Postharvest Control of *Botrytis cinerea* on Cut Roses with Picro-cupric-ammonium Formate

P. E. HAMMER, Department of Vegetable Crops, and J. J. MAROIS, Department of Plant Pathology, University of California, Davis 95616

ABSTRACT

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A new copper-based fungicide, a soluble tannate complex of picro-cupric-ammonium formate (PCAF), was evaluated for postharvest control of infections of *Botrytis cinerea* on rose (*Rosa hybrida*) flowers. Dip treatments of PCAF significantly reduced disease severity on cut rose cultivars Sonia, Royalty, and Gold Rush during storage at 2.5 C and promoted poststorage fresh weight gain (an index of flower quality). Treatment with 1,000 mg/L of PCAF reduced disease severity to 3% of that on control flowers. PCAF was phytotoxic at 4,000 mg/L. Disease control (depending on the cultivar) was comparable to or greater than that achieved with 1,800 mg/L of vinclozolin. Vinclozolin formulations left visible residue on the flowers, but PCAF did not.

Botrytis cinerea Pers. is a serious pathogen of roses and other cut flower crops. The infections often cannot be detected at harvest but develop rapidly under the moist conditions encountered during storage and transit, even at temperatures as low as 0 C (5). Such infections cause major postharvest losses and are considered a limiting factor in the storage and shipment of cut flowers (2,3,10).

Many rose growers dip cut flowers in fungicide solutions to prevent postharvest development of *B. cinerea*. Several materials are registered for this application, but most leave unsightly residues on the flowers and foliage, even after removal of outer petals (10). Although McCain and Welch (10) stated that treatment with vinclozolin left minimal residue, commercial growers and shippers say that customers find even this amount of residue objectionable (W. Walker, *personal communication*).

In preliminary screenings, a new copper-based fungicide, a soluble tannate complex of picro-cupric-ammonium formate (PCAF) (Phyton-27, Source Technology Biologicals, Minneapolis, MN), was effective against *B. cinerea* and left no visible residue. PCAF is described by the manufacturer as a copper-based, solubilized, systemic fungicide-bactericide and is registered for vascular injection to control Dutch elm disease. This report describes research undertaken to determine the usefulness of PCAF for postharvest dip application to control *B. cinerea* on cut rose flowers.

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MATERIALS AND METHODS

B. cinerea was isolated from infected roses found in commercial growers' cold-storage facilities. Three isolates were selected and grown on potato-dextrose agar in darkness at room temperature. Conidia were washed from 9- to 12-day-old cultures of each isolate, combined, and diluted to approximately 1,000 conidia per milliliter. Flowers were inoculated by spraying to the point of incipient runoff using a Chromist spray unit (Gelman Sciences, Ann Arbor, MI). Noninoculated controls, sprayed with deionized water, were included to monitor background disease levels (those infections not resulting from laboratory inoculation).

Three cultivars of cut roses (*Rosa hybrida* L.)—Royalty, Sonia, and Gold Rush—were obtained from a commercial grower. The flowers were harvested at the normal growth stage, when sepals begin to split and petals become visible. The stems were recut 30 cm below the receptacles, and all except the distal two or three leaves were removed. Each experimental unit consisted of three flowers in a 500-ml bottle containing 200 ml of a preservative solution composed of 10 mg of glucose, 0.33 mg of $CaCl_2$, 0.72 mg of $MgSO_4$, 60 mg of 8-hydroxyquinoline citrate, and 4 mg of sodium hypochlorite per liter of deionized water. Sufficient citric acid (about 100 mg) was added to reduce the pH to 3.50.

Fungicides were diluted in deionized water, and all treatment solutions also contained 0.5 ml/L of Tween 20. Flower buds were immersed in the solutions for 3–4 sec, then gently shaken to remove the excess. The upper leaves of each stem were wetted to check for possible foliar phytotoxicity. After treatment, the flowers were allowed to dry, inoculated as described above, and placed in humidified storage chambers (relative humidity ranged from 95 to 100%) at 2.5 ± 1 C. Condensation was present on the petals throughout the storage period.

