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Use of 1-Methylcylopropene in cyclodextrin-based nanosponges to control grey mould caused by *Botrytis cinerea* on *Dianthus caryophyllus* cut flowers

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Abstract

Botrytis cinerea is one of the pathogens resulting in the heaviest commercial losses in ornamental cut flowers, and the severity of grey mould disease partly depends on the presence of ethylene in the storage environment. The efficacy of β-cyclodextrin-based nanosponge 1:8 (NS) - 1-methylcyclopropene (1-MCP) complex was evaluated as a novel control agent in protecting carnation (*Dianthus caryophyllus* L. 'Idra di

Muraglia') cut flowers against *B. cinerea* infection. Two concentrations of this innovative, non-volatile 1-MCP formulation (NS complex, 0.25 and 0.5 μL L^{-1}, a. i) were compared to the commercial gaseous 1-MCP (0.25 μL L^{-1}, a. i.), and to an inoculated control. Furthermore, a not-inoculated control was used to assess the natural infection level. Eleven days after inoculation, the development of grey mould on carnation was significantly reduced (59.9% of flower surface) in cut stems treated with the NS complex at low dosage, compared to the high dosage of the NS complex (91.5%), the commercial gaseous 1-MCP formulation (76.2%) and to the inoculated control (100.0%). Endogenous ethylene production was correlated to the symptoms development. Results showed a reduced ethylene production in 1-MCP treated flowers (0.25 μL L^{-1}, a. i., both suspended and gaseous formulation). NS complex could therefore be an effective alternative to conventional chemicals to protect ornamental cut flowers.

Keywords: carnation, grey mould, nanocarriers, postharvest, ethylene antagonist.

Abbreviations: CD, cyclodextrin; 1-MCP, 1-methylcyclopropene; CD-NS, β-CD-based nanosponge 1:8; CD-NS complex, β-CD-based NS 1:8 - 1-MCP complex

1. Introduction

The flower trade worldwide is marked by an increasing competitiveness in cut flower offer (Serra, 2003). An important cut-flower crops grown internationally is carnation (*Dianthus caryophyllus* L.). Increasing attention is paid to postharvest vase life, that plays a crucial role in the quality of cut flowers. The senescence process is induced by several factors, among these are ethylene and pathogens (Woltering and van Doorn, 1988; Serek et al., 1995a,b).

Botrytis cinerea is an airborne pathogen for a wide variety of cut flower crops, causing grey mould responsible of severe economic losses. During the last few decades, the introduction of new ornamentals, and the improved growing techniques caused significant changes that contributed to aggravate the severity of diseases (Daughtrey and Benson, 2005). Therefore, a high priority on the research of sound management of greenhouse- and nursery-grown ornamentals, and of a more effective disease control, is required.

Considering the increasing restrictions on the use of pesticides (European Regulation 1107/2009 and Directive 2009/128, US Food Quality Protection Act), the development of new eco-friendly disease management strategies is needed. In a previous study, the use of the ethylene antagonist 1-methylcyclopropene (1-MCP) resulted effective in reducing damages caused by *B. cinerea* in cut flowers of several ornamental species (Seglie et al., 2009). However, difficulties in the application of this gaseous compound, such as the necessity of enclosed areas to prevent gas leakage, the need of continuous or repeated treatments, and the low action at temperatures (0–5 °C), complicate its commercial use (Serek and Sisler, 2005; Serek et al., 2006).

The inclusion of 1-MCP in cyclodextrin-based nanosponges structures (CD-NS, patented by Trotta et al. ultrasound-assisted synthesis of cyclodextrin-based nanosponges patent WO2006/002814) can reduce these practical limitations, and already showed to be effective in prolonging cut flower vase life (Seglie et al., 2011a). CD-NS is a delivery system able to induce an extended release of the 1-MCP, leading to benefits such as reduced active ingredient dosages required and reduced number of delivery times as compared to the gaseous commercial product.

In the present study, the effectiveness of the non-volatile formulation of 1-MCP included in CD-NS (CD-NS complex) in controlling *B. cinerea* damage on carnation cut flowers was evaluated.

2. Materials and methods

For the experiments, carnations (*Dianthus caryophyllus* L. 'Idra di Muraglia') were grown in standard greenhouse conditions in Sanremo, Liguria, Italy. Cut flowers were harvested at maturity stage (vertical sepals, vivid petal and stem colour) and taken to the postharvest laboratory within 24 h, where they were re-cut and labelled. The experiment was performed three times, each treatment included 3 repetitions of six cut flowers (stems 30 cm long). Twelve carnation cut flowers, inoculated or not with the pathogen, were kept in tap water as controls.

