

Use of Methyl Jasmonate for Suppression of Botrytis Rot in Various Cultivars of Cut Rose Flowers

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Abstract

Gray mold, caused by *Botrytis cinerea* on flower petals, is a common disease of greenhouse roses (*Rosa hybrida* L.). The disease develops rapidly after harvest and causes a significant reduction of cut flowers value. Methyl jasmonate (MJ), known to induce plant defense responses, was examined for postharvest control of this disease in various cultivars of cut roses. We have previously demonstrated (Meir et al., 1998, Postharvest Biol. Technol. 13: 235) that under laboratory conditions, application of MJ to cut roses by either pulsing (24 h at 20°C) or spraying, provided six rose cultivars with a systemic or local protection against *B. cinerea*, respectively. Based on these results, a practical application of MJ consisting of simultaneous pulsing and spraying, was developed for growers handling conditions. The treatment included MJ pulsing for 4 h at 20°C and MJ spraying, followed by continuation of the pulsing for additional 20 h at 6°C. The flowers were then packed and incubated for two days at 6°C for air transport simulation, transferred subsequently to water cylinders placed at 20°C, and artificially inoculated with *B. cinerea* spore suspension. Experiments performed under these conditions with four rose cultivars ('Frisco', 'Jazz', 'Mercedes' and 'Red Charm') showed that the optimal treatment was pulsing with 350 µM MJ and spraying with 500 µM MJ. This combined MJ treatment neither increased ethylene production in petals, nor it was phytotoxic. The MJ treatment effectively suppressed gray mold development following both natural and artificial infection in seven additional rose cultivars ('Eskimo', 'Profita', 'Tamara', 'Sun Beam', 'Pink Tango', 'Carmen', 'Golden Gate'). In yellow, orange and pink cultivars, the MJ treatment improved petal color by inhibiting color fading during vase life, as assayed visually and by color measurements. Collectively, our findings suggest a possible commercial application of MJ as a useful and environmentally friendly means for suppressing Botrytis rot in cut roses.

INTRODUCTION

Botrytis rot, caused by the ubiquitous pathogen *Botrytis cinerea* Pers.:Fr., is a widespread disease of greenhouse roses and many other flower crops (Coyier, 1985). Since flower petals infected with *B. cinerea* significantly reduce the ornamental value of cut roses, susceptibility to this pathogen is an important factor in determining vase life. Initially, the symptoms appear on infected petals as restricted lesions (Elad, 1988). Subsequently, these lesions become necrotic and spread to the whole petals and the receptacle, finally resulting in collapse of the flower head and petal drop (Elad, 1988). The problem is aggravated by latency of the petal infection, which may not present visible symptoms at the time of flower harvest, but would become apparent under humid (RH above 93%) and high-temperature (18-25°C) conditions prevailing during storage and transport (Elad, 1988). Various cut rose cultivars show different susceptibility to Botrytis (Hammer and Evensen, 1994), and various methods were developed for determining the relative susceptibility of the cultivars to the disease, based on various inoculation treatments (Hazendonk et al., 1995). Currently, chemical fungicides are being used either as pre-harvest sprays in the greenhouse or as postharvest dips of rose flowers to prevent disease development. However, the efficacy of this control strategy is limited, since latent infections are not controlled efficiently by the various fungicide treatments (Elad, 1988;

Elad et al., 1992).

The natural growth regulator jasmonic acid and its derivative methyl jasmonate (MJ) are postulated to induce plant defense responses (Creelman and Mullet, 1995; Reymond and Farmer, 1998), and to increase shelf life of various commodities (Buta and Moline, 1998; Droby et al., 1999). We have previously demonstrated (Meir et al., 1998) that pulsing of cut roses with 200 μM MJ provides systemic protection against Botrytis rot by inducing resistance mechanisms in the treated cut roses (6 cultivars) without impairing flower quality. Also, a direct antifungal effect of 100-400 μM MJ on spore germination and germ-tube elongation of *B. cinerea* was obtained in vitro, with complete inhibition at 400 μM MJ (Meir et al., 1998). These results suggest that a combined treatment of spraying and pulsing with MJ should be very efficient in controlling Botrytis infection in cut roses. Preliminary experiments examining this treatment with 2 rose cultivars ('Frisco' and 'Red Charm') gave excellent results (data not shown).

