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Use of 1-methylcyclopropene for the control of *Botrytis cinerea* on cut flowers

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Summary. Cut flowers are marketed for their ornamental characteristics making post-harvest flower life an important determinant of crop value. *Botrytis cinerea* is one of the most significant post-harvest fungal pathogens causing losses in ornamental plants. Disease caused by this fungus seems to be enhanced by the presence of ethylene, a hormone, that both the plant and the fungus are known to synthesize. The aim of the experiment was to determine if 1-methylcyclopropene (1-MCP), an ethylene antagonist, could be used to reduce *B. cinerea* damage to cut flowers. Six cultivars of four ornamental species: *Dianthus caryophyllus* 'Idra di Muraglia', *Rosa* × *hybrida* 'White Dew' and 'Ritz', *Ranunculus asiaticus* 'Saigon' and 'Green', and *Cyclamen persicum* line 'Halios Bianco Puro Compatto' were given three concentrations of 1-MCP (0.38 μl L^{-1}, 1.14 μL L^{-1}, and 3.62 μL L^{-1}) for 24 hours. Subsequently, 10 petals per cultivar were treated with a *B. cinerea* conidial suspension (5×10^3 conidia cm^{-2}) and stored in air-tight vases. To evaluate *B. cinerea* development, an arbitrary damage scale (1–7) was used. A high concentration of 1-MCP significantly reduced B. cinerea damage on *D. caryophyllus* 'Idra di Muraglia' and C. persicum 'Halios Bianco Puro Compatto' petals. In carnation, 1-MCP treatment slowed *B. cinerea* infection; its threshold level was reached three days after that of the control. In cyclamen, treated petals and control petals remained aesthetically good until day 53 and day 28 respectively. At low concentrations, 1-MCP slowed grey mould on *R.* × *hybrida* 'Ritz' for up to three days beyond the control. On the two buttercup cultivars 'Green' and 'Saigon', 1-MCP treatments were not effective. In conclusion, 1-MCP limited pathogen development; its effect depended on the species and the 1-MCP concentration. Further investigations will be performed to improve methods to reduce *B. cinerea* development on the petals of cut flowers.

Key words: grey mould, post-harvest decay, vase life.

Introduction

The floricultural export trade worldwide has a value of nearly 20 million euros, and consists of 50% cut flowers, 35% potted foliage and bedding plants, 7.5% bulbs, and 7.5% cut cultivated greens (Heinrichs, 2005). Not surprisingly, Italy devotes a large agricultural area to floriculture under greenhouse and open-air conditions (4.25 thousand million pieces produced) (ISTAT, 2005: http://www.istat.it/agricoltura/datiagri/fiori/flo22005.html).

Critically important to the cut flower market are the ornamental characteristics of form, shape, and colour of the flowers. Equally important and a key determinant of flower quality is longevity, not only to the end-user, but also to the producer and marketer, who must subject the flowers to sometimes lengthy handling and transportation processes.

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A number of parameters are used to characterize longevity and to define life span termination in cut flowers. Flowers become unacceptable because of sepal yellowing, petal wilting, petal blueing, petal abscission, lack of bud opening or peduncle bending.

Cut flower deterioration is determined mostly by environmental conditions. The most adverse effects on several species of cut flowers are caused by ethylene in the storage atmosphere (Halevy and Mayak, 1979). By their post-harvest longevity cut flowers are divided into two groups: flowers that have low ethylene sensitivity and that have no flower buds or leaf abscission, and flowers that are highly sensitive to ethylene. Chrysanthemum, tulip, iris and lily do not increase ethylene production or respiration during flower senescence; and even exogenous ethylene has little or no effect on the senescence of their flowers (Elgar *et al*., 1999). On the other hand, *Campanulaceae*, *Caryophyllaceae*, *Geraniaceae*, *Ranunculaceae,* and *Rosaceae* are highly sensitive to ethylene (Serek *et al*., 2006a). These tendencies generally persist across a single plant family (Woltering and van Doorn, 1988).

