Postinfection and Antisporulant Activities of Selected Fungicides in Control of Blossom Blight of Sour Cherry Caused by *Monilinia fructicola*

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ABSTRACT

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Nine fungicides—captan, fenarimol, flusilazole, iprodione, myclobutanil, propiconazole, tebuconazole, triforine, and vinclozolin—were tested for ability to prevent infection of sour cherry (*Prunus cerasus*) blossoms when applied 24, 48, or 72 hr after inoculation with different levels of *Monilinia fructicola*. When applied 24 hr after inoculation with a suspension containing 5,000 conidia per milliliter, all fungicides gave 86–100% control of blossom blight (relative to untreated checks), and all fungicides except captan gave 97–100% control following inoculation with 50,000 conidia per milliliter. However, when sprays were applied 48 hr after inoculation with 500, 5,000, and 50,000 conidia per milliliter, the degree of control was 84–100%, 57–98%, and 7–82%, respectively. When sprays were applied 72 hr after inoculation with 500 conidia per milliliter, control was only 16–64%, and when the inoculum level was 5,000 conidia per milliliter, virtually no control was provided. When blossoms were sprayed with the same fungicides 72 hr after inoculation with 50,000 conidia per milliliter and incubated an additional 4 days at 95–100% relative humidity, all became necrotic, but conidium production was reduced by 51% (captan) to 100% (vinclozolin), relative to unsprayed blossoms. At the dosage rates tested, vinclozolin, iprodione, tebuconazole, and propiconazole were the most effective fungicides in both the postinfection and antisporulant modes, whereas captan was the least effective.

Additional keywords: brown rot, ergosterol biosynthesis-inhibitor fungicides, sterol-demethylation-inhibitor fungicides, sterol-inhibitor fungicides, triazole fungicides

The "American" brown rot fungus, *Monilinia fructicola* (Wint.) Honey, is a pathogen of primary economic importance throughout North American stone fruit production regions and also causes losses on these crops in South America, Oceania, and Africa (2). Although management programs for brown rot typically include several horticultural practices designed to minimize disease severity, applications of fungicides during the preharvest and/or blossom periods of crop development usually are required to obtain commercially acceptable levels of control (6,19,20).

Blossom infections may cause a reduction in fruit set or lead to the development of twig cankers, yet the production of conidia from infected flowers and their spread to ripening fruit is generally regarded as the most damaging consequence of the blossom blight phase of brown rot (13,19). Thus, fungicides that not only protect blossoms from infection by *M. fructicola* but also suppress sporulation on those that do become diseased could provide a significant secondary means of attaining the ultimate objective, control of fruit rot. Furthermore, blossom infections occur relatively infrequently in some regions of the United States (1,17) and may not need to be controlled in all orchards every year (13). In such regions, the need for fungicidal control of blossom blight might be determined each season based on an assessment of environmental conditions conducive to disease development (17). Management programs of this type would be greatly aided by the identification of fungicides with significant postinfection activity, which would provide an option to spray after the occurrence of a probable infection period rather than before an anticipated weather event. Accordingly, this study was initiated: 1) to determine the postinfection and antisporulant properties of several fungicides recently registered or under current investigation for control of brown rot on stone fruits and 2) to compare their activities with those of a traditional standard, captan.

MATERIALS AND METHODS

Fungicides. All fungicides were applied as aqueous suspensions of formulated product. The active ingredients, their concentrations, and the rates of formulated product were: captan, 1,198 μg/ml (908 g [2 lb] Captan 50W/379 L [100 gal]); fenarimol, 28 μg/ml (88.7 ml [3 fl oz] Rubigan 1E/379 L [100 gal]); flusilazole, 23 μg/ml (49.7 g [1.5 oz] Nustar 20DF/379 L [100 gal]); iprodione, 449 μg/ml (341 g [12 oz]

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Rovral 50WP/379 L [100 gal]); myclobutanil, 52 μg/ml (49.7 g [1.75 oz] Nova 40WP/379 L [100 gal]); propiconazole, 44 μg/ml (88.7 ml [3 fl oz] Orbit 3.6E/379 L [100 gal]); tebuconazole, 67 μg/ml (56.8 g [2 oz] Elite 45DF/379 L [100 gal]); triforine, 180 μg/ml (355 ml [12 fl oz] Funginex 1.6EC/379 L [100 gal]); and vinclozolin, 449 μg/ml (341 g [12 oz] Ronilan 50WP/379 L [100 gal]).