It should be noted that all the experiments described above were performed under laboratory conditions consisting of pulsing the flowers with MJ in the presence of 0.15 mM silver thiosulfate (STS) and 8-hydroxyquinoline citrate (HQC) for 24 h at 20°C (Meir et al., 1998). However, the commercial conditions of the Israeli rose growers are different, and include pulsing of the cut roses for 4 h at 20°C in the presence of 50 $\mu\text{l l}^{-1}$ chlorine and without STS, following by additional pulsing for 20 h at 6°C.

The aim of the present research was to develop a practical solution for control of the Botrytis infection in various cultivars of cut rose flowers, by application of MJ under commercial conditions used by the growers.

MATERIALS AND METHODS

Rose cultivars were obtained from commercial growers in Israel, brought to the laboratory on the day of harvest and treated as previously described (Meir et al., 1998) with slight modifications. STS and HQC were omitted from the pulsing solution, which contained TOG-6 (Milchan Bros. Ltd., Israel) composed of active chlorine (5 mg ml^{-1}) complexed as sodium dichloroisocyanurate. MJ was added to this pulsing solution as the product TOG-MJ-1 (Milchan Bros. Ltd., Israel), which consists of a 2% MJ (Aldrich Chemical Company Inc., USA) solution formulated with surfactants. This formulation enabled MJ application as a water-based solution. Pulsing in various MJ concentrations ranging between 200 and 600 μM was performed for 4 h at 20°C followed by an additional 20 h at 6°C. Spraying of flowers with various MJ concentrations ranging between 200 and 600 μM (prepared in water) was performed immediately following pulsing and the flowers were allowed to dry before their transfer to 6°C. The flowers were then packed and incubated for two days at 6°C for air transport simulation, transferred subsequently to water cylinders placed at 20°C, and artificially inoculated with *B. cinerea* spore suspension. All other procedures, including vase life conditions, natural and artificial inoculation with Botrytis, preparation of spore suspension, disease assessment and statistical analyses were performed as outlined previously (Meir et al., 1998). The decay index was based on visual rating, ranging between a relative scale of 0-5, defined as follows: 0 = no symptoms; 1 = appearance of one to four lesions on the petals; 2 = appearance of more than four lesions; 3 = appearance of fully necrotic petals; 4 = necrosis of the petals and receptacle; 5 = collapse of flower head and petal drop.

Color of 'Frisco' rose petals was assessed with a hand-held tristimulus reflectance colorimeter (Minolta CR-200), based on the CIE- $L^* a^* b^*$ uniform color space values (Clydesdale, 1987). Total color difference function, ΔE based on CIE- Lab values at day 0 was calculated as previously described (Meir et al., 1992). Color measurements were recorded on the outer flower petals no. 3-6 sampled from 4 different flowers in each treatment (total of 16 measurements per treatment at every sampling day).

RESULTS AND DISCUSSION

In the present study we have modified the MJ pulsing conditions as compared to our previous report (Meir et al., 1998), to simulate the commercial conditions used by the

Israeli growers. Additionally, we have used a combined treatment of MJ pulsing and spraying. Therefore, we have assumed that the optimum MJ concentration required for pulsing would be different. To calibrate the MJ pulsing concentrations required for the commercial conditions, we have pulsed the flowers with various MJ concentrations ranging between 0-500 μM , and sprayed them with a constant MJ concentration of 300 μM . Representative results of this kind of experiments are presented in Fig. 1 with 'Mercedes' and 'Red charm' cut roses. The data show that both MJ concentrations of 350 and 500 μM , combined with MJ spraying, significantly reduced Botrytis infection after both natural (Fig. 1A, 1C) and artificial (Fig. 1B, 1D) inoculation, as compared with control only sprayed flowers. In most experiments, increasing MJ concentration above 350 μM in the pulsing solution did not improve MJ effect in reducing the Botrytis decay. Based on these results we have concluded that the optimal MJ pulsing concentration combined with MJ spraying under these conditions is 350 μM .

The second set of experiments was performed in the reciprocal way, namely pulsing the flowers in a constant concentration of 350 μM MJ and spraying them with various MJ concentrations ranging between 0-500 μM MJ. Representative results of this kind of experiments are presented in Fig. 2 with 'Frisco' and 'Jazz' roses. The data show that the optimal MJ spray concentration under these conditions was 500 μM (Fig. 2A, 2B, 2C), except for 'Jazz' roses after artificial inoculation (Fig. 2D).

