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Activity of Captan and Prochloraz on Benomyl-Sensitive and Benomyl-Resistant Isolates of *Monilinia fructicola*

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ABSTRACT

Dijkhuizen, J. P., Ogawa, J. M., and Manji, B. T. 1983. Activity of captan and prochloraz on benomyl-sensitive and benomyl-resistant isolates of *Monilinia fructicola*. Plant Disease 67:407-409.

A benomyl-resistant *Monilinia fructicola* isolate grew as fast as a sensitive isolate on a medium free of fungicides but grew more slowly on a medium containing 10 μg/ml of captan or 1 μg/ml of prochloraz. Conidial germination was inhibited by captan but not by prochloraz. Yet when conidia exposed to fungicides were transferred onto a fungicide-free potato-dextrose agar (PDA) medium, spores exposed to 10 μg/ml of captan germinated and formed colonies, whereas conidia germinating in contact with prochloraz made no further growth. Benomyl-resistant or -sensitive conidia germinated on PDA were not affected by exposure to captan for 16 hr, but exposure to prochloraz for 4 hr severely reduced further germ-tube growth. Blossom blight on peaches was not reduced with a single spray of captan applied at pink bud or initial petal fall, but applications at pink bud followed by a spray at 75% petal fall reduced blossom blight equivalent to that of benomyl spray or combination of benomyl and captan at pink bud. Effective disease control was provided by a single spray of prochloraz at pink bud but not at initial petal fall. Blighted blossoms sprayed with prochloraz had the fewest conidia. Peach fruits dipped in prochloraz failed to develop *Monilinia* decay when flesh surrounding the pit was inoculated with conidia, indicating systemic activity.

Captan was the most widely used fungicide to control brown rot blossom blight and fruit rot of peaches caused by *Monilinia fructicola* (Wint.) Honey until the introduction of benomyl and thiophanate methyl (3,4). Development of benomyl resistance in *M. fructicola* in California (5) resulted in testing of alternative chemicals and comparing their efficacy to captan treatments.

This study was made to compare the activity of captan with that of an unrelated fungicide, prochloraz, on *M. fructicola*. Disease control of each was compared with benomyl and a combination of benomyl and captan.

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MATERIALS AND METHODS

In vitro reaction to fungicides. Benomyl-resistant (1-81) and sensitive (2-65) isolates of *M. fructicola* with similar mycelial growth rates on potato-dextrose agar (PDA) were compared on PDA amended with captan or prochloraz. To determine mycelial growth rates, 4-mm mycelium plugs were transferred onto the center of a PDA medium incorporated with 10–1200 μg a.i./ml captan or 1–10 μg a.i./ml prochloraz. To prevent reduction of activity, captan and prochloraz were added to the medium after sterilization and the medium was cooled to about 55 C before pouring. Ten plates of each concentration of fungicide were inoculated and incubated at 24 C and colonies were measured daily for 14 days.

To provide conidia for all other laboratory studies, two *M. fructicola* isolates collected from peach orchards were used. One isolate was benomyl-resistant (78-4) and the other was benomyl-sensitive (1-65). These two isolates were grown on V-8 juice agar and incubated at room temperature for 5–7 days to induce conidial production. Cellophane disks (Du Pont 215 PD-65) 6 mm in diameter were placed on a PDA medium amended with fungicides (1,2). Conidia were brushed onto these disks with a camel's hair brush. Concentrations of fungicides (a.i.) used were: captan 50W at 0, 10, and 1,200 μg/ml and prochloraz 50W (BTS 40452 WP) at 0, 1, 3, 5, and 10 μg/ml. The suggested concentration for field use of captan is 1,200 μg/ml and for prochloraz, 250 μg/ml. To establish whether ungerminated conidia were inactivated, conidia on cellophane disks were placed in contact with the various concentrations of captan and prochloraz for 6 or 8 hr and then transferred to the fungicide-free medium for 40 hr. One hundred conidia were observed for germination and germ-tube length was measured on 10 randomly selected germinated conidia. If germ tubes failed to develop or grow after transfer onto the fungicide-free PDA medium, the conidia or germ tubes were considered to be inactivated.

To establish whether germinated conidia were inactivated, conidia germinated on cellophane in contact with PDA with a germ-tube length of 30 μm or more were exposed to the various concentrations of captan for 16 hr and to prochloraz for 4 hr and then transferred onto the fungicide-free PDA medium for 24 hr. If germ tubes did not grow to more

than 973 μm, they were considered inactivated.

Evaluation on the host. To determine the effectiveness of the fungicides in blossom blight control, a test was conducted in a Lockeford, CA, Loadel peach orchard where the first incidence of benomyl-resistant *M. fructicola* in California was recorded in 1977. By spring of 1980, about 40% of the *M. fructicola* population in this orchard was resistant to benomyl from 0.5 to 5.0 μg/ml (5). Six single-tree replicates were handgun sprayed to drip stage at various stages of bloom with benomyl, benomyl plus captan, captan, and prochloraz. Blossom blight was counted randomly by examining 175 blossoms on four quadrants of the trees. To determine numbers of conidia produced on each blossom, 10 blighted blossoms were picked at random. Each blossom was placed in a vial with 1 ml of distilled water with a Tween 20 (Atlas Powder Co., Dallas, TX 75251) wetting agent, stirred with a mechanical vibrator for 1 min, and three samples of each conidial suspension were examined for conidial numbers with a hemacytometer.