The flowers were removed from storage 7 days after inoculation, and disease severity was quantified as the number of lesions on each flower. Subsequently, vase life was evaluated for 10 days at 21 ± 1 C. Irradiance from fluorescent lamps was continuous at 5.5 ± 1 $\mu E \cdot m^2 \cdot sec$. Flower fresh weight (a quantitative index of cut flower quality) and visual observations were recorded daily. These are standard measures of flower quality (1,7,8,13).

Sonia flowers were used in a preliminary experiment to determine the relationship between PCAF concentration and disease control. The experiment was a six concentration by two inoculation complete factorial in a completely randomized design with two replicates of each treatment and three flowers per replicate.

To test further for efficacy and phytotoxicity, flowers were treated with 250–4,000 mg/L of PCAF and inoculated with *B. cinerea*. This experiment was a three cultivar by six concentration complete factorial with noninoculated controls included to monitor the background infection levels. There were two replicates of each treatment with three flowers per replicate. The experiment was repeated, and the data were pooled using time as the blocking factor in a randomized complete block design.

Comparison of PCAF with vinclozolin. Treatments of 250 or 1,000 mg/L of PCAF were compared with 900 or 1,800 mg a.i./L of vinclozolin (Ronilan), the minimum and maximum label rates for postharvest dip application. Inoculated and noninoculated controls received no dip treatment. There were two replicates of each treatment with three flowers per replicate. The experiment was repeated, and the data were pooled using time as the blocking factor in a randomized complete block design.

RESULTS

Concentrations of 62.5–1,000 mg/L of PCAF reduced the severity of infections of *B. cinerea* on Sonia flowers (Fig. 1). Thirty-five to 50 lesions developed on inoculated controls, compared with zero to eight lesions on inoculated flowers treated with 1,000 mg/L of PCAF, for an average disease reduction of 92% at that

rate. No phytotoxicity symptoms or adverse effects on fresh weight gain or vase life were observed.

Of the three cultivars used in subsequent tests, Sonia was the most susceptible to infection by *B. cinerea*, followed by Royalty, then Gold Rush. There was a significant interaction between concentration and cultivar ($P = 0.0001$) (Fig. 2). Dip treatments of 250–4,000 mg/L of PCAF reduced disease severity on all three cultivars. Approximately 97% control was achieved at 1,000 mg/L, and higher concentrations increased disease control only slightly (Fig. 2). Because of the low level of infection in the noninoculated flowers, none of the concentrations tested reduced disease severity significantly below that found on noninoculated control flowers.

Phytotoxicity symptoms were observed after storage on some Royalty and Sonia flowers treated with 4,000 mg/L of PCAF. The visible symptoms were bleached or blackened areas, 1–3 mm wide, along the margins of sepals and on petals of the more open flowers. These areas became necrotic within 1–2 days at 21 C. Slight necrosis of sepal margins also was noted at 2,000 mg/L, but there were no other visible phytotoxicity symptoms on the flowers or foliage at concentrations below 4,000 mg/L.

The peak fresh weights of inoculated flowers treated with 250–2,000 mg/L of PCAF were significantly higher than those of inoculated control flowers but were not significantly different from those of noninoculated controls (Fig. 3). The peak fresh weights of flowers treated with 4,000 mg/L were not significantly different from those of flowers in either control group (Fig. 3).

Comparison of PCAF with vinclozolin. Postharvest dip treatment with vinclozolin left a visible wettable powder residue on the flower buds, but no residue was visible on PCAF-treated flowers (Fig. 4). Both vinclozolin and PCAF significantly reduced disease severity. In preliminary experiments using Royalty and Gold Rush, disease severity on inoculated