Ethylene is a plant growth regulator with an important role in the post-harvest environment, controlling abscission and senescence in the fruit and floral organs (Broekaert *et al*., 2006). Depending on the species, ethylene (either endogenous or from an external source) induces various processes (Woltering and van Doorn, 1988). In the *Caryophyllaceae*, ethylene hastens the senescence of petals which normally stay attached to the flower. Burchi *et al*., (1999) reported that ethylene bound to a normal ligand-receptor interaction, and was both saturable and reversible. In the *Ranunculaceae* and the *Rosaceae*, ethylene may actually cause the abscission of fully turgescent, non-senescent petals or of whole corollas. In species insensitive to ethylene, however, premature petal abscission seems extremely rare in the plant kingdom as compared with normal petal senescence in these same species (Sexton *et al*., 2000).

Ageing petals become more sensitive to exogenous ethylene by initiating autocatalytic ethylene production and by gradually de-restricting the ethylene biosynthetic pathway (Macnish *et al*., 2000). This change is related to either a decline in a hypothetical inhibitor of ethylene biosynthesis, or an accumulation of another natural agent in petals. The transition to autocatalytic ethylene production during petal ageing occurs in response to a change in tissue sensitivity to ethylene (Hassan and Gerzson, 2002).

The critical role that ethylene plays in regulating petal senescence (Serek *et al*., 2006b; van Doorn and Woltering, 2008) is species-dependent. Pre-climacteric flowers produce a low and constant rate of this phytohormone until they reach a critical stage at which a concomitant increase in the activities of the enzymes S-adenosyl-methionine (SAM) and 1-aminocyclopropane-1-carboxilic acid (ACC) begins to cause ethylene biosynthesis (Bleecker and Kende, 2000; Sharon *et al*., 2004; Lurie, 2005). In many sensitive herbaceous species, biotic or abiotic stresses promote ethylene production by accelerating its biosynthesis (Müller *et al*., 2000).

Higher levels of ethylene are considered to be an early response of plants to pathogen attack (Malathrakis and Goumas, 1999). Balestra *et al*. (2005) suggested that ethylene concentrations change in response to bacterial levels in gerbera cut flowers, and thus contribute to stem break. Little direct evidence is available on how ethylene is involved in vegetative and pathogenic *Botrytis cinerea* development. Most of the data on this subject come from studies on ethylene production in infected plants, on the possible effect of ethylene on grey mould, and on the production of ethylene by *B. cinerea* (Taylor *et al*., 2001). Qadir *et al*. (1997) reported that *B. cinerea* produces ethylene in shake culture via a specific ethylene biosynthetic pathway in which methionine is de-amined to yield α-keto-γ-methylthiobutyric acid (KMBA) which, in turn, produces ethylene by spontaneous or enzymatic oxidation (Chagué *et al*., 2002).

Ethylene is reported to stimulate conidial germination, germ tube elongation and appressorium formation in several fungi, including *B. cinerea* (Prusky, 1996); this is worth noting since *B. cinerea* infects not only dying plant tissues but also healthy tissues, such as developing rose petals. On living tissue, *B. cinerea* infections usually remain inactive until the cut flowers are harvested and start to deteriorate physiologically. When *B. cinerea* senses the deterioration of the host, the latent or quiescent infection becomes active, with tan-coloured lesions and superficial white and grey fungal matting as typical symptoms.

Previous studies carried out on cut roses showed that a fungistatic storage atmosphere reduces the se-

verity of *B. cinerea* infections (Hammer *et al*., 1990). It has also been found that pre-harvest calcium sulphate ($CaSO_4$) applications had a positive effect in slowing grey mould development (De Capdeville, 2005).

An additional strategy to restrict *B. cinerea* development may be the application of compounds that inhibit ethylene biosynthesis or activity. Recent studies have confirmed that 1-MCP antagonises ethylene action by competing with it for binding sites in many plants (Serek *et al*., 2004).

The physical and chemical properties of low ethylene levels and respiration, and the synthesis and activity of 1-MCP, were reviewed by Blankenship and Dole (2003); however, the exact mechanism of interaction between grey mould and 1-MCP remains unclear. Nonetheless, a correlation seems to exist, though still undefined, between ethylene synthesis, pathogen attack, and the use of ethylene inhibitors.

