Postharvest Control of *Botrytis cinerea* on Cut Rose Flowers with Pyrrolnitrin

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ABSTRACT

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Pyrrolnitrin, an antibiotic isolated from *Pseudomonas cepacia,* was tested for postharvest control of *Botrytis cinerea* infections on cut Sonia and Royalty rose flowers. After pyrrolnitrin was applied as a bud dip, buds were inoculated with conidia of *B. cinerea* and stored for 7 days at 2 C. Dip treatments of 12–200 mg/L significantly reduced lesion development during storage at 2 C and promoted poststorage fresh weight gain (an index of cut flower quality). No phytotoxicity was observed on leaves or petals at concentrations of pyrrolnitrin up to 200 mg/L. Dip treatment with 100 mg/L reduced lesion development by about 90% compared to inoculated control flowers and prevented poststorage flower rot. This degree of disease control was comparable to that achieved with 1,800 mg of vinclozolin per liter (the maximum label rate).

Botrytis blossom blight, caused by *Botrytis cinerea* Pers.:Fr., is a widespread and destructive disease on greenhouse-grown roses and many other cut flower crops. *B. cinerea* infections often escape detection at the time of harvest but develop rapidly under moist conditions during storage and shipment. Currently, many rose growers use fungicide sprays or postharvest flower dips to prevent disease development. Several materials are registered for this purpose, but their effectiveness often is compromised by the development of fungicide-resistant pathogen populations. Resistance of *B. cinerea* to benomyl is widespread (16), and resistance to dicarboximides (e.g., vinclozolin and iprodione) has been documented in Europe (14,20,24), Israel (12,13), Australia (18), New Zealand (2), and the United States (17).

Janisiewicz and Roitman (10) identified a strain of *Pseudomonas cepacia* Burkh. that effectively controlled blue mold (caused by *Penicillium expansum* Link) and gray mold (caused by *B. cinerea*) infections on apple and pear fruit after harvest. They concluded that the principal mode of action of this biocontrol organism is antibiosis by production of pyrrolnitrin, an antibiotic originally isolated from *Pseudomonas pyrrocinia* Imanaka, Kousaka, Tamura, and Arima (1,9). Preharvest sprays of purified pyrrolnitrin applied to raspberries reduced postharvest gray mold caused by *B. cinerea* (5). Postharvest treatments of pyrrolnitrin have been used to delay rot of strawberry fruit caused by *B. cinerea* and *Rhizopus* spp. (23) and to control rots caused by *B. cinerea* and *Penicillium expansum* on apple and pear fruits (11). Our objective was to determine the usefulness of pyrrolnitrin for postharvest control of *B. cinerea* on cut rose flowers.

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MATERIALS AND METHODS

B. cinerea, isolated from infected rose petals found in a commercial greenhouse, was grown at 21 C with a 12-hr photoperiod under fluorescent lamps (photosynthetically active radiation [PAR] = 25 $\mu E \cdot m^{-2} \cdot s^{-1}$). The medium contained 1 g of K_2HPO_4, 0.5 g of $MgSO_4$, 0.5 g of KCl, 0.01 g of $FeSO_4 \cdot 7H_2O$, 2 g of asparagine monohydrate, 20 g of anhydrous α-D-glucose, and 20 g of agar per liter (19). Conidia were washed from 10- to 15-day-old cultures of three separate isolates with autoclaved deionized water, combined, strained through cheesecloth, vortexed with one drop of Tween 20, and centrifuged for 10 min at 2,000 *g*. The supernatant was discarded, the pelleted conidia were resuspended in autoclaved deionized water, and conidial concentration was determined with a hemacytometer. The conidial suspension was diluted to approximately 2,000 conidia per milliliter with deionized water.

Pyrrolnitrin was purified from cultures of *Pseudomonas cepacia* as described previously (23). The crystalline material was dissolved in methanol and diluted in deionized water. Vinclozolin (Ornalin 50WP, BASF Corp., Parsippany, NJ) was suspended in deionized water. All dip treatment solutions also contained 0.5 ml of Tween 20 per liter and a final methanol concentration of 10 ml/L.

