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β-cyclodextrin-based nanosponges improve 1-MCP efficacy in extending the postharvest quality of cut flowers

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Abstract

Investigations of the effect of the β-cyclodextrin-based nanosponge-1-methylcyclopropene (1-MCP) complex (β-CD-NS complex) on six ethylene-sensitive cut flowers were performed. *Anemone coronaria* L. multicolor, *Ranunculus asiaticus* L. 'Minou Abrown', *Helianthus annuus* L. 'Sunrich Orange', *Rosa hybrida* L. 'Jupiter', *Paeonia lactiflora* L. 'Sarah Bernhardt', and *Papaver nudicaule* L. multicolor underwent four different treatments: a vase placement in a suspension of β-CD-NS complex (0.25 μL L^{-1}), exposure to gaseous 1-MCP (0.25 μL L^{-1}) for 6 h, exposure to exogenous ethylene (1 ± 0.2 μL L^{-1}) for 24 h, and placement in tap water.

β-cyclodextrin-based nanosponges proved to enhance 1-MCP efficacy in all the tested species. According to the species, senescence symptoms were decreased or delayed. Anemone and poppy had a longer aesthetic flower quality and vase life, and a reduced

petal abscission. In ranunculus, β-CD-NS complex improved longevity. In sunflower, it also maintained longer chlorophyll leaf content and cut flower ornamental value. Peony opened more slowly and rose maintained its ornamental quality longer than 1-MCP treated flowers. This study opens possibilities for commercial use of the β-CD-NS complex in the floriculture industry.

Keywords: Ethylene inhibitor; Flower longevity; Nano-carrier; Preservative; Senescence; Vase life.

Abbreviations: CD, cyclodextrin; 1-MCP, 1-methylcyclopropene; β-CD-NS, β-CD-based-nanosponge; β-CD-NS complex, 1-MCP included in β-CD-NS

1. Introduction

Postharvest performance is of crucial importance to the value of cut flowers. The life span of flowers is generally determined by the time to abscission of petals that are still turgid, or by the time to petal wilting or withering. In many species, these phenomena are regulated by ethylene (Woltering and Van Doorn, 1988; van Doorn, 2001). Premature senescence and abscission resulting from exposure to exogenous or endogenous ethylene can be controlled several ways including ethylene action inhibitors (Martínez-Romero et al., 2007) quite commonly.

In particular, while 1-methylcyclopropene (1-MCP), has proved itself to be a very effective preventer of negative ethylene responses, its gaseous nature makes treatment difficult (Serek and Sisler, 2005; Serek et al., 2006).

Recently, we developed a non-volatile formulation of 1-MCP in a β-cyclodextrin-based nanosponge (β-CD-NS 1:8) structure. Supplied in the conservation solution, this formulation (β-CD-NS complex) prolonged the vase life of carnation cut flowers (Seglie et. al., 2011a, b), and controlled *B. cinerea* damage (Seglie et al., 2012) better than gaseous 1-MCP.

While carnation is generally used in studies on postharvest preservation of ethylene-sensitive flowers (Woltering and van Doorn, 1988; Serek et al., 1995a, b); it is not a universal paradigm for flower senescence. The response to ethylene varies widely according to species (Reid and Wu, 1992). Therefore, our study evaluated the effectiveness of the β-CD-NS complex to improve cut flower vase life on a number of ethylene-sensitive species.

2. **Materials and methods**

The flower market of Sanremo (UCFlor, Liguria, Italy) seasonally provided cut flowers of *Anemone coronaria* L. multicolor, *Ranunculus asiaticus* L. 'Minou Abrown', *Helianthus annuus* L. 'Sunrich Orange', *Rosa hybrida* L. 'Jupiter', *Paeonia lactiflora* Pall. 'Sarah Bernhardt', and *Papaver nudicaule* L. multicolor.

Stems were transferred to the postharvest laboratory of the Department of Agricultural, Forest and Food Sciences at the University of Turin. Experimental evaluations of vase life were performed in an imitated inside environment (IE) maintained at 20±2° C, 60 % RH, and 12 h daily of 46 μmol m^{-2} s^{-1} cool white light (meter model HT307; HT, Faenza, Italy).