Determination of postinfection activity. Inoculation procedures were similar to those detailed previously (17). Briefly, potted sour cherry trees (*Prunus cerasus* L. 'Montmorency') 6–12 yr old were maintained outdoors during the growing season, stored indoors at 1 C during the winter, and induced to bloom in the spring by repotting and transfer to a greenhouse. Unopened blossom buds were removed immediately before inoculation, leaving about 100–200 fully opened blossoms per tree. A spray paint gun was used to uniformly mist all blossoms with a suspension containing 500, 5,000, or 50,000 conidia per milliliter of *M. fructicola*; conidia were obtained by rinsing the surface of a canned peach fruit inoculated 1 wk previously with a virulent strain of the fungus originally isolated from a rotting peach fruit in the 1970s. The mean volume of conidial supension retained on each blossom was 0.20 ml, as determined in six different experiments by excising 10 blossoms immediately after inoculation, placing them on an analytical balance, and determining the weight of water that had evaporated at the point the blossoms became visibly dry.

Immediately after inoculation, trees were moved into a mist chamber maintained at 20 C. After either 23 or 47 hr, depending upon the experiment, half of the trees in each inoculation group were removed from the mist chamber and allowed to dry for 1 hr. A spray paint gun was then used to thoroughly spray individual trees with each fungicide (check trees were sprayed with water, using a different gun). This procedure was repeated with the remaining trees 24 hr after the initial group was treated. Once the fungicide sprays had dried, trees were moved to a lighted chamber maintained at 20 C and 95–97% relative humidity (RH). Blossoms were examined daily from 3–7 days after inoculation and rated as diseased if a necrotic lesion expanded into the base of the calyx. Diseased blossoms were recorded and excised after each examination, and a final count of uninfected blossoms was made to determine disease incidence. Two experiments were conducted, each of which was repeated four times to provide four replicate data points per treatment. Data were analyzed using a variance stabilizing (arcsin) transformation, standard analysis of variance for random block design, and the Waller-Duncan k-ratio t test variable for mean separation.

Antisporulant activity. Using the procedures outlined above, trees were induced to bloom and inoculated with a suspension containing 50,000 conidia per milliliter of *M. fructicola*. The trees were then subjected to a 48-hr period in a mist chamber at 20 C and transferred after a brief drying period to another chamber maintained at 20 C and 95–97% RH. Seventy-two hours after inoculation, a spray paint gun was used to thoroughly spray individual trees with each fungicide suspension. After the sprays had been allowed to dry for 3–5 hr, 45 individual blossoms and supporting pedicels were detached from each tree and divided into five groups of nine blossoms each. After all petals were removed, each nine-blossom sample was placed into a 10 × 10 × 5 cm plastic freezer box by inserting individual pedicels through a wire screen into a 1% sucrose solution at the bottom of each container; the remaining floral parts rested on the screen above the solution such that individual flowers were not touching. The boxes were returned immediately to the 20 C, 95–97% RH chamber, then were sealed by attaching the snap-on lids 24 hr later. After 3 days of additional incubation, (i.e., 7 days after inoculation), all blossoms in a box were transferred to a test tube containing 10 ml of distilled water plus one drop of surfactant (Tween 20) and shaken vigorously for 30 sec; the concentration of conidia was then determined using a hemacytometer. The readings from all five subsamples of each treatment were bulked and expressed as a percentage of the number of conidia recovered from