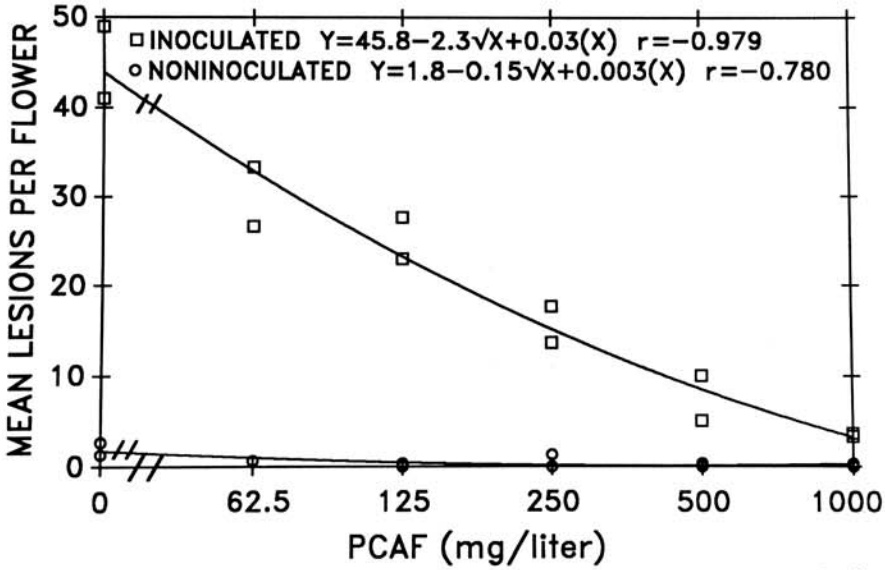

Fig. 1. Control of *Botrytis cinerea* infections of inoculated and noninoculated rose cv. Sonia flowers by picro-cupric-ammonium formate (PCAF) dip treatment. Each point represents the mean from three flowers. The main effects of inoculation and PCAF concentration and the inoculation by concentration interaction were highly significant ($P = 0.0001$).

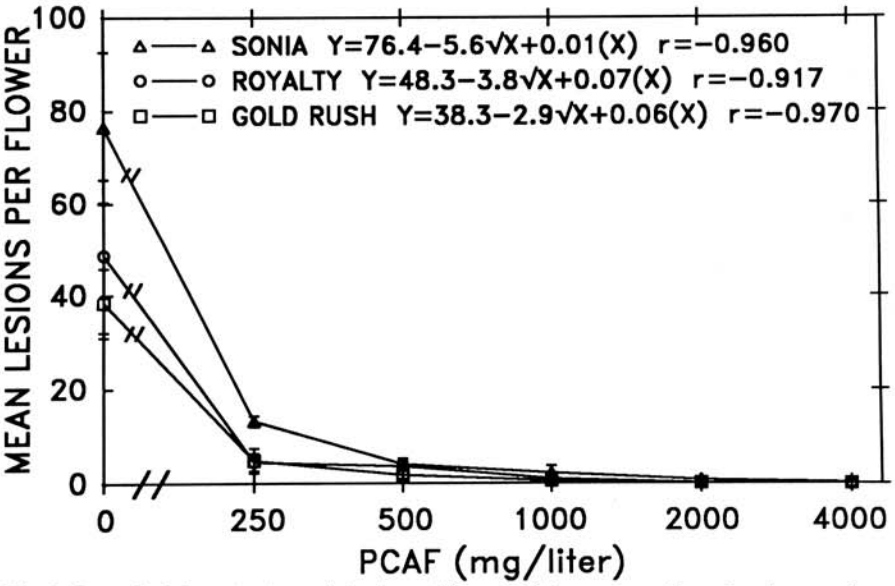

Fig. 2. Control of *Botrytis cinerea* infections of flowers of three rose cultivars by picro-cupric-ammonium formate (PCAF) dip treatment followed by inoculation with the pathogen. The main effects of cultivar and PCAF concentration and the cultivar by concentration interaction were highly significant ($P = 0.0001$). The regression equations are for the range of 0–1,000 mg/L only. Error bars are ± SD. Average background infection levels were 3.3, 3.0, and 0.7 lesions per flower on Royalty, Sonia, and Gold Rush, respectively. None of the concentrations tested reduced disease severity significantly below that on noninoculated controls, according to two-sided Dunnett tests ($\alpha = 0.05$).