The present study investigated the role of 1-MCP in reducing *B. cinerea* growth on plants. The specific objective of the experiment was to determine whether 1-MCP treatment could be used to slow *B. cinerea* damage on cut flowers of various species and cultivars.

Materials and methods

Plant material

Six experimental trials were carried out on four species of cut flowers (Table 1). The ornamental flowers selected were chosen for their species criteria: a model species on senescence (*Dianthus caryophyllus* L., 'Idra di Muraglia'), an economically important species that has long been common (*Rosa* × *hybrida* L., 'Ritz' and 'White Dew'), a product new to the cut flower market (*Ranunculus asiaticus* L., 'Green' and 'Saigon'), and a species not widely used as a cut flower until now (*Cyclamen persicum* L., line 'Halios Bianco Puro Compatto').

Cut stems were provided by commercial companies in Sanremo (Liguria, north-western Italy), and kept in the greenhouse under optimal growth conditions, avoiding previous *B. cinerea* infection and without preservative treatments.

Flowers were cut in the morning with a sterile scalpel.

Experimental trials

The study was undertaken in the spring-to-winter (March–February) flowering seasons of 2006 and 2007.

After stem selection, which was based on aesthetic and health parameters, cut flowers at commercial maturity (sepals standing vertically, petal colours and stems very vivid) were transported from Liguria to the post-harvest laboratory of the Department of Agronomy, Forest and Land Management of the University of Turin, where they were kept in a standard vase life room at 20±2°C, 60% RH, and 46 μmoL m^{-2} s^{-1} cool white light as measured at flower height with a light meter (model HT307; HT, Faenza, Italy) with a 12 h day, from 6 am to 6 pm (Scariot *et al*., 2008).

Within 24 h, flowering stems were re-cut, labelled, weighed (after removing the leaves, except for two leaves close to the flower), and immediately placed in clean tap water.

To determine the effect of 1-MCP on *B. cinerea*, different concentrations of 1-MCP were applied. As a control, cut flowers were maintained in tap water without any preservative treatment.

For each trial and cultivar, 10 cut flowers (stems

Table 1. Flower species, cultivars (and their breeders, in brackets, where known) and one trade-line of cut flowers (Halios Bianco Puro Compatto) used in the *Botrytis cinerea* control study, colour and diameter of petals.

Species	Cultivar or trade-line	Petal colour	Petal diameter (cm)[a]
Dianthus caryophyllus	Idra di Muraglia	White	1.50±0.05
Rosa × *hybrida*	Ritz (De Raiter)	Yellow	2.00±0.10
Rosa × *hybrida*	White Dew (Marchese)	White	2.20±0.09
Ranunculus asiaticus	Green	Green	1.30±0.04
Ranunculus asiaticus	Saigon	Dark red	1.10±0.03
Cyclamen persicum	Halios Bianco Puro Compatto	White	1.70±0.08

10–30 cm long according to the species) were placed in an air-tight cabinet (112 L) and treated for 24 h with the commercial formula SmartFresh™ (0.14% w:w, active ingredient; AgroFresh, Inc. Philadelphia, PA, USA) to produce 0.38 μl L^{-1}, 1.14 μl L^{-1}, and 3.62 μl L^{-1} (the last two being triplicate and duplicate doses respectively) of 1-MCP, by injecting twice-distilled water through a suitable valve to free the active ingredient (Scariot *et al*., 2008).

Preliminary tests were carried out to establish the most effective concentration of 1-MCP. The lowest concentration was tested only on *D. caryophyllus* to determine if this concentration was enough to control *B. cinerea* (Philosoph-Hadas *et al*., 2003). After opening the cabinet, the stems were ventilated and the petals collected. Subsequently, petals gathered from treated and untreated cut flowers were inoculated with a mixed *B. cinerea* suspension (5×10^3 conidia cm^{-2}) to improve fungal growth.