Cut flowers were re-cut, labeled and re-watered. Each species sample was divided into sub-groupings of six cut stems (each 30 cm long after re-cutting). Each sub-group was then treated according to the following schemes: in vases with an aqueous suspension of β-CD-NS complex to supply 0.25 μL L^{-1} of active ingredient; in an air tight cabinet (112 L) exposed to equal concentrations of volatile 1-MCP (3.3% a. i., SmartFresh™, AgroFresh Inc., USA) for 6 h; in another air tight cabinet exposed to exogenous ethylene (1 ± 0.2 μL L^{-1}) for 24 h; and in vases of tap water as a control. The experiment was performed twice for each species.

Endogenous ethylene production of the control flowers was measured using a digital Agilent Technologies gas chromatograph, 7890A Network GC system (Santa Clara, California). N_2 at 40 mL min^{-1} was used as the gas carrier at a column temperature 60 °C and calibration range between 0.5-3 ppm. For each species, six samples were considered.

Flower postharvest performance was measured and visual checks for symptoms of variation in senescence were performed daily. In particular, we monitored senescence level (1 = initial senescence, 2 = complete senescence; Seglie et al., 2011a), flower opening stage (1 = initial opening, 2 = half opening, 3 = full opening; Guo et al., 2004, modified), and abscission. Chlorophyll content was indirectly measured in leaves through the Chlorophyll Meter SPAD-502 instrument (Konica Minolta Sensing Inc., Osaka, Japan).

Analysis of variance (ANOVA) was performed to assess statistical significance among mean values using the Ryan–Einot–Gabriel–Welsch's multiple step-down F (REGW-F) test ($p \leq 0.05$) with SPSS Software (Chicago, USA).

3. **Results and Discussion**

Senescence performance and ethylene sensitivity are highly species related (van Doorn, 2001). Large variabilities have been observed even among cultivars, such as the Asian buttercup (Kenza et al., 2000; Scariot et al., 2009), rose (Chamani et al., 2005), and peony (Hoffman et al., 2010; Eason et al., 2002). Flowers that respond to low concentrations of exogenous ethylene are probably those in which ethylene is naturally involved in senescence (Reid and Wu, 1992). In this study, ethylene hastened the symptoms of natural senescence in all species. However, a climacteric pattern was observed only in poppy (ethylene up to 12 $\mu L\ L^{-1}$ at day 5) while in peony and sunflower ethylene production was not detected (data not shown).

Anemone cut flowers lose their decorative value in a very short period. Pedicels elongate excessively and petals wilt, lose their color, become translucent, and abscise (Meir, 2007). Previous studies have already demonstrated the ethylene sensitivity of the anemone (Armitage, 1993) and the efficacy of ethylene antagonists (silver nitrate and silver thiosulphate, STS) in extending its vase life and preventing petal abscission (Sharifani et al., 2005). In this study, the application of β-CD-NS complex was found to prolong cut flower aesthetic quality and longevity for up to 1.5 additional days, (Fig. 1), slow stage 1 and 2 of flower opening (Tab. 1), and completely prevent abscission (Fig. 2) more effectively than gaseous 1-MCP. Petal rolling over and stem folding (Sharifani et al., 2005), which usually occur in anemone cut flowers, were also limited.

Asian buttercup flower senescence is characterized by loss of turgor and petal colour change followed by petal wilting and drop when lightly touched. Previous tests of the effects of silver thiosulphate (STS) and aminooxyacetic acid (AOA) on buttercup postharvest performance have yielded unsatisfactory results in enhancing vase life (Shahri et al., 2011). In this study, ethylene antagonists were effective in delaying senescence symptoms with β-CD-NS complex; they outperformed gaseous 1-MCP as demonstrated by delay of complete senescence (9.7 *versus* 8 days; Fig. 1) and original flower colour maintenance (data not shown).

