μL/L)	0.75 (μL/L)				
1-MCP	3.3A	3.6A	3.3A	3.3A	3A				
1-MCP+Ethylene	EC ¹	IC¹	1-MCP (0.75 μL/L)	Ethylene (100 μL/L)	1-MCP+ethylene (0.75+100 μL/L)				
	4A	3.6A	4A	2.3B	3.6A				

*Means followed by the same letter in the line did not differ significantly from each other, Tukey, p<0.05 (médias seguidas de mesmas letras nas linhas não diferem significativamente entre si, Tukey, p<0.05). Scores correspond to 1= totally enclosed bud, with visible petal tips, turgid; 2= expanded buds, with visible although closed petals, turgid; 3= newly open buds, turgid; 4= fully open flowers. turgid; -1= wilted flowers; -2= start of necrosis at the petal edge; -3= necrosis up to half of the petals; -4= completely dried petals (notas correspondentes a 1= botões totalmente fechados, apresentando projeção da ponta das pétalas, túrgidos; 2= botões bastante expandidos, porém fechados, apresentando projeção de toda a pétala, túrgidos; 3= botões recém-abertos, túrgidos; 4= flores totalmente abertas, túrgidas; -1= flores murchas; -2= início de necrose nas bordas das pétalas; -3= necrose até metade das pétalas; -4= pétalas completamente secas). ¹EC: external control (controle externo); ²IC: internal control (controle interno).

petal wilting and blocks normal bud development, inducing heterogeneity in flower opening and/or in morphology of opened flowers.

Ethylene does not seem to be decisively involved with floral opening in nasturtium. In roses, it is totally different. In one hand, in some rose cultivars, exogenous ethylene led to a considerable reduction in flower opening, followed by fast and severe petal wilting (MacNish et al., 2010). In the other hand, in cultivar Osiana, ethylene stimulated flower bud opening without reducing flower post-harvest longevity (Cordeiro et al., 2011). These results confirm that, regarding to flower opening, responses to ethylene is cultivar-specific (MacNish et al., 2010). In addition, depending on the cultivar and management conditions, flower opening may be hampered and ethylene would then have a role as an important regulating factor. Thus, flower opening is dependent on ethylene concentration, which depending on the cultivar, can either inhibit or accelerate the process, or just does not affect it.

In conclusion, our results demonstrate the sensitivity of nasturtium flowers to ethylene and the efficiency of 1-MCP in extending nasturtium flowers postharvest shelf life.

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