Botrytis cinerea strains were isolated from naturally-infected ornamental plants (*Rhododendron*, white and red cyclamen, geranium, and hydrangea) in the pathogen collection of Agroinnova (Grugliasco, Turin, Italy) and were stored in test tubes containing 39 g L^{-1} of potato dextrose agar (PDA) (Merck, Darmstadt, Germany) + 50 μg L^{-1} streptomycin. After 10 days growth in Petri dishes, the mycelium was used to prepare the conidial suspension. A Ringer solution (Merck, Darmstandt, Germany) with Tween 20® (Merck surface-active agent) was used to harvest the pathogen conidia. The conidial suspension was brought to a final concentration of 10^5 conidia mL^{-1}, after which it was sprayed on the lower surface of each petal.

For each trial, two inoculated petals were placed in air-tight vases (0.5 L) previously prepared with moistened blotting paper (3 ml twice-distilled water) and the *B. cinerea* infection was determined. Each experiment was repeated twice.

Data collection and statistical analysis

Every two days the extent of *B. cinerea* mould was evaluated on each petal using an arbitrary 7-point scale in which: 0, 0%; 1, 0–2%; 2, 2–5%; 3, 5–10%; 4, 10–25%; 5, 25–50%; 6, 50–75%; and 7, 75–100% of mould area (Araújo, 1995, with modifications).

Data were recorded until petals were completely damaged (point 7 on the scale). Petals were considered unsaleable when they reached level 5 on the scale.

Data were statistically analysed through SPSS software Inc. (Chicago, IL, USA) and analysis of variance (ANOVA) was established through the SNK Test ($P<0.05$).

Results

The effectiveness of 1-MCP in restricting the severity of *B. cinerea* infection on cut stems of different ornamental flowers was investigated. Disease severity was therefore evaluated first on petals from untreated cut flowers, and then 1-MCP treatments were compared at different concentrations.

The highest dose of 1-MCP (3.62 μL L^{-1}) reduced *B. cinerea* damage on *D. caryophyllus* 'Idra di Muraglia' petals (Fig. 1). Until day 4, petals collected from 1-MCP treated cut stems of this flower showed no statistical differences compared to the control kept in tap water, and petal health remained stationary at level 0. 1-MCP treatment significantly slowed *B. cinerea* damage, which first became visible on day 6. Up to day 11, petal quality remained stationary; the threshold level 5 was reached on day 12. Petals picked from the stem in tap water, on the other hand, reached the threshold level after day 9.

Neither of the lower concentrations of 1-MCP produced results statistically different from the control (data not shown). A level of 7 (completely damaged) was registered 18 days after the appearance of the petals.

The *Cyclamen persicum* line 'Halios Bianco Puro Compatto' also displayed the best results with the highest dose of 1-MCP (3.62 μL L^{-1}) (Fig. 2). Petals of this species remained in aesthetically good condition (level 5) until day 53, while untreated petals already attained the threshold level on day 28. Level 7 was reached on day 56 and day 44 for treated and untreated petals respectively.

However, with this line the lowest concentration of 1-MCP did not statistically deter *B. cinerea* petal infection when compared to inoculated petals from the stems and kept in tap water (data not shown).

The lowest dose of 1-MCP (1.14 μL L^{-1}) improved control of *B. cinerea* in the two cultivars of *R.* × *hybrida* 'Ritz' and 'White Dew', as compared to the non-treated specimens (Fig. 3). In 'Ritz', *B. cinerea* infection was contained until day 9 (level 5) on the treated petals, 3 days later than on the control petals. Treated petals were totally damaged on day 12, 2 days later than on the control petals. In 'White Dew', the threshold damage level 5 was reached on day 15, and complete

damage on day 18, on both 1-MCP treated petals and control petals. In this case 1-MCP treated petals did not differ significantly from untreated petals.

Both treated (1.14 μL L^{-1}) and control petals of *R. asiaticus* 'Green' reached the threshold level 5 on day 19. Not until day 32 was the surface of treated petals completely damaged (level 7); 10 days later than the control (day 22).