Previous studies of sunflower have shown exogenous ethylene applications result in short vase life, flower abscission (Redman et al., 2002), and in largely, wilted basal foliage immediately after anthesis (Pallez et al., 2002; Castro et al., 2011). Furthermore, ethylene antagonist treatment has failed to limit or delay senescence (Redman et al., 2002). In this study, chlorophyll degradation was restrained by ethylene antagonist application. At day 5, the SPAD value was 26.2 ± 0.63 units for control flowers and on average 33.7 ± 0.19 units for flowers treated with ethylene antagonists. β-CD-NS complex was more effective than gaseous 1-MCP at prolonging vase life (9 *versus* 7 days, respectively; Fig. 1) and at slowing flower opening (stages 1 and 2; Tab. 1).

In rose, flower opening seemed to be affected by exogenous ethylene (Reid et al., 1989). Ethylene induced abscission of fully turgescent, non-senescent petals or the entire corolla (Serek et al., 2006), which suggests that anti-ethylene compounds could retard the behavior (Serek et al., 1995b) and extend vase life (Mor et al., 1989). Precedent studies have noted decreased leaf and bud abscission by application of 1-MCP along with increased display life in both ethylene-contaminated and ethylene-free air (Muller et al., 2000; Serek et al., 1994). This study showed β-CD-NS complex performed better than commercial 1-MCP as evidenced by extension of ornamental rose quality (5.0 and 3.5 days, respectively; Fig. 1), and slowed flower opening (stages 2 and 3; Tab. 1).

Peony has been found by Jia et al. (2008) to have a high ethylene sensitivity; they correlated it to an increased flower diameter during senescence. On the other hand, Hoffman et al. (2010) have found 1-MCP ineffective at increasing storage longevity and quality of cut peony flowers. Our study results show that both 1-MCP applications significantly increased the longevity of peony to almost 2 days (7 days) beyond that of the control (5 days; Fig. 1), and limited abscission (Fig. 2). Also, β-CD-NS complex outperformed the commercial product in slowing flower opening (Fig. 2).

The vase life of poppy is usually 5 days in untreated flowers; it ends when petals shatter or crinkle, discolor or turn brown, and the stem collapses (Dole et al., 2009).

This research indicated that gaseous 1-MCP was ineffective at delaying these symptoms of senescence while β-CD-NS complex, on the other hand, was shown to extend ornamental flower quality for up to 5.8 days and increased the vase life to as long as 8.7 days (Fig. 1). Moreover, this compound significantly reduced petal abscission (Fig. 2) and retarded flower opening (Tab. 1). Therefore, β-CD-NS complex use could increase the commercial market for poppy cut flowers, albeit one that will continue to be limited by high production costs (Dole and Greer, 2009).

In conclusion, β-CD-NS 1:8 was shown to enhance the efficacy of 1-MCP in six ethylene-sensitive species, as was previously seen in carnation (Seglie et al., 2011a, b). 1-MCP is a high unstable and reactive gas that very quickly dimerizes even at room temperature. This dimer has not antiethylenic activity. Most likely β-CD-NS stabilize to a great extent the included 1-MCP thus preserving its properties.

The present study opens the possibilities of commercial use of the β-CD-NS complex in the floriculture industry.

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Figure captions

Fig. 1 Longevity of six species of cut flowers according to four treatments: placement in a vase suspension of β-CD-NS complex (0.25 µL L^{-1}), exposure to gaseous 1-MCP (0.25 µL L^{-1}) for 6 h, exposure to exogenous ethylene (1 ± 0.2 µL L^{-1}) for 24 h, and placement in tap water. The count of days to reach the initial stage of senescence (Level 1, line) and complete senescence (Level 2, dotted line) was measured.

*Mean separation within bars by the Ryan-Einot-Gabriel-Welsch's multiple stepdown F (REGW-F) test, $P \leq 0.001$.

Fig. 2 Petal abscission in six flower species according to four treatments: placement in a vase suspension of β-CD-NS complex (0.25 µL L^{-1}), exposure to gaseous 1-MCP (0.25 µL L^{-1}, for 6 h, exposure to exogenous ethylene (1 ± 0.2 µL L^{-1}) for 24 h, and placement in tap water. For each species, abscission (%) was calculated as the total number of abscised petals/the mean number of petals in three flowers * 100.

*Mean separation within bars by the Ryan-Einot-Gabriel-Welsch's multiple stepdown F (REGW-F) test, $P \leq 0.001$.