Rose flowers (*Rosa hybrida* L.) were immersed in the treatment solutions for 2–3 sec, then gently shaken to remove excess solution and allowed to dry. The flowers were then sprayed with the conidial suspension with a Chromist spray unit (Gelman Sciences, Ann Arbor, MI). Noninoculated flowers sprayed with deionized water were included in each experiment to monitor natural infections. After inoculation, the roses were placed in a humidified storage chamber at 2 ± 1 C. Roses were removed from storage 7 days after inoculation, and disease development was quantified as the number of lesions on each flower.

Subsequently, the roses were evaluated for 10 days in a simulated consumer environment at 21 C with a 12-hr photoperiod from cool-white fluorescent lamps (PAR = 32 $\mu E \cdot m^{-2} \cdot s^{-1}$) (vase life evaluation). Fresh weights and visual observations were recorded daily. Roses were discarded during the 10-day period if *B. cinerea* macerated the entire receptacle, causing it to fall off the stem, or induced petal abscission.

Throughout storage and evaluation, roses were maintained in a preservative solution containing 35.8 mg of 8-hydroxyquinoline hemisulfate, 81.5 mg of citric acid monohydrate, 441 mg of $CaCl_2 \cdot 2H_2O$, 1.47 g of $MgSO_4 \cdot 7H_2O$, and 4 mg of NaOCl per liter of deionized water (6).

Determination of the effective concentration range. In the first experiment, Sonia roses were grown in the Horticulture Department greenhouses at The Pennsylvania State University. The roses were harvested at commercial maturity (when the sepals were partially reflexed and the outer two petals were starting to unfurl) and stored at 4 C until needed (1–6 days). The stems were recut 15 cm below the receptacles, and all foliage was removed. The flowers were treated with 0, 2.5, 5, 10, 20, 40, or 80 mg of pyrrolnitrin per liter. They were then inoculated, stored, and evaluated as described above.

Each experimental unit consisted of a single flower in a 125-ml flask with 100 ml of preservative. Six replicates of each treatment were arranged in a completely randomized design. Regression functions were fit after the reciprocal transformation was applied to pyrrolnitrin concentration to linearize the relationships between concentration and the response variables. The experiment was performed twice. Data from the first run of the experiment are presented.

Further testing for efficacy and

phytotoxicity. In the second experiment, Sonia and Royalty roses were obtained as described above. The stems were recut 30 cm below the receptacles, all except the top two leaves were removed, and pyrrolnitrin dip treatments of 0, 12.5, 25, 50, 100, or 200 mg/L were applied. During dip treatment, the top leaf of each rose was wetted to check for possible foliar phytotoxicity. Inoculated and noninoculated control roses received no dip treatment.

Each experimental unit consisted of three roses in a 0.9-L jar with 300 ml of preservative solution. There were two replicates (jars) of each factor-level combination, with three observations (roses) per replicate. The experiment was performed twice, and the data were combined for analysis with the two runs as blocks in a randomized complete block design. Because no meaningful regression relationships were found in this concentration range, analysis of variance with pairwise and multiple comparison procedures was used to compare the means.

Comparison of pyrrolnitrin with vinclozolin. In the third experiment, roses were obtained and treated as described for experiment 2. Pyrrolnitrin treatments of 25 or 100 mg/L were compared with vinclozolin treatments of 900 or 1,800 mg/L (applied as Ornalin 50WP at 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 roses per replicate. The experiment was performed twice, and the data were combined for analysis of variance with the two runs as blocks in a randomized complete block design.

RESULTS

Effective concentration range. Dip treatments with 2.5–80 mg of pyrrolnitrin per liter reduced disease severity on Sonia rose flowers (Fig. 1A). Disease severity was linearly related to the reciprocal of the pyrrolnitrin concentration (Fig. 1B). In the first run of this experiment, between 56 and 93 lesions developed on inoculated control roses, compared to zero to four lesions on inoculated flowers treated with pyrrolnitrin at 80 mg/L, which represents an average disease reduction of 97% at that concentration. No pyrrolnitrin treatment reduced disease severity significantly below the level found on noninoculated control roses.