In 'Saigon', both control petals and 1-MCP treated petals (1.14 μL L^{-1}) showed extensive infections on

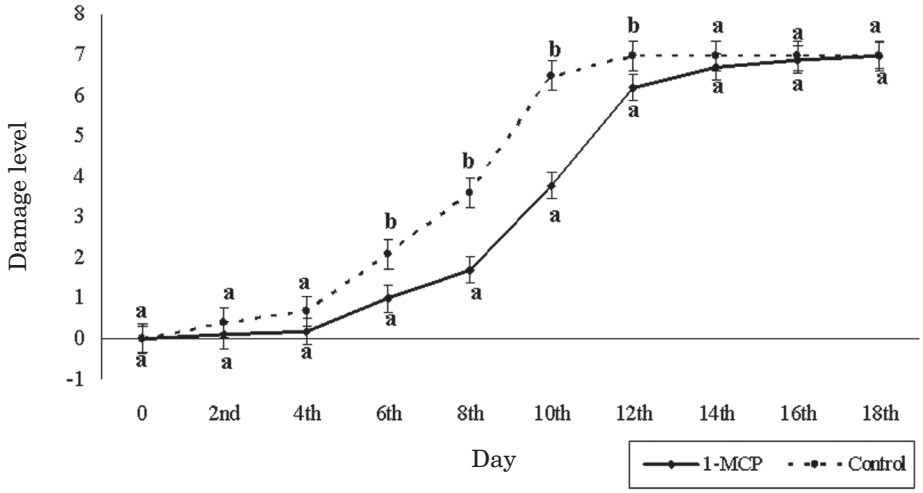

Fig. 1. Development of *Botrytis cinerea* infection on petals of *Dianthus caryophyllus* 'Idra di Muraglia', treated or not treated with 1-MCP (dosage 3.62 μL L^{-1}), level of damage. Data points show treatment means with standard errors. Mean values showing the same letter are not statistically different at $P \leq 0.05$ according to the SNK test.

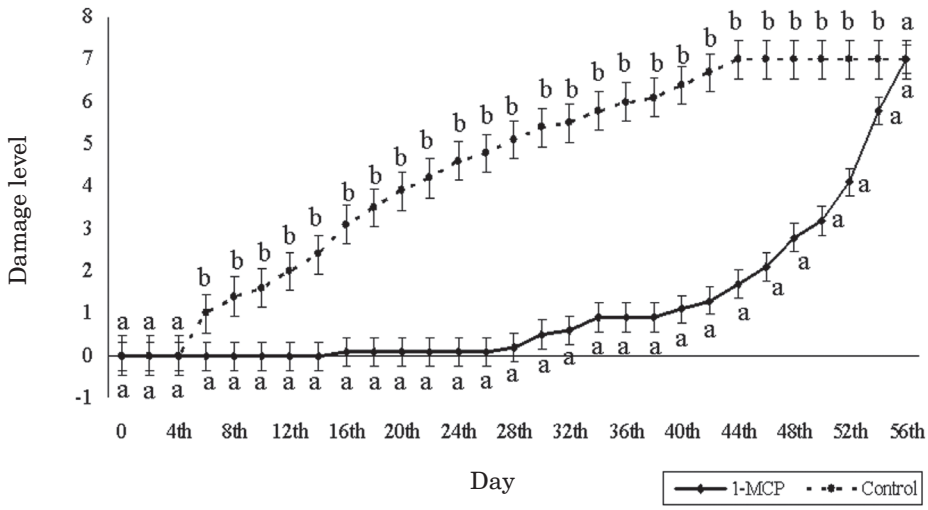

Fig. 2. The two lines with the error bands represent the development of *Botrytis cinerea* infection on petals of *Cyclamen persicum* 'Halios Bianco Puro Compatto', treated or not treated with 1-MCP (dosage 3.62 μL L^{-1}). Data points show treatment means with standard errors. Mean values showing the same letter are not statistically different at $P \leq 0.05$ according to the SNK test.