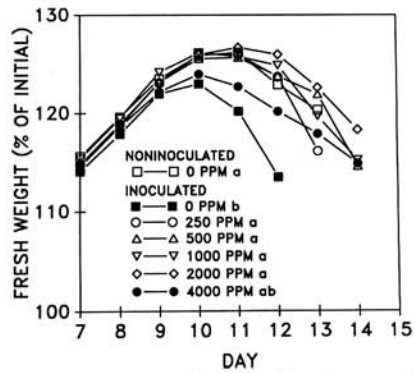

Fig. 3. Poststorage fresh weight changes of flowers treated with picro-cupric-ammonium formate (PCAF) followed by inoculation with *Botrytis cinerea* and storage for 7 days at 2.5 C. The main effect of treatment on peak fresh weight was significant ($P = 0.014$). Mean peak fresh weights of treatments followed by the letter "a" were not significantly different from that of the noninoculated control, and those followed by the letter "b" were not significantly different from that of the inoculated control, according to two-sided Dunnett tests ($\alpha = 0.05$).

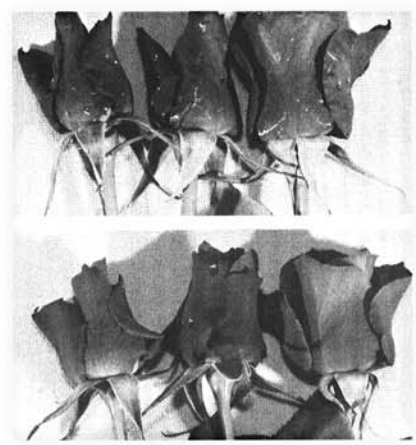

Fig. 4. Appearance of cv. Royalty roses after postharvest dip treatments with (top) vinclozolin 50WP at 1,800 mg/L or (bottom) picro-cupric-ammonium formate at 1,000 mg/L followed by storage for 7 days at 2.5 C.

flowers treated with 250–2,000 mg/L of PCAF or 1,800 mg/L of vinclozolin was significantly lower than that on non-inoculated controls (Table 1).

In subsequent experiments using all three cultivars, 1,000 mg/L of PCAF was the most effective treatment tested, but none of the treatments reduced disease severity significantly below the already low background levels (Table 2). These dip treatments also reduced the number of flowers that were discarded prematurely (before the end of the vase life evaluation period) because of maceration of the receptacles or petal abscission induced by *B. cinerea* infection (Table 2). Vinclozolin was substantially more effective than PCAF in reducing the percentage of flowers discarded.

Flowers treated with either vinclozolin or PCAF opened normally, and no petal color changes or other phytotoxicity symptoms were observed on petals or leaves. Flowers treated with either rate of either fungicide gained significantly more fresh weight (Fig. 5) and reached peak fresh weight significantly later (Table 2) than inoculated control flowers.

DISCUSSION

Postharvest dip treatment with PCAF significantly reduced the severity of infections by *B. cinerea* and helped maintain the quality of cut roses during storage. Preliminary experiments and those of other workers (12) indicated that treatments of vinclozolin and iprodione, fungicides commonly used for postharvest control of *B. cinerea* on cut roses, result in 70–80% disease reduction under laboratory conditions. Comparable or greater control was achieved with PCAF at 250–1,000 mg/L without leaving visible residue on the petals. Higher PCAF rates did not reduce disease severity further, and the disease pressure applied experimentally was high relative to naturally occurring disease levels observed in 1986 (typically five to 30 lesions per flower, depending on the cultivar and season) and to those reported elsewhere (10,12). Thus, in most commercial situations, 1,000 mg/L would be the highest PCAF rate necessary, and lower rates may provide satisfactory control.

None of the treatments reduced disease severity significantly below that observed on noninoculated control flowers when the background infection levels were low. When higher naturally occurring infection levels were encountered in preliminary experiments, both PCAF and vinclozolin reduced the disease severity significantly below the background level even though the flowers also were inoculated artificially. Thus, PCAF, like vinclozolin, should control the latent infections encountered commercially.