Pyrrolnitrin also reduced poststorage flower rot. In the first run of this experiment, all of the inoculated control roses and 67% of the noninoculated control roses were discarded before the end of the vase life evaluation period because of petal abscission or receptacle maceration induced by *B. cinerea*. None of the roses treated with pyrrolnitrin at 40 or 80 mg/L were discarded prematurely (Fig. 2). As with disease severity (Fig. 1B), the incidence of poststorage flower rot was linearly related to the reciprocal of the pyrrolnitrin concentration (Fig. 2).

In the second run of this experiment (*data not presented*), overall disease levels were substantially lower. For example, 12–51 lesions developed on inoculated control roses. The effects of pyrrolnitrin on disease were the same as in the first run. Disease severity and poststorage flower rot were linearly related to the reciprocal of pyrrolnitrin concentration ($r^2 = 0.74$ and 0.98, respectively), and none of the flowers treated with pyrrolnitrin at 40 or 80 mg/L were discarded prematurely.

Efficacy and phytotoxicity. Pyrrolnitrin at 12.5–200 mg/L controlled *B. cinerea* infections on Royalty and Sonia roses (Fig. 3). Rates of pyrrolnitrin higher than 12.5 mg/L did not result in further reductions in disease severity (as evaluated immediately after storage) but did reduce further the incidence of poststorage flower rot (Table 1). Poststorage