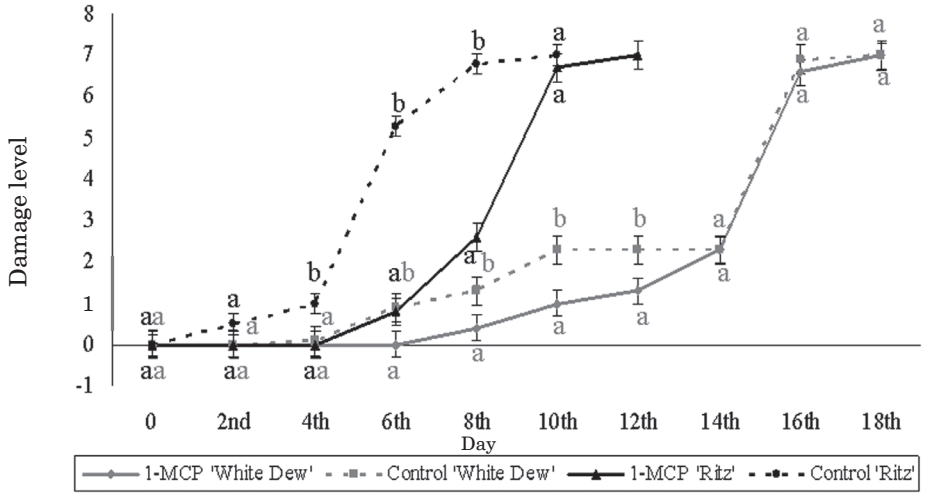

Fig. 3. Development of *Botrytis cinerea* infection on petals of *Rosa* × *hybrida* 'Ritz' and 'White Dew', treated or not treated with 1-MCP (dosage 1.14 μL L^{-1}). Data points show treatment means with standard errors. Mean values showing the same letter are not statistically different at $P \leq 0.05$ according to the SNK test.

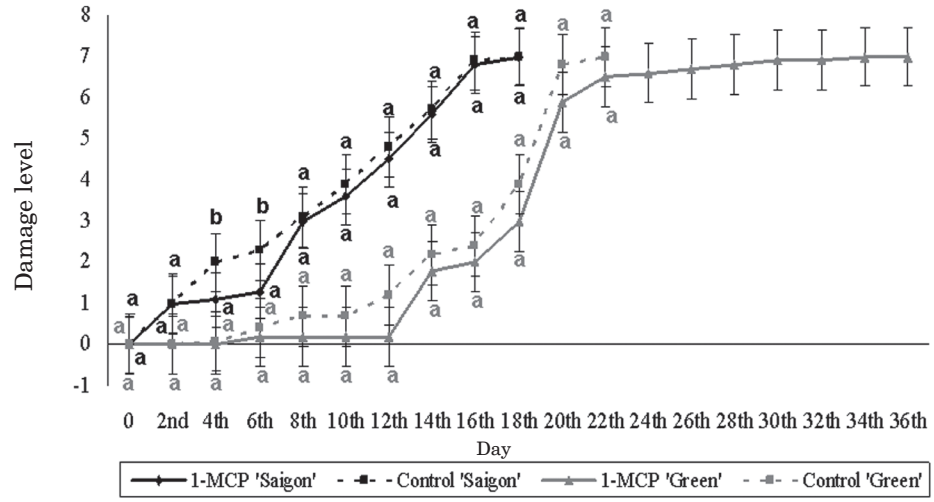

Fig. 4. Lines with error bands represent the comparison between the development of *Botrytis cinerea* infection on petals of *Ranunculus asiaticus* 'Green' and 'Saigon', treated or not treated with 1-MCP (dosage 1.14 μL L^{-1}). Data points show treatment means with standard errors. Mean values showing the same letter are not statistically different at $P \leq 0.05$ according to the SNK test.

their surface (level 5) on day 13, reaching the complete damage threshold on day 18 (Fig. 4). Only during the first days was the reduction of *B. cinerea* infection statistically significant (day 4–6) using 1-MCP treatment when compared with the control.

In both cultivars, even the highest dosage of 1-MCP yielded no improvement in petal health (data not shown).

Discussion

1-methylcyclopropene is known to be effective as an anti-ethylene substance in lengthening the life of cut flowers, with or without the presence of *B. cinerea* (Serek *et al.*, 2006).

The present study found that the species tested reacted differently to treatment with 1-MCP slowing *B. cinerea* damage.