According to the results presented in Figs. 1 and 2 we have concluded that the standard MJ application treatment for most rose cultivars is spraying with 500 μM MJ and pulsing with 350 μM MJ. The combined MJ application was very effective in reducing the decay development in four rose cultivars following artificial infection (Fig. 3), and in eight cultivars following natural (Fig. 4A) and artificial infection (Fig. 4B). The only exceptions from this pattern were found in 'Carmen' roses after natural infection (Fig. 4A) and in 'Golden Gate' roses after artificial infection (Fig. 4B). The effect of the combined MJ application on the appearance of three rose cultivars following artificial infection with Botrytis after 7 days of vase life is presented in Fig. 5. While control untreated flowers were severely infected with Botrytis, the MJ-treated flowers were barely affected.

No visible injuries or damage were observed in the MJ-treated rose flower petals, leaves or stems, but this does not exclude the occurrence of possible invisible physiological damage. It is well known that stressed tissues are characterized with increased ethylene production rates (Abeles, 1973), and MJ was reported to increase ethylene production rates in petunia flowers (Porat et al., 1993). This suggests that MJ may increase ethylene production rates, which could therefore serve as a physiological marker for possible invisible stress or damage. Our results show that MJ treatment had no effect on ethylene production rates in petals of 'Frisco' cut rose flowers during seven days of vase life (data not shown). It may therefore be concluded that MJ treatment does not cause any deleterious effect in cut roses, neither visual nor physiological.

Apart of increasing tissue resistance to Botrytis, MJ had a beneficial effect in several pink and yellow rose cultivars, such as 'Europa', 'Golden Gate' and 'Frisco'. In these cultivars we have observed an improvement in petal color during vase life and delay in their typical color fading following MJ treatment. Color fading can be assessed by the total color difference function (ΔE) calculated according to the CIE-*Lab* color space values of petals measured at day 0 (Clydesdale, 1978). Higher ΔE values during vase life are correlated with increased color change, which denote increased color fading. A delay of six days in color fading followed by a moderate increase in the fading rate could be observed in the MJ-treated 'Frisco' flowers, as expressed by reduced ΔE values (Fig. 6A). The MJ treatment also prevented for six or eight days of vase life the decrease of CIE-*Lab* b^* value, which reflects the intensity of the yellow color (Fig. 6B). The effect of MJ treatment on delaying petal color fading could be related to its effect on increasing anthocyanin production and/or delaying anthocyanin breakdown. MJ has already been reported to stimulate anthocyanin accumulation in different plant systems (Franceschi and Grimes 1991; Feys et al., 1994; Saniewski et al., 1998), including flower petals (Tamari et al., 1995).

Taken together, our results suggest that MJ can be commercially applied as a useful and environmentally friendly means for suppressing Botrytis rot in cut roses without impairing flower quality. In some rose cultivars MJ application even improved flower color. The most efficient treatment of MJ included a combined application of pulsing (350 μ M) and spraying (500 μ M), which was effective in all rose cultivars examined in the present study. This suggests that this promising treatment can be used as a standard treatment which should be commercialized.

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Figures

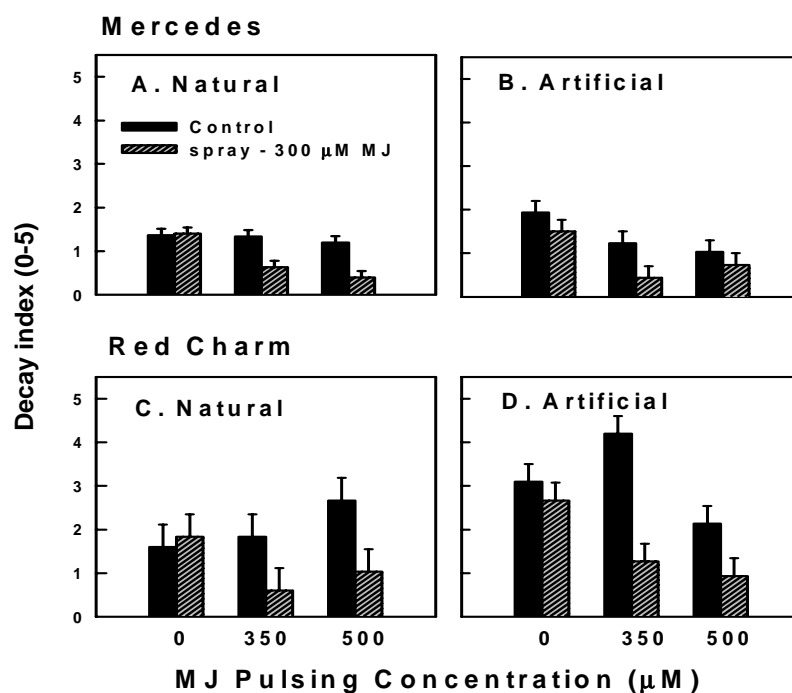


Fig. 1. Effect of combined MJ application by various pulsing concentrations (350 or 600 μM) and spraying with 300 μM , on decay index after 6 days of vase life in cut 'Mercedes' (A, B) and 'Red Charm' (C, D) roses, following natural (A, C) or artificial (B, D) inoculation with *Botrytis*. Means \pm S.E. of 20 replicates are presented.