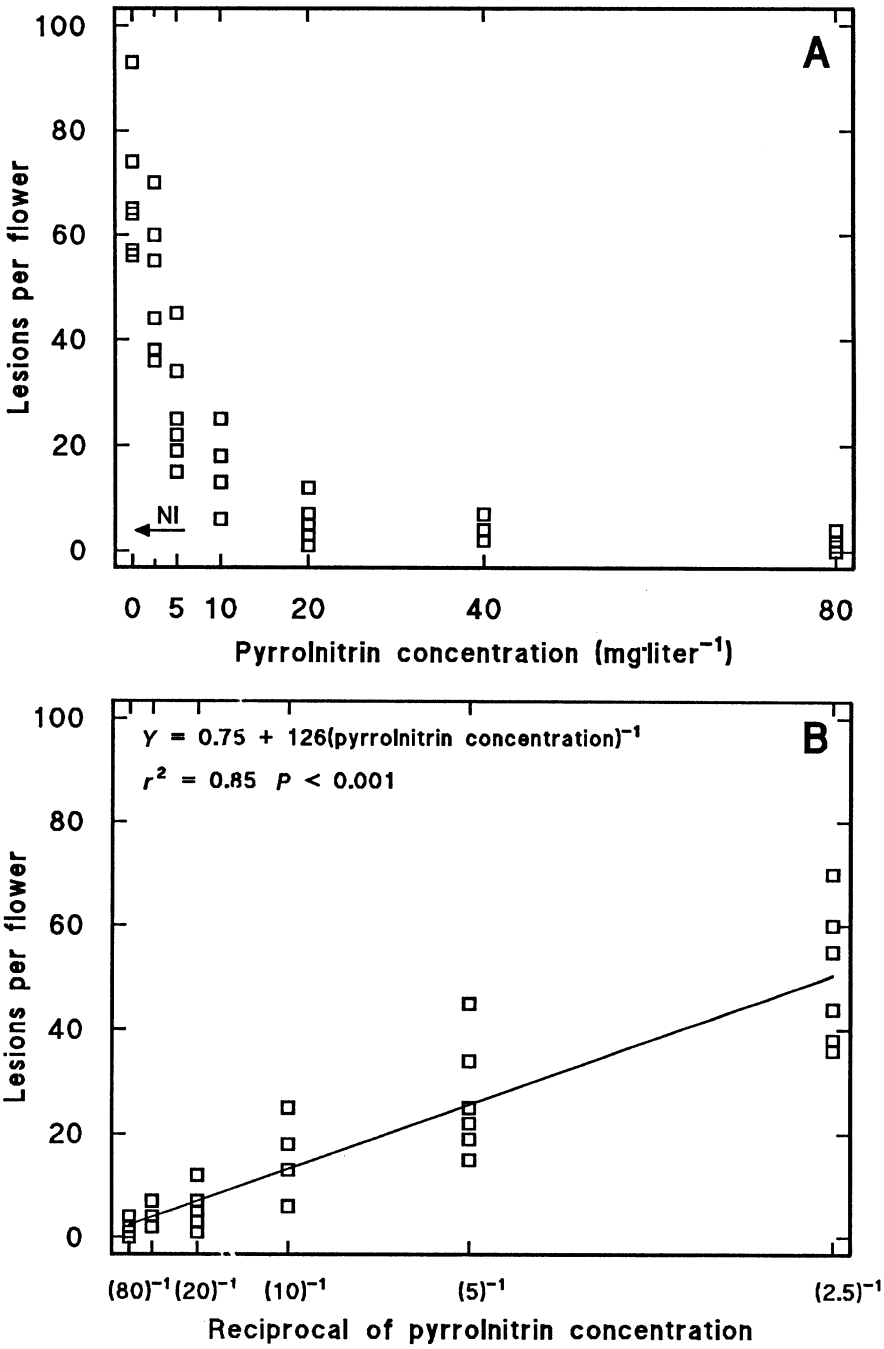

Fig. 1. Control of *Botrytis cinerea* infections on Sonia roses with dip treatments of of pyrrolnitrin at 2.5–80 mg/L (experiment 1). **(A)** Scatter plot. The arrow indicates the mean disease severity on noninoculated (NI) control flowers. No pyrrolnitrin treatment reduced disease severity below that found on the noninoculated controls, according to two-sided Dunnett's tests ($\alpha = 0.05$). **(B)** The regression function was linearized by reciprocal transformation of pyrrolnitrin concentration.

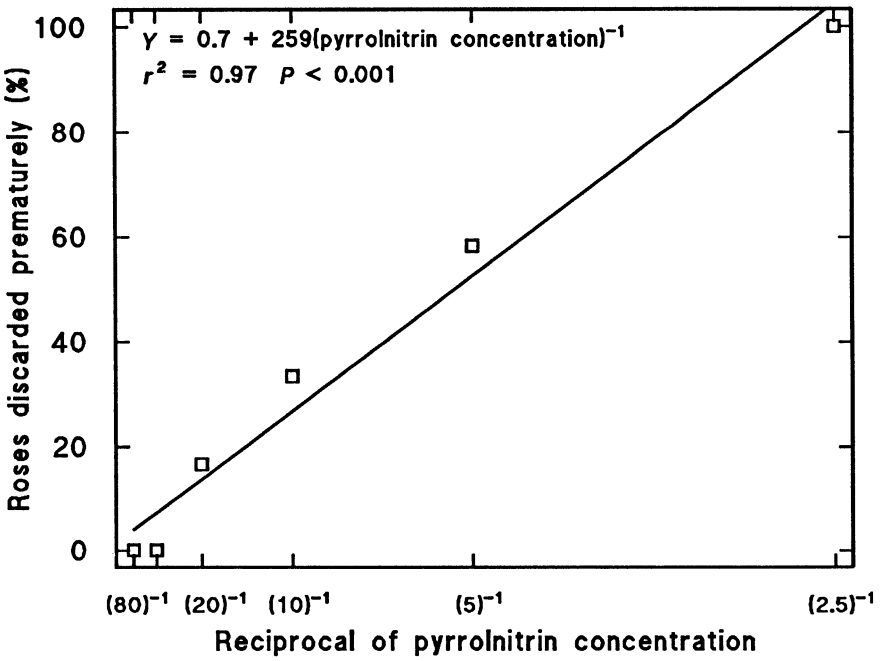

Fig. 2. Control of poststorage rot of Sonia rose flowers with pyrrolnitrin dip treatments of 2.5–80 mg/L (experiment 1). The vertical axis is the percentage of roses discarded before the end of vase life evaluation because of *Botrytis cinerea*-induced petal abscission or maceration of the receptacles. The regression function was linearized by reciprocal transformation of pyrrolnitrin concentration. All of the inoculated control roses and 67% of the noninoculated control roses were discarded prematurely.