Flower fresh weight is an index of cut flower quality. Increased weight gain and longer time to peak fresh weight are correlated with longer vase life and other more subjective measures of quality (1,7,8,13). Inoculation with *B. cinerea* resulted in lower peak fresh weight, shorter time to peak fresh weight, and higher percentages of flowers discarded because of maceration of the receptacles before the end of the vase life evaluation period (Figs. 3 and 5, Table 2). The higher and later peak fresh weights (i.e., higher quality) of flowers treated with vinclozolin or PCAF are probably secondary effects of disease control. Vinclozolin had a more pronounced effect on weight gain than PCAF, even though more lesions developed on vinclozolin-treated flowers (Fig. 5, Table 1). Also, fewer vinclozolin-treated flowers succumbed to *B. cinerea*, indicating that vinclozolin had greater activity than PCAF against established infections.

A disease control measure would be of little value if it damaged or shortened the vase life of the flowers. Concentrations of PCAF required to control disease ranged from 250 to 1,000 mg/L and were not phytotoxic. Visible phytotoxicity caused by PCAF treatment was evident only at 4,000 mg/L, four times the estimated highest practical rate. Necrosis of the sepal margins at 2,000 mg/L was visible only upon close examination and probably would not lower the aesthetic value of the flowers. This visual evidence of safety to the flowers is corroborated by the fresh weight data. The peak fresh weights of flowers treated with 250–2,000 mg/L of PCAF or either rate of vinclozolin were not significantly different from (and in most cases higher than) the peak fresh weights of non-inoculated controls, indicating that the treatments did not damage the flowers physiologically.

Table 1. Control of *Botrytis cinerea* infections on experimentally inoculated cut roses in the presence of high natural infection levels by postharvest dip treatment with picro-cupric-ammonium formate (PCAF) or vinclozolin

Treatment	Mean lesions per flower[z] cv. Royalty	cv. Gold Rush	Av.
Noninoculated control	19.9 b	15.0 b	17.4
Inoculated Control	83.2 a	65.0 a	74.1
Vinclozolin, 900 ppm	18.7 bc	9.7 bc	14.2
Vinclozolin, 1,800 ppm	9.8 cd	4.0 cd	6.9
PCAF, 250 ppm	4.2 de	3.0 cd	3.6
PCAF, 2,000 ppm	0.1 e	0.3 d	0.2

[z] Values represent the means from two replicates of three flowers each. The main effects of treatment and cultivar and the treatment by cultivar interaction were highly significant ($P = 0.0001$). Within each column, means followed by the same letter are not significantly different according to Duncan's multiple range test ($\alpha = 0.05$).

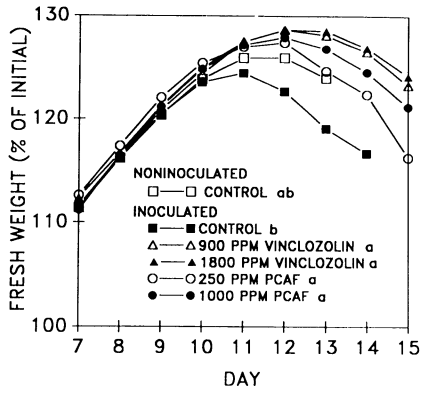

Fig. 5. Daily fresh weight changes of flowers treated with two rates of vinclozolin or picro-cupric-ammonium formate (PCAF) followed by inoculation with *Botrytis cinerea* and storage for 7 days at 2.5 C. The main effect of treatment on peak fresh weight was highly significant ($P = 0.0001$). Peak fresh weights of treatments followed by the same letter were not significantly different, according to Tukey's Studentized t test ($\alpha = 0.05$).

Table 2. Effects of dip application of picro-cupric-ammonium formate (PCAF) or vinclozolin, followed by inoculation with *Botrytis cinerea* conidia and storage for 7 days at 2.5 C, on disease severity and postharvest behavior of three cultivars of cut roses[w]

Treatment	Mean lesions per flower[x] cv. Royalty	cv. Sonia	cv. Gold Rush	Days to peak fresh weight[y]	Percent flowers discarded prematurely[z]
Noninoculated control	5.6 cd	3.2 c	4.0 bc	11.7 ab	36 b
Inoculated Control	51.4 a	72.6 a	41.3 a	11.0 a	82 a
Vinclozolin, 900 ppm	24.0 b	21.6 b	9.1 b	12.6 c	0
Vinclozolin, 1,800 ppm	12.8 c	12.3 c	3.8 bc	12.4 bc	0
PCAF, 250 ppm	5.4 cd	11.1 c	4.7 bc	12.1 bc	39 b
PCAF, 1,000 ppm	2.1 d	2.3 c	0.7 c	12.2 bc	19 b

[w] Natural infection levels were low.