To determine penetration of prochloraz into the fruit flesh, 10 peach fruits were dipped for 1 min in 250 μg/ml of water suspension, held in cold storage (1–2 C) for 24 hr, and injected with 5 μl of suspension (40×10^3 conidia per milliliter) into the pit area on each side of the seed and incubated for 5 days at 25 C.

RESULTS

In vitro reactions to fungicides. The benomyl-sensitive *M. fructicola* isolate (2-65) and the benomyl-resistant isolate (1-81) grew at the same rate on PDA. On the medium with 10 μg/ml of captan and 1 μg/ml of prochloraz, the benomyl-resistant isolate was more restricted in mycelial growth than the benomyl-sensitive isolate (Fig. 1).

Captan at 10 and 1,200 μg/ml inhibited germination of conidia within 8 hr. Upon transfer to the medium without fungicide, conidia exposed to 10 μg/ml germinated and made extensive growth within 40 hr but those exposed to 1,200 μg/ml failed to germinate (Table 1). Prochloraz at 1–10 μg/ml allowed conidia germination and growth up to 84 μm, but upon transfer to fungicide-free PDA, the germ tubes failed to make further growth (Table 1).

Conidia of both isolates germinated on PDA. Conidia exposed to captan for 16 hr followed by 24 hr on fungicide-free medium continued to grow. Benomyl-resistant conidia exposed to 1 and 3 μg/ml of prochloraz for 4 hr followed by 24 hr on fungicide-free PDA medium resulted in reduced germ-tube growth compared with the benomyl-sensitive isolate. Germ-tube growth of conidia exposed to 5 and 10 μg/ml of prochloraz for 4 hr and then placed on fungicide-free medium was about equal for both isolates. Both *Monilinia* isolates not exposed to captan or prochloraz grew extensively during the same period (Table 2).

Evaluation on the host. Field sprays were applied at pink bud (27 February), initial petal fall (7 March), and 75% petal fall (10 March) to ascertain the benefits of earlier and later applications than recommended for protectant fungicides such as captan. Differences were shown in blossom blight control, sporulation on blighted blossoms, and percentage of blighted blossoms with conidia. The

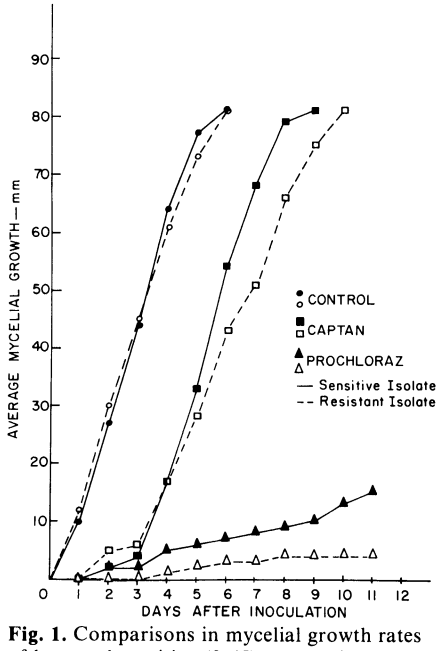

Fig. 1. Comparisons in mycelial growth rates of benomyl-sensitive (2-65) and -resistant (1-81) *Monilinia fructicola* isolates on PDA medium incorporated with 10 μg/ml of captan and 1 μg/ml of prochloraz. Greater sensitivity of benomyl-resistant isolates to captan and prochloraz is shown.

Table 1. Germination and germ-tube elongation of benomyl-resistant *M. fructicola* conidia on cellophane exposed to various concentrations of captan and prochloraz

	Conidial germination and germ-tube growth[w]			
	On medium with fungicide		On medium free of fungicide	
Fungicide (μg/ml)	Germinated[x] (%)	Growth[y] (μm)	Germinated[x] (%)	Growth[y] (μm)
	Captan for 8 hr		Fungicide-free PDA for 24 hr	
Control	100 a[z]	67 a	100 a	⩾973 a
Captan, 10	0 b	0 b	100 a	⩾973 a
Captan, 1,200	0 b	0 b	0 b	0 b
	Prochloraz for 6 hr		Fungicide-free PDA for 40 hr	
Control	100 a	141 a	100 a	⩾973 a
Prochloraz, 1	100 a	71 b	100 a	70 b
Prochloraz, 3	100 a	72 b	100 a	60 b
Prochloraz, 5	100 a	84 b	100 a	72 b
Prochloraz, 10	100 b	84 b	100 a	60 b

[w] Conidia brushed onto cellophane, exposed to fungicide, and transferred to fungicide-free PDA.
[x] Percentage germination of 100 conidia based on exposure to fungicide amended medium first and then transferred on fungicide-free medium for evaluating germination and growth.
[y] Mean length of 10 germ-tube measurements. Germ tubes were considered inactivated if further growth after transfer onto fungicide-free medium did not occur.
[z] Values in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Growth of germinated *Monilinia fructicola* conidia on cellophane exposed to captan and prochloraz and transferred to fungicide-free PDA