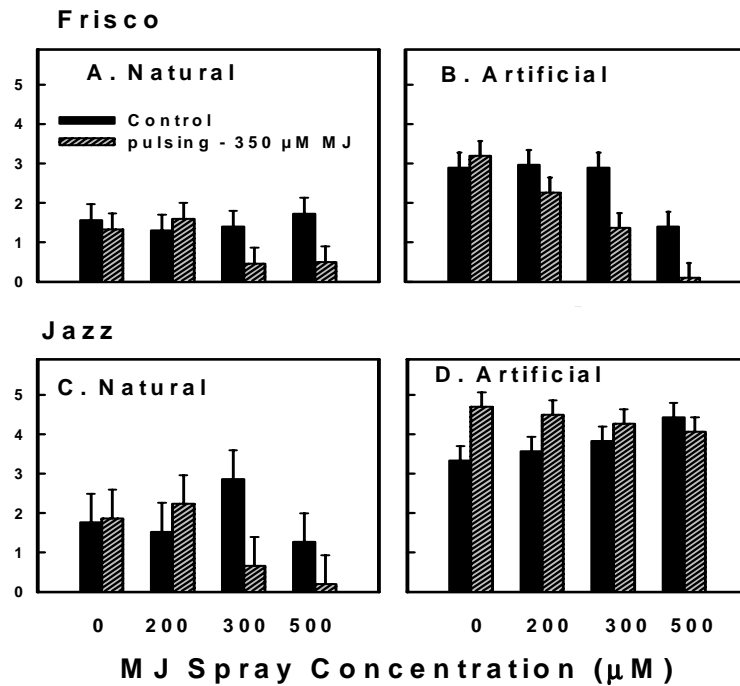


Fig. 2. Effect of combined MJ application by various spraying concentrations (200-500 μM) and pulsing with 350 μM , on decay index after 6 days of vase life in cut 'Frisco' (A, B) and 'Jazz' (C, D) roses, following natural (A, C) or artificial (B, D) inoculation with *Botrytis*. Means \pm S.E. of 20 replicates are presented.

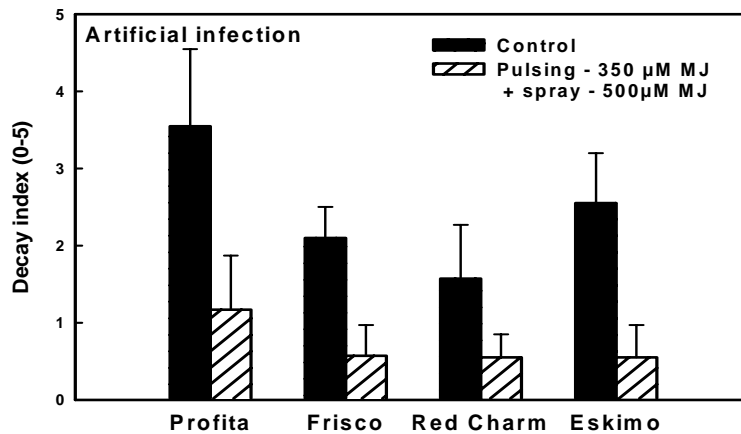


Fig. 3. Effect of combined MJ application by pulsing (350 μM) and spraying (500 μM), following artificial inoculation with *Botrytis* on decay index in various cut rose cultivars after 3 days in vase. Means \pm S.E. of 20 replicates are presented.