On *R.* × *hybrida* 'Ritz', 1-MCP provided very good protection, preventing grey mould even at low concentrations. This could be linked to an effective response by the flower species to the treatment and to the tendency of this species to adsorb anti-ethylene substances (Chamoni *et al.*, 2005).

However, the capacity of 1-MCP to slow down pathogen development increased when it was applied at higher doses, as shown by *D. caryophyllus* 'Idra di Muraglia' and *C. persicum* 'Halios Bianco Puro Compatto'. In these species, low doses of 1-MCP did not reduce *B. cinerea* damage effectively, but at the higher concentrations the positive effects were evident. Moreover, high doses of 1-MCP showed no toxic effects, corroborating Porat *et al.* (1995).

These findings are important since both carnation and cyclamen are extremely susceptible to fungal diseases, and new methods of biological and chemical control are essential. Petals of *C. persicum* 'Halios Bianco Puro Compatto', treated with 1-MCP, were highly resistant to *B. cinerea*, thanks to the effectiveness of this anti-ethylene substance. After almost two months, the petals of this species appeared healthy except for a few slight blemishes (level 2 or 3 on the damage scale). At present, *C. persicum* is not widely produced as a cut flower, but is mostly sold as a potted plant. The effectiveness of 1-MCP is an interesting finding that may increase the marketability of this species, which has great ornamental potential (Halevy and Mayak, 1984).

Treatment with 1-MCP was not effective on either *R. asiaticus* cultivar. The cultivar 'Green' showed no difference between the control and the treated petals, suggesting that this cultivar has an innate resistance. Araújo *et al.* (2005), found that the rose petals of 'Green' were more sensitive to *B. cinerea* than the leaves and suggested that this greater resistance was due to the particular morphology, since the petals are similar in shape and colour to the leaves. These authors also found that *B. cinerea* does not survive as long on the stems and leaves as on the petals.

By contrast the cultivar 'Saigon' showed extensive damage and petals lasted only a very short time. On this cultivar, 1-MCP did not significantly affect *B. cinerea* resistance. Lastly, botrytical perfusion treatments did not cause immediate damage to the 'Saigon' petals.

In general, these findings contradict earlier studies reporting that ethylene enhances resistance to *B. cinerea* and that anti-ethylene substances are ineffective against *B. cinerea* mould. Treatment with 1-MCP, a strong, irreversible ethylene perception inhibitor significantly increased *B. cinerea* mould of disease in different tomato cultivars (Díaz *et al.*, 2002). The unexpected findings here reported on flowers are probably related to the lowering of the petal defence mechanisms against *B. cinerea* during petal senescence, and the hastening effect that ethylene has on petal senescence.

The study suggests that the practical use of 1-MCP to prevent *B. cinerea* infection is a very promising possibility. The research considered healthy plant material first since it was assumed that flowers in import and export situations do not normally suffer from infections. However, during the practical processes of handling, transporting, and retailing, *B. cinerea* conidia may develop on flower petals before they can be gassed with 1-MCP. It will be interesting to carry out further investigations under such circumstances.

Measures to control grey mould on petals must be socially and environmentally acceptable as well as effective. Combinations of such measures must be as effective as traditional fungicides. The future of chemical control has become a topic of particular interest to many growers who have witnessed the failure of grey mould control in several ornamental plants due to resistance problems, consumers who have concerns over possible effects of toxic residues on human health, and governmental authorities who impose environmental restrictions on the use of agro-chemicals (Droby and Lichter, 2004).

In conclusion, the study demonstrates that 1-MCP applications may be an effective substitute for many chemical treatments by inhibiting the deleterious effects of *B. cinerea* on the petals of some cut flower species. 1-MCP has many practical advantages over other chemical treatments.

In recent years, the number of chemical pesticides approved for use on ornamentals has strongly

decreased (Gullino and Garibaldi, 2005). Moreover, even when pesticides are available, they are often only partially effective, because the plants have developed resistance to them (Gullino and Garibaldi, 2007). Since 1-MCP is non-toxic, odourless, and efficient at low doses, it can also be used to protect a wide variety of plant life, including cut flowers, potted plants, and other horticultural commodities, against ethylene-driven senescence and, at the same time, provide protection from pathogens.

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