Table 1. Postinfection activities of nine fungicides when applied to sour cherry blossoms 24 and 48 hr after inoculation with two different levels of *Monilinia fructicola* in greenhouse experiments

		Percent blossom blight[z]			
		24 hr Postinoculation		48 hr Postinoculation	
Fungicide	Dosage (μg/ml)	5,000 conidia/ml	50,000 conidia/ml	5,000 conidia/ml	50,000 conidia/ml
Iprodione	449.4	0.0 e	0.3 d	4.3 c	18.5 c
Vinclozolin	449.4	0.0 e	1.0 d	11.3 c	21.8 c
Propiconazole	43.9	0.0 e	0.3 d	6.7 c	29.0 bc
Tebuconazole	67.4	0.3 de	0.3 d	4.3 c	29.8 bc
Myclobutanil	52.4	2.0 cd	1.5 cd	16.0 c	38.0 bc
Flusilazole	22.5	12.5 b	2.7 cd	14.5 c	40.7 bc
Triforine	180.2	2.3 cd	2.3 cd	15.3 c	51.8 b
Fenarimol	28.1	3.0 c	3.3 c	13.3 c	55.0 b
Captan	1,198.0	4.7 c	27.3 b	42.0 b	93.3 a
Check (water)	···	91.0 a	98.0 a	99.3 a	99.3 a

[z] Mean incidence of blossom blight 7 days after inoculation with the indicated conidial suspensions, obtained from four replicate experiments. Potted trees were inoculated during full bloom, placed in a mist chamber at 20 C, then sprayed with aqueous suspensions of fungicide formulations either 24 or 48 hr later. Trees were subsequently incubated at 20 C and 95–97% RH before assessment of disease incidence. Means within a column not followed by a common letter are significantly different ($P = 0.05$) according to the Waller-Duncan exact Bayesian k-ratio LSD rule.

Table 2. Postinfection activities of nine fungicides when applied to sour cherry blossoms 48 and 72 hr after inoculation with two different levels of *Monilinia fructicola* in greenhouse experiments

		Percent blossom blight[z]			
		48 hr Postinoculation		72 hr Postinoculation	
Fungicide	Dosage (μg/ml)	500 conidia/ml	5,000 conidia/ml	500 conidia/ml	5,000 conidia/ml
Vinclozolin	449.4	0.0 d	0.0 e	42.6 d	93.6 b
Iprodione	449.4	0.0 d	0.8 de	52.0 cd	95.4 ab
Propiconazole	43.9	0.0 d	0.3 e	56.3 cd	95.8 ab
Tebuconazole	67.4	0.0 d	0.4 de	65.0 bcd	98.7 ab
Myclobutanil	52.4	0.8 cd	0.2 e	66.0 bc	98.7 ab
Flusilazole	22.5	0.1 d	1.0 de	56.4 cd	97.6 ab
Fenarimol	28.1	0.3 cd	3.7 cd	68.5 bc	98.9 ab
Triforine	180.2	3.0 c	7.9 c	59.5 cd	99.6 ab
Captan	1,198.0	15.5 b	44.8 b	77.9 ab	98.7 ab
Check (water)	···	93.0 a	99.2 a	92.7 a	100.0 a

[z] Mean incidence of blossom blight 7 days after inoculation with the indicated conidial suspensions, obtained from four replicate experiments. Potted trees were inoculated during full bloom, placed in a mist chamber at 20 C, then sprayed with aqueous suspensions of fungicide formulations either 48 or 72 hr later. Trees were subsequently incubated at 20 C and 95–97% RH before assessment of disease incidence. Means within a column not followed by a common letter are significantly different ($P = 0.05$) according to the Waller-Duncan exact Bayesian k-ratio LSD rule.

the water-sprayed (check) blossoms. The experiment was repeated four times to provide four replicate data points per treatment. Data were analyzed using a variance stabilizing (arcsin) transformation, standard analysis of variance for random block design, and the Waller-Duncan *k*-ratio *t* test variable for mean separation.