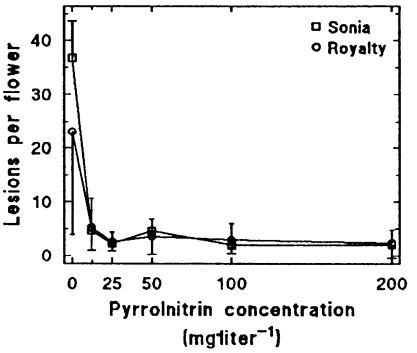

Fig. 3. Control of *Botrytis cinerea* infections on inoculated Royalty and Sonia roses with pyrrolnitrin at 12.5–200 mg/L (experiment 2). Error bars indicate the standard error of the mean. The mean disease severities on inoculated control flowers (no dip treatment) were 23 and 40 lesions per flower for Royalty and Sonia, respectively. These means did not differ significantly from those of the 0 mg/L dip treatment, according to two-sided Dunnett's tests ($\alpha = 0.05$). The mean disease severities on noninoculated control flowers were 6.2 and 7.4 lesions per flower for Royalty and Sonia, respectively. For Sonia flowers treated with pyrrolnitrin at 25, 100, or 200 mg/L, the mean disease severity was lower than that on noninoculated control flowers, according to two-sided Dunnett's tests ($\alpha = 0.05$).

flower rot was prevented on both cultivars by pyrrolnitrin at 100 mg/L or higher.

All flowers treated with pyrrolnitrin opened normally, and no phytotoxicity symptoms were observed on petals or leaves at any concentration tested. Roses treated with 25 mg/L or higher rates of pyrrolnitrin gained more fresh weight during vase life evaluation and reached peak fresh weight later than both inoculated and noninoculated control roses (Table 1).

Comparison of pyrrolnitrin with vinclozolin. The level of disease control (as evaluated immediately after storage) achieved with pyrrolnitrin at 25 or 100 mg/L was comparable to that achieved with vinclozolin at 900 or 1,800 mg/L (Table 2). Fewer than 17% of the roses treated with pyrrolnitrin at 25 mg/L and none of the flowers treated with pyrrolnitrin at 100 mg/L or with either rate of vinclozolin were discarded before the end of vase life evaluation because of petal abscission or maceration of the receptacles, compared to more than 90% of the inoculated control roses (Table 2). Roses treated with either concentration of either fungicide reached peak fresh weight later than roses in both control groups (Table 2).

Table 1. Effects of pyrrolnitrin dip treatments on poststorage flower rot and weight gain of cut roses[x]

Pyrrolnitrin treatment (mg/L)	Poststorage flower rot[y]		Peak fresh weight (% of initial)[z]	Days to peak fresh weight[z]
	Sonia	Royalty		
Noninoculated control	58 a	67 ab	132 ab	5.1 bc
Inoculated				
Control	92 a	100 a	129 a	4.2 a
0	92 a	83 a	131 ab	4.9 b
12.5	8 b	33 bc	135 bc	5.8 cd
25	17 b	8 c	135 bc	6.5 e
50	8 b	0 c	138 c	6.7 e
100	0 b	0 c	136 bc	6.4 de
200	0 b	0 c	137 c	6.2 de

[x] After pyrrolnitrin treatment, the flowers were inoculated with conidia of *Botrytis cinerea* and stored for 7 days at 2 C (experiment 2).

[y] Percentage of roses discarded before the end of vase life evaluation because of *B. cinerea*-induced petal abscission or maceration of the receptacles. Data were transformed with the arcsine-square root transformation for statistical analysis. Nontransformed means are presented. The main effect of treatment and the treatment-by-cultivar interaction were highly significant ($P < 0.004$). Mean separation within columns by t tests.

[z] The main effect of treatment was highly significant ($P < 0.001$). The treatment-by-cultivar interaction was significant for days to peak fresh weight ($P = 0.003$) but not for peak fresh weight ($P > 0.7$). Mean separation within columns by Tukey's studentized range test at $\alpha = 0.05$.

DISCUSSION

Pyrrolnitrin dip treatment of rose flowers effectively controlled lesion development and flower rot caused by *B. cinerea*. Concentrations as low as 25 mg/L controlled lesion development as effectively as label rates of vinclozolin, but rates of 100 mg/L or higher were required to eliminate poststorage flower rot consistently. These disease reductions are comparable to those achieved with label rates of vinclozolin, iprodione, or picro-cupric ammonium formate (PCAF) in similar laboratory experiments (6,21). Our results also are similar to those of Janisiewicz and Roitman (10), who found antifungal activity by pyrrolnitrin at concentrations as low as 1 mg/L in agar diffusion tests, whereas a concentration of 10 mg/L was required to consistently protect apple and pear fruits from infection by *B. cinerea*.