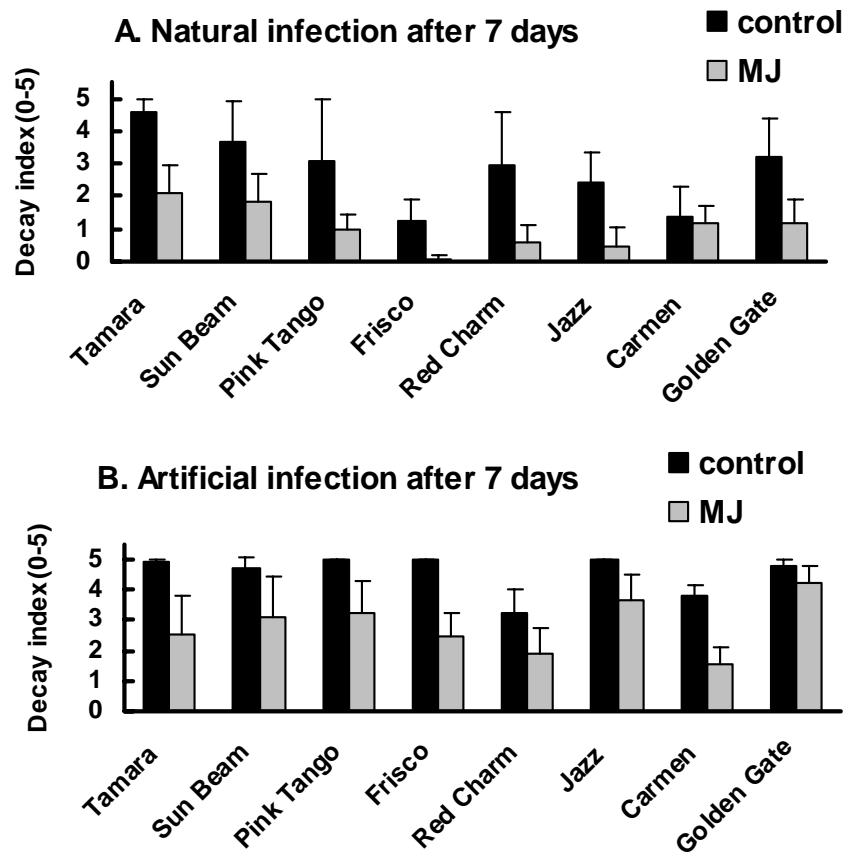


Fig. 4. Effect of combined MJ application by pulsing (350 μ M) and spraying (500 μ M) following natural (A) or artificial (B) inoculation with *Botrytis*, on decay index in various cut rose cultivars after 7 days in vase. Means \pm S.E. of 20 replicates are presented.



Fig. 5. Effect of combined MJ application by pulsing (350 μ M) and spraying (500 μ M), following artificial infection with *Botrytis*, on appearance of 'Sun Beam' (A), 'Tamara' (B) and 'Frisco' (C) cut roses after 7 days in vase.

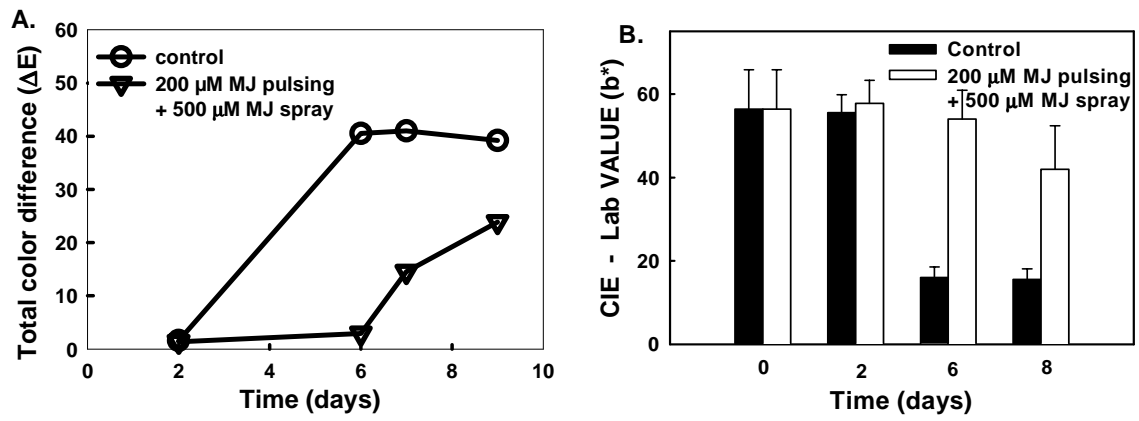


Fig. 6. Effect of combined MJ application by pulsing (200 μM) and spraying (500 μM) on changes in total color difference function - ΔE (A) and CIE- b^* value (B) in petals of 'Frisco' cut roses during vase life. Means \pm S.E. of 20 replicates are presented.