RESULTS

Postinfection activity. When applied 24 hr after inoculation with 5,000 conidia per milliliter of *M. fructicola*, all fungicides gave 86–100% control of blossom blight (relative to disease incidence on the check trees), and all fungicides except captan gave 97–100% control with an inoculum concentration of 50,000 conidia per milliliter (Table 1). When sprays were applied 48 hr after inoculation, however, the degree of control was strongly influenced by both the inoculum concentration and the fungicide used. For instance, triforine provided a mean of 88% control in two different experiments when blossoms were inoculated with suspensions containing 5,000 conidia per milliliter (Tables 1 and 2), provided 97% control when the inoculum concentration was 500 conidia per milliliter (Table 2), but gave only 52% control when blossoms were inoculated with suspensions containing 50,000 conidia per milliliter (Table 1). Similarly, there was little difference in efficacy (84–100% control) among all materials other than captan when sprays were applied 48 hr after inoculation with 5,000 conidia per milliliter, but at 50,000 conidia per milliliter, control ranged from 81% for iprodione down to 45% for fenarimol (Table 1). Control was only fair to poor when fungicides were applied 72 hr after inoculation with 500 conidia per milliliter, and virtually no control was obtained when applications were made 72 hr after inoculation with 5,000 conidia per milliliter (Table 2). In general, vinclozolin, iprodione, propiconazole, and tebuconazole showed the most pronounced postinfection activities, whereas captan showed the least; triforine and fenarimol often were marginally less effective than the other sterol-demethylation inhibitor (DMI) fungicides.

Antisporulant activity. All fungicides showed significant antisporulant activity, with the relative activities among fungicides similar to the relative postinfection activities showed by the same materials. For instance, although a 100% incidence of blossom blight occurred regardless of which fungicide was applied 72 hr after inoculation with 50,000 conidia per milliliter, vinclozolin completely suppressed production of conidia from diseased blossoms incubated in a saturated or near-saturated environment for 7 days following inoculation; iprodione, tebuconazole, and propiconazole also were extremely effective at suppressing production of conidia under these conditions (Table 3). Consistent with trends evidenced in the postinfection experiments, captan had the least effect on production of conidia from blighted blossoms (51% reduction relative to the check treatment), and fenarimol and triforine provided less reduction than all remaining materials (Table 3).

Table 3. Activities of nine fungicides in suppressing the formation of conidia of *Monilinia fructicola* on infected sour cherry blossoms when applied before symptom expression

Fungicide	Dosage (μg/ml)	Percent reduction[y]
Vinclozolin	449.4	100.0 f
Iprodione	449.4	99.6 ef
Tebuconazole	67.4	95.4 def
Propiconazole	43.9	93.2 de
Myclobutanil	52.4	78.4 cd
Flusilazole	22.5	77.9 cd
Triforine	180.2	67.8 bc
Fenarimol	28.1	57.7 bc
Captan	1,198.0	51.3 b
Check (water)	···	0.0 a[z]

[y] Percent reduction relative to the number of conidia recovered from check blossoms in the same experiment. Potted trees were inoculated in full bloom with a suspension containing 50,000 conidia per milliliter of *M. fructicola*, placed in a mist chamber at 20 C for 72 hr, then sprayed with aqueous suspensions of fungicide formulations. Blossoms were excised, then incubated at 95–100% RH for an additional 4 days until all were necrotic, when the number of conidia produced was determined with a hemacytometer. Values represent the means from four replicate experiments, 45 blossoms per treatment observed in each experiment. Means not followed by a common letter are significantly different ($P = 0.05$) according to the Waller-Duncan exact Bayesian *k*-ratio LSD rule.

[z] An average of 1.12×10^6 conidia was recovered from each blossom in the check treatment.