In these experiments we applied fungicides to rose flowers before inoculating them with conidia of *B. cinerea*, but a postharvest control measure must also be effective against inoculum that is present on flower petals at harvest. None of the pyrrolnitrin treatments eliminated lesion development altogether or consistently reduced lesions below the levels observed on noninoculated control flowers. These results are similar to those achieved with other fungicides (vinclozolin and PCAF) when naturally occurring disease levels were low (6). When natural infection rates were higher (17–20

Table 2. Effects of pyrrolnitrin and vinclozolin dip treatments on disease development, poststorage flower rot, and weight gain of cut roses[w]

Treatment	Lesions per flower[x]		Poststorage flower rot[y]		Peak fresh weight[z] (% of initial)	Days to peak fresh weight[z]
	Sonia	Royalty	Sonia	Royalty		
Noninoculated control	3.6 b	7.9 b	58 b	83 a	140 ab	5.1 a
Inoculated						
Control	25.5 a	21.4 a	92 a	96 a	137 a	4.8 a
25 ppm pyrrolnitrin	2.4 b	5.3 bc	17 c	8 b	142 ab	6.4 b
100 ppm pyrrolnitrin	1.8 b	3.1 c	0 c	0 b	143 b	6.4 b
900 ppm vinclozolin	3.8 b	5.6 bc	0 c	0 b	143 b	6.9 b
1,800 ppm vinclozolin	3.4 b	2.8 c	0 c	0 b	140 ab	6.6 b

[w] After fungicide treatment, roses were inoculated with conidia of *Botrytis cinerea*, then stored at 2 C for 7 days (experiment 3).

[x] The main effect of treatment and the treatment-by-cultivar interaction were highly significant ($P < 0.007$). Mean separation within columns by Tukey's studentized range test.

[y] Percentage of roses discarded before the end of vase life evaluation because of *B. cinerea*-induced petal abscission or maceration of the receptacles. Statistical analysis was performed on data transformed by the arcsine-square root transformation. Nontransformed means are presented. Mean separation within columns by *t* tests.

[z] The main effect of treatment was highly significant ($P < 0.001$), but the treatment-by-cultivar interactions were not ($P > 0.1$). Mean separation within columns by Tukey's studentized range test.

lesions per flower), both vinclozolin and PCAF provided effective disease control. Furthermore, pyrrolnitrin, like vinclozolin, reduced poststorage flower rot of inoculated flowers to levels below that observed on noninoculated control flowers (Tables 1 and 2). Thus, we conclude that pyrrolnitrin can effectively control Botrytis blight under commercial conditions.

Flower fresh weight is a sensitive index of quality and vase life. Higher peak fresh weights and longer times to peak fresh weight are correlated with other, more subjective measures of flower quality (3,15,22). In these experiments, as in previous reports, infection by *B. cinerea* inhibited weight gain and shortened time to peak fresh weight (6–8). Treatment with either pyrrolnitrin or vinclozolin prevented these effects. These results, together with the poststorage flower rot data, demonstrate that pyrrolnitrin effectively controlled the deleterious effects of disease on flower quality.

No phytotoxicity symptoms were observed on rose flowers or foliage at any pyrrolnitrin concentration tested. This evidence of safety was corroborated by the fresh weight data. Flowers treated with pyrrolnitrin gained more fresh weight and reached peak fresh weight later than the noninoculated controls, indicating that the flowers were not damaged physiologically. We interpret these effects of fungicide treatment on weight gain as secondary benefits of disease control rather than direct effects on flower physiology.

Pyrrolnitrin dip treatment of 25–100 mg/L effectively controlled infections by *B. cinerea* on cut rose flowers without phytotoxicity. A synthetic phenylpyrrole (CGA 173506, Ciba-Geigy Ltd., Basel, Switzerland) chemically similar to pyrrolnitrin gave good control of *B. cinerea* on grapes (4). Gehmann et al (4) found no cross-resistance to CGA 173506 by pathogens that are resistant to benzimidazoles, dicarboximides, or guanidines. Neither pyrrolnitrin nor CGA 173506 is available commercially or currently registered for use on roses. However, they are potential alternatives to the fungicides now in use and may be particularly useful for preventing the development of fungicide-resistant pathogen populations.