	Germ-tube growth in μm[y]	
Fungicide (μg/ml)	Benomyl-resistant (78-4)	Benomyl-sensitive (1-65)
	Captan for 16 hr	Fungicide-free PDA for 24 hr
Control	⩾973 a[z]	⩾973 a[z]
Captan, 10	⩾973 a	⩾973 a
Captan, 1200	⩾973 a	⩾973 a
	Prochloraz for 4 hr	Fungicide-free PDA for 24 hr
Control	⩾973 a	⩾973 a
Prochloraz, 1	51 b	255 b
Prochloraz, 3	36 b	230 b
Prochloraz, 5	43 b	55 c
Prochloraz, 10	38 b	54 c

[y] Before transfer to fungicide-amended agar, germination percentage on cellophane in contact with PDA was about 95% and germ-tube length about 30 μm for benomyl-resistant and benomyl-sensitive strains of *M. fructicola*. Germ tubes were considered inactivated when the length did not reach more than 973 μm.
[z] Values in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Comparison of captan, benomyl, and prochloraz in control of blossom blight in a Loadel peach orchard with benomyl-sensitive and -resistant *Monilinia fructicola*

Treatment[v]	Timing[w]			Blossom blight[x] (%)	Blighted blossoms with conidia (%)	Conidia per blighted blossom[y] ($\times 10^3$)
		Petal fall				
	Pink bud	Initial	75%			
Control	···	···	···	57 e[z]	100	35.4 b
Benomyl	X	···	···	33 bc	80	11.4 ab
Benomyl plus Captan	X	···	···	26 bc	···	···
Captan	X	···	···	51 e	85	26.4 ab
Captan	···	X	···	48 de	95	75.9 c
Captan	X	···	X	36 bc	88	34.1 b
Prochloraz	X	···	···	1 a	48	7.5 a
Prochloraz	···	X	···	28 bc	60	3.9 a

[v] Chemical concentrations per liter are 0.5 g of prochloraz 50W, 0.6 g benomyl 50W, and 2.4 g captan 50W applied on 27 February 1980 (pink bud), 7 March 1980 (initial petal fall), and 10 March 1980 (75% petal fall). Blossoms were read and collected on 9 April 1980.
[w] Six single-tree replicates sprayed with handgun to drip stage.
[x] Mean of 175 blossoms per tree.
[y] Mean of 10 blighted blossoms on each of four trees.
[z] Values in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

unsprayed trees had 57% blossom blight. No reduction in blossom blight was experienced with a captan spray applied at pink bud before the anthers were showing or at the initial petal fall stage of bloom. A benomyl spray at pink bud significantly reduced blossom blight. The combination of captan plus benomyl did not provide any added benefits. Two applications of captan at pink bud and 75% petal fall significantly reduced blossom blight and control was equivalent to benomyl. The best treatment was prochloraz applied at pink bud, with only 1% blossom blight. A single prochloraz application at petal fall provided control equivalent to a single benomyl or benomyl plus captan spray at pink bud. Reduction in conidial production was significant with prochloraz but not with captan or benomyl treatments (Table 3).

Penetration of prochloraz from the epidermis through the mesocarp tissue to the pit area was indicated when *Monilinia* decay failed to occur on fruit dipped in prochloraz and injected with conidia.

DISCUSSION

The control of brown rot blossom blight in a peach orchard with benomyl-sensitive isolates of *M. fructicola* had been achieved with a single spray at pink bud stage of bloom in California. With the onset of benomyl-resistant isolates in 1977, growers had been using an alternative fungicide such as captan or a combination such as captan and benomyl. Because growers were reluctant to change spray timing from pink bud for benomyl to 30–40% bloom for captan, disease control was not satisfactory in many instances. Data presented here help explain the failure of the early captan application; growth of germinated conidia exposed to captan was not inhibited or suppressed on the captan-free medium. Furthermore, captan is not considered a fungicide with systemic activity capable of moving from the outer blossom parts to the inner susceptible anthers. Blossom petals or sepals are not sites for infection-causing blossom blight. Prochloraz, on the other hand, controlled blossom blight with a single pink-bud spray. The better disease control by prochloraz is also explained by its ability to suppress mycelial growth at 1 μg a.i./ml concentration and restrict germ-tube growth on conidia or germinated conidia that had been exposed to prochloraz and later transferred onto prochloraz-free medium. There is further indication that prochloraz is more active in reducing mycelial growth of benomyl-resistant isolates than benomyl-sensitive isolates. In addition, prochloraz-treated blossoms provided less inoculum for further infections than captan-treated blossoms. These mechanisms of action by prochloraz on benomyl-sensitive and -resistant *M. fructicola* mycelium and conidia and on the peach blossoms explain the superiority of prochloraz over captan. The bases for unsatisfactory control with captan or the combination of captan and benomyl in an orchard with high populations of benomyl-resistant isolates appears to be related to the timing of sprays and the relative ineffectiveness of captan.

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