DISCUSSION

The ability of various DMI fungicides to: 1) prevent development of lesions caused by *Venturia inaequalis* (Cke.) Wint. when applied to apple leaves within one to several days after inoculation and 2) suppress production of conidia from developing lesions when applied slightly beyond these limits (i.e., "presymptom" activity) has been well documented (9,10,15). The results from the present study indicate that a number of DMI fungicides show analogous postinfection and antisporulant activities with respect to control of sour cherry blossom infections caused by *M. fructicola*, although the relatively short incubation period for blossom blight (about 3–5 days under these test conditions) apparently restricts the period for effective postinfection activity. For instance, although some DMI fungicides provide good postinfection control of apple scab lesions when applied up to 120 hr after inoculation (9,10), all six DMI materials examined in the present study provided poor control of blossom blight when applied more than 48 hr after inoculation under the conditions of these experiments.

The pronounced postinfection and antisporulant activities showed by iprodione and vinclozolin are somewhat contradictory of the view that "a general feature of the dicarboximides is an essentially protective activity" (11). Nevertheless, systemicity is a property that was recognized early in the development of the newer dicarboximides, i.e., those materials such as iprodione and vinclozolin whose chemical structure includes a benzene ring (in contrast to captan, a dicarboximide whose structure does not) (8). For instance, in 1977, Hisada et al (5) showed that C-14-labeled procymidone was acropetally translocated when applied to either the leaves or the roots of cucumber plants, and disease control resulting from the systemic movement of procymidone and iprodione was demonstrated shortly thereafter on strawberries (4) and potatoes (3), respectively. Limited examples of the postinfection capabilities of these materials also exist, e.g., 1) the claim of Hisada et al (5) that "very successful" control of gray mold was obtained when procymidone was sprayed onto cucumber cotyledons after inoculation with *Botrytis cinerea* Pers. ex Fries. (no experimental details provided); 2) the report of Lambert et al (7) that infections of lowbush blueberry blossoms were controlled when vinclozolin was applied 51 hr after inoculation with *B. cinerea*; and 3) the report by Szkolnik (16), in an experiment similar to those reported herein, of control of sour cherry blossom blight by applying iprodione 24 hr after inoculation with *M. fructicola*. Furthermore, Ritchie and Bennett (12) also have demonstrated the antisporulant properties of iprodione and vinclozolin upon application of these materials to peach tree cankers infected with *M. fructicola*.

Just as the wetness period necessary to initiate blossom infections by *M. fructicola* must be defined in terms of temperature and inoculum density (17), so must the same variables be considered when determining the effective period during which a fungicide can eradicate nascent blossom blight infections. Although the effect of temperature was not examined in these experiments, the effect of inoculum density was clearly demonstrated, questioning the relationship between the experimental inoculum densities employed and those likely to be encountered under field conditions. Sources of field inoculum during bloom are incompletely understood and largely unquantified and undoubtedly vary among stone fruit crops, geographic locations, and individual years; neverthe-

less, a 3-yr study of overwintered sour cherry mummies in New York orchards showed that an average of approximately 40,000 conidia could be washed from each infected mummy removed from a tree just before bloom (17). At such a level, one mummy produces enough conidia to inoculate 400, 40, or 4 blossoms with the same number of conidia delivered to each blossom in the experiments described herein using 0.20 ml of suspension containing 500, 5,000, or 50,000 conidia per milliliter, respectively.

Interpretation of the experimental data presented should include a recognition of the effect of fungicide dose on subsequent activity and the possibility that a wide range of sensitivities to various fungicides may exist among field populations of *M. fructicola*. For instance, a wide range in sensitivities to flusilazole recently was demonstrated among wild-type populations of *V. inaequalis* (14). Nevertheless, the data strongly suggest that certain DMI and dicarboximide fungicides can be used to control brown rot blossom blight through a combination of protectant (16,18) and postinfection activities and that these fungicides furthermore may provide indirect protection against fruit infection by suppressing the production of secondary inoculum from blighted blossoms. The duration of such suppressive activity, the postinfection and antisporulant properties of these materials when applied to fruits, and the analogous activities of the dicarboximides in other host-pathogen systems remain to be determined.

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