LITERATURE CITED

1. Arima, K., Imanaka, H., Kousaka, M., Fukuta, A., and Tamura, G. 1964. Pyrrolnitrin, a new antibiotic substance, produced by *Pseudomonas*. Agric. Biol. Chem. 28:575-576.
2. Beever, R. E., and Brien, H. M. R. 1983. A survey of resistance to dicarboximide fungicides in *Botrytis cinerea*. N.Z. J. Agric. Res. 26:391-400.
3. Brantly, R. K. 1975. A new postharvest chemical treatment for roses. HortScience 10:178-179.
4. Gehmann, K., Nyfeler, R., Leadbeater, A. J., Nevill, D., and Sozzi, D. 1990. CGA 173506: A new phenylpyrrole fungicide for broad-spectrum disease control. Proc. Brighton Crop Prot. Conf. 2:399-405.
5. Goulart, B. L., Hammer, P. E., Evensen, K. B., Janisiewicz, W., and Takeda, F. 1992. Pyrrolnitrin, captan + benomyl, and high CO_2 enhance raspberry shelf life at 0 or 18 C. J. Am. Soc. Hortic. Sci. 117:265-270.
6. Hammer, P. E., and Marois, J. J. 1988. Postharvest control of *Botrytis cinerea* on cut roses with picro-cupric-ammonium formate. Plant Dis. 72:347-350.
7. Hammer, P. E., and Marois, J. J. 1989. Nonchemical methods for postharvest control of *Botrytis cinerea* on cut roses. J. Am. Soc. Hortic. Sci. 114:100-116.
8. Hammer, P. E., Yang, S. F., Reid, J. J., and Marois. J. J. 1990. Postharvest control of *B. cinerea* infections on cut roses using fungistatic storage atmospheres. J. Am. Soc. Hortic. Sci. 115:102-107.
9. Imanaka, H., Kousaka, M., Tamura, G., and Arima, K. 1965. Studies on pyrrolnitrin, a new antibiotic. III. Structure of pyrrolnitrin. J. Antibiot. Ser. A 18:207-210.
10. Janisiewicz, W. J., and Roitman, J. 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78:1697-1700.
11. Janisiewicz, W., Yourman, L., Roitman, J., and Mahoney, N. 1991. Postharvest control of blue mold and gray mold of apples and pears by dip treatment with pyrrolnitrin, a metabolite of *Pseudomonas cepacia*. Plant Dis. 75:490-494.
12. Katan, T. 1982. Persistence of dicarboximide-fungicide resistance in populations of *Botrytis cinerea* in a warm, dry temperate agroclimate. Phytoparasitica 10:209-211.
13. 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.
14. Leroux, P., and Clerjeau, M. 1985. Resistance of *Botrytis cinerea* Pers. and *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni to fungicides in French vineyards. Crop Prot. 4:137-160.
15. 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. 96:38-43.
16. 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.
17. Moorman, G. W., and Lease, R. J. 1992. Benzimidazole- and dicarboximide-resistant *Botrytis cinerea* from Pennsylvania greenhouses. Plant Dis. 76:477-480.
18. O'Brien, R. G., and Glass, R. J. 1986. The appearance of dicarboximide resistance in *Botrytis cinerea* in Queensland. APP, Australas. Plant Pathol. 15:24-25.
19. Phillips, D. J., Margosan, D. A., and Mackey, B. E. 1987. Size, nuclear number, and aggressiveness of *Botrytis cinerea* spores produced on media of varied glucose concentrations. Phytopathology 77:1606-1608.
20. Pommer, E.-H., and Lorenz, G. 1982. Resistance of *Botrytis cinerea* Pers. to dicarboximide fungicides—A literature review. Crop Prot. 1:221-230.
21. 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.
22. 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.
23. Takeda, F., Janisiewicz, W. J., Roitman, J., Mahoney, N., and Abeles, F. B. 1990. Pyrrolnitrin delays postharvest fruit rot in strawberries. HortScience 25:320-322.
24. Wang, Z.-N., Coley-Smith, J. R., and Wareing, P. W. 1986. Dicarboximide resistance in *Botrytis cinerea* in protected lettuce. Plant Pathol. 35:427-433.