[x] The main effects of treatment and cultivar and the treatment by cultivar interaction were highly significant ($P = 0.0001$). Mean separation within columns by Tukey's Studentized t test at the 5% level.

[y] The main effects of treatment and cultivar were highly significant ($P = 0.0001$), but there was no significant interaction ($P = 0.899$). Mean separation by Tukey's Studentized t test at the 5% level.

[z] Percentage of flowers discarded before the end of vase life evaluation because of *B. cinerea*-induced petal abscission or maceration of the receptacles.

Current postharvest *B. cinerea* control programs are hampered by unsightly residues (10) and by the threat of fungicide resistance (4,6,9,11). In contrast, residue was not visible after treatment with PCAF and copper-based fungicides have been used for over 100 yr to control numerous fungal diseases without the development of resistant pathogen populations.

This research indicates that postharvest dip applications of PCAF at 250–1,000 mg/L are effective for control of *B. cinerea* on cut rose flowers, are not phytotoxic, and do not leave the visible residues associated with other materials currently in use. Registration by the Environmental Protection Agency for the use of PCAF for postharvest control of *B. cinerea* on cut roses has been obtained, and further testing may demonstrate the utility of PCAF for controlling *B. cinerea* on other flower crops.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Brantley, R. K. 1975. A new postharvest chemical treatment for roses. HortScience 10:178-179.
2. Carre, D. D. 1984. The influence of atmospheric humidity and free water on germination and germ tube growth of *Botrytis cinerea* Pers. M.S. thesis. Oregon State University, Corvallis.
3. Cline, M. N., and Bardsley, C. E. 1984. Postharvest treatment of cut flowers for Botrytis blight. Proc. Br. Crop Prot. Conf. 3:1169-1173.
4. Dennis, C., and Davis, R. P. 1979. Tolerance of *Botrytis cinerea* to iprodione and vinclozolin. Plant Pathol. 28:131-133.
5. Jarvis, W. R. 1980. Epidemiology. Pages 219-250 in: The Biology of *Botrytis*. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, London. 318 pp.
6. Katan, T. 1982. Resistance to 3,5-dichlorophenyl-N-cyclic imide ('dicarboximide') fungicides in the grey mould pathogen *Botrytis cinerea* on protected crops. Plant Pathol. 31:133-141.
7. Marousky, F. J. 1969. Vascular blockage, water absorption, stomatal opening, and respiration of cut 'Better Times' roses treated with 8-hydroxyquinoline citrate and sucrose. J. Am. Soc. Hortic. Sci. 94:223-226.
8. Marousky, F. J. 1971. Inhibition of vascular blockage and increased moisture retention in cut roses induced by pH, 8-hydroxyquinoline citrate, and sucrose. J. Am. Soc. Hortic. Sci. 96:38-41.
9. Maude, R. B. 1980. Disease control. Pages 275-308 in: The Biology of *Botrytis*. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, London. 318 pp.
10. McCain, A., and Welch, K. E. 1982. Controlling gray mold of rose flowers. Page 6 in: Univ. Calif. Coop. Ext. Serv. Flower Nursery Rep. (Spring).
11. Northover, J., and Matteoni, J. A. 1986. Resistance of *Botrytis cinerea* to benomyl and iprodione in vineyards and greenhouses after exposure to the fungicides alone or mixed with captan. Plant Dis. 70:398-402.
12. Redmond, J. C., Marois, J. J., and MacDonald, J. D. 1987. Biological control of *Botrytis cinerea* on roses with epiphytic microorganisms. Plant Dis. 71:799-802.
13. Sacalis, J. N. 1974. Inhibition of vascular blockage and extension of vase life in cut roses with an ion exchange column. HortScience 9:149-151.