Stems were then placed in vases with a suspension of 1-methylcyclopropene (1-MCP) included in β-cyclodextrin-based nanosponges 1:8 (CD-NS complex, 6% a. i.) at two different concentrations of active ingredient (0.25 and 0.5 $\mu L\ L^{-1}$, a. i.), or exposed to 6 h treatment with the commercial gaseous 1-MCP (3.3% a. i., SmartFresh™, AgroFresh Inc., USA). Treated flowers were inoculated with a *B. cinerea* conidial suspension (10^4 conidia mL^{-1}) to favour the mould development.

Daily, the extent of *B. cinerea* development on each flower was monitored, counting the number of infected petals in relation to a mean total number of petals (61.9), calculated on 10 flowers.

Ethylene production was daily measured by keeping single treated flowers in air tight vases (250 mL) containing 50 mL of the different preservative solutions, or tap water for the controls. The ethylene concentration was measured using a digital Agilent Technologies gas chromatograph, 6890N Network GC system (Santa Clara, California).

The gas carrier was N_2 at 40 mL min^{-1}, and the column temperature was 60 °C. For each treatment, three samples were considered.

Statistical significance among mean values was assessed performing the analysis of variance (ANOVA), and the Ryan-Einot-Gabriel-Welsch's multiple stepdown F (REGW-F) test ($p \leq 0.05$), with the SPSS software Inc. (Chicago, United States).

3. Results and discussion

In order to prolong health and quality product, investigations on the effect of anti-ethylene compounds on the disease development on cut flowers were performed. All the 1-MCP treatments significantly slowed down the development of grey mould, compared to the inoculated control. The not inoculated control did not show any disease symptom during the experiment, indicating the health of the plant material used. Significant differences ($p \leq 0.05$) were denoted among the three 1-MCP applications/concentrations (Fig. 1). Treatment with the lower CD-NS complex concentration (0.25 μL L^{-1}) performed similarly or better than the commercial gaseous 1-MCP until day 13. At day 14, the lower dose of CD-NS complex resulted the best effective. At day 15, the pathogen infection reached 100% value in all the inoculated flowers. Application of the higher NS complex concentration (0.5 μL L^{-1}) was less active than the other two 1-MCP treatments. Seglie et al. (2011a) already reported a lower activity of the higher concentration of NS complex in extending the vase life of carnation cut flower. This result might be explained by considering that the increase of the total amount of nanosponge decreases the antagonist release (Seglie et al., 2011b).

Data about endogenous ethylene production were strictly related to the development of grey mould on flowers. The lowest ethylene production was measured in flowers treated with 0.25 μL L^{-1} NS complex (0.53 μL L^{-1}), followed by gaseous 1-MCP-treated flowers (0.70 μL L^{-1}). The not inoculated control and the flowers treated with the higher concentration of NS complex produced the same ethylene concentration (1.15 μL L^{-1}). As expected, the highest endogenous ethylene production was observed in the inoculated control (1.77 μL L^{-1}) (Fig. 2). It could be assumed that NSs are able to reduce ethylene production, by slowly releasing the ethylene antagonist, reducing the senescence process, and, simultaneously, by adsorbing the phytohormone, and other trap targeted organic compounds (Li and Ma, 1999). Ethylene antagonists could maintain membrane integrity of plant tissues (Elad, 1997), by reducing *Botrytis* blight of rose (Elad, 1995) and other plants (Elad et al., 1993). Anyway, the relationship between ethylene synthesis, pathogen infection, and ethylene antagonists, such as NS complex, should be further elucidated.

In conclusion, 1-MCP included in nanosponges can be a promising formulation to be developed to control fungal diseases of cut flowers in the postharvest environment, though the mechanism of action needs further elucidation.

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Figure captions

Fig. 1 Effect of two different concentrations (0.25 and 0.5 μL L^{-1}) of β-CD-based-nanosponge 1:8 - 1-MCP complex (NS complex) on grey mould development of carnation cut flowers, compared to commercial gaseous 1-MCP (0.25 μL L^{-1} for 6 h), and to an inoculated (B^{+}) and not inoculated (B^{-}) controls. Vertical bars show the confidence intervals (95%) of mean values.

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

Fig. 2 Endogenous ethylene production in cut flowers of *Dianthus caryophyllus* 'Idra di Muraglia'. Flowers were treated with β-CD-based nanosponge 1:8 - 1-MCP complex (NS complex; 0.25 and 0.5 μL L^{-1}, a. i.), and gaseous 1-MCP treatment (0.25 μL L^{-1} for 6 h). Controls were inoculated (B^{+}) or not (B^{-}) with *Botrytis cinerea*.