Mycelial Growth, Peach Fruit-Rotting Capability, and Sporulation of Strains of *Monilinia fructicola* Resistant to Dichloran, Iprodione, Procymidone, and Vinclozolin

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ABSTRACT

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Among 22 dicarboximide-resistant and four sensitive strains of *Monilinia fructicola*, resistant strains produced smaller lesions or sporulated less, or both, on fungicide-treated and untreated peach fruits than did sensitive strains. Mixed inocula containing equal numbers of propagules of resistant and sensitive strains produced at least threefold more sensitive conidia than resistant conidia. Sensitive and resistant strains sporulated on untreated fruit, whereas no sporulation by sensitive or resistant strains was observed on fruit treated with iprodione. Mycelial growth of resistant strains on fungicide-amended (25 μg a.i. per milliliter) potato-dextrose agar was less on plates containing dichloran than of those containing iprodione, procymidone, or vinclozolin.

Additional key words: brown rot, dicarboximide fungicides, dichloronitroaniline fungicides.

In the past decade, fungal resistance to fungicides has become an increasingly important problem (1,3,4). Current recommendations for the prevention or delay of the development of resistant populations are to use fungicide mixtures or to alternate fungicides; however, the effectiveness of either strategy has not been experimentally confirmed (7,16). Success of those strategies may depend on parasitic fitness of resistant strains that may develop. With the exception of a few isolates, benomyl-resistant strains generally have been as fit as sensitive isolates (2,6,9,14,15,18). Similarly, strains of *Rhizopus stolonifer* (Fr.) Lind, and *Botrytis cinerea* Pers. ex Fr., resistant to 2,6-dichloro-4-nitroaniline (dichloran) did not differ significantly from sensitive strains in their ability to rot fruit (19,20).

Resistance in *Monilinia fructicola* (Wint.) Honey, to the dicarboximide fungicides iprodione, procymidone, and vinclozolin has been reported (12,17). Dicarboximide- and dichloran-resistant strains of *M. fructicola* can be readily isolated in the laborabory (12). Gullino and Garibaldi (5) reported that strains of *B. cinerea* resistant to vinclozolin produced fewer conidia and were less pathogenic to grapes than were sensitive strains, whereas Mariate et al (8) found similar pathogenicity between dicarboximide-sensitive and resistant strains of *B. cinerea*. Sztejnberg and Jones (17) reported that dicarboximide-resistant strains of *M. fructicola* were pathogenic to and sporulated on apricot and sweet cherry blossoms. Otherwise, the parasitic fitness of dicarboximide-resistant strains of *M. fructicola* has not been reported.

The purpose of the studies reported here was to investigate the parasitic fitness of laboratory strains of *M. fructicola* resistant to dichloran, iprodione, procymidone, and vinclozolin.

MATERIALS AND METHODS

Fungicides and media. The following fungicides were used: dichloran (Botran 75 W, the Upjohn Company, Kalamazoo, MI 49001); iprodione (Rovral 50W, RP 26019, Rhône-Poulenc Inc., Monmouth Junction, NJ 08852); procymidone (DPX-4424 50W, E. I. du Pont de Nemours & Co., Wilmington, DE 19898); and vinclozolin (Ronilan 50W, BAS 352-04F, BASF Wyandotte Corp., Parsippany, NJ 07054). Fresh fungicide suspensions were prepared in sterile distilled water and appropriate dilutions were added to autoclaved, warm (45–50 C) potato-dextrose agar (PDA) immediately prior to dispensing it into petri dishes. Cultures were grown and maintained on PDA (Difco Laboratories, Detroit, MI 48232).

Fungal strains. Four fungicide-sensitive isolates (S8, S10, S14, and S20) were isolated as single conidia from sporulating lesions on peach fruits (cultivar Redhaven) obtained from trees at the Sandhills Research Station, Jackson Springs, NC, and compared with 22 fungicide-resistant strains. The resistant strains were selected from the four fungicide-sensitive strains as previously described (12). The resistant strains derived from these sensitive isolates are designated by a letter R preceding the number of the parent isolate from which it was derived.

Mycelial growth on PDA. Three fungicide-sensitive and 16 fungicide-resistant strains of *M. fructicola* were tested for growth on fungicide-free PDA and on PDA amended with 25 μg a.i./ml of dichloran, procymidone, vinclozolin, or iprodione. Four 5-mm-diameter plugs of mycelium were cut from the margin of 7-day-old PDA cultures and transferred to the test media. These were incubated at 24 C without light and colony diameters were measured after 7 days.

Fungal fruit-rotting capability and sporulation. Four sensitive and 22 resistant strains were compared for ability to rot fungicide-free peach fruits (cultivar Correll). Fruits were surface disinfested by washing them in 10% Chlorox (0.525% NaOCl) for 30 sec then rinsing them in tap water. Four semi-ripe peaches were inoculated with each fungal strain by placing a 5-mm mycelial plug, taken from the margin of a 7-day-old PDA culture, into a 4-mm-diameter well in each fruit. The fruit were incubated on trays in plastic bags at 24 C in the dark. The lesion diameter was measured after 4 days, and an estimate of the surface area was calculated. The fruits were incubated an additional 2 days, then the number of conidia per lesion was determined by washing the surface of each sporulating lesion with 50 ml of distilled water containing 1% Tween-20. Conidia were counted with a hemacytometer. Sporulation on some lesions either was absent or too sparse to be detected by this

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method. Therefore, each lesion was observed for sporulation prior to washing.

Sporulation of resistant strains in mixed inoculations. Sporulation of fungicide-resistant strains in the presence of a sensitive strain was studied by applying mixed inocula to semi-ripe peach fruits (cultivar Clayton). The fruits were surface disinfested in 10% Chlorox (0.525% NaOCl) for 30 sec then rinsed in tap water. Three sensitive and five resistant strains were used with three replications per strain or strain combination. Inoculations were done by removing a 4-mm-diameter plug from each fruit and placing a 5-mm-diameter mycelial plug in the well. For the mixed inoculations, the 5-mm-diameter mycelial plug was cut in half, half of the well was inoculated with a resistant strain and the adjoining half was inoculated with the sensitive strain. The fruit were incubated for 8 days on trays in plastic bags at 24 C in the dark. The conidia were washed from the lesion surface and plated on PDA and PDA amended with 25 μg a.i. of iprodione per milliliter. After 12–15 hr of incubation at 24 C, 100 conidia per plate were randomly selected, and the number of germinating conidia was counted.

Fruit-rotting capability and sporulation of resistant strains on iprodione-treated fruits. Semi-ripe peaches (cultivar Clayton) were inoculated with 7-day-old mycelial plugs as described above. Six fruits were used per strain; prior to inoculation three fruits were dipped in a suspension of iprodione (0.469 g a.i./L) for 30 sec. Fruit were incubated on trays in plastic bags for 7 days at 24 C in the dark. Lesion diameter was measured and the amount of sporulation was rated. The rating system was 0–4 with 0 = no conidia observed and 4 = lesion completely covered with conidia.

RESULTS

Mycelial growth on fungicide-amended PDA. All sensitive and resistant strains grew on fungicide-free PDA. Except for S8 and S14 on procymidone-amended PDA, none of the sensitive strains grew on the amended media (Fig. 1). Growth, as measured by colony diameter, was variable among the resistant strains, being most evident on fungicide-amended PDA (Fig. 1). Although all resistant strains grew on the four fungicide-amended media, none grew as well on fungicide-amended PDA as on nonamended PDA except strains R10-1 on procymidone, R10-4 on all but dichloran, and R10-3 on procymidone and iprodione (Fig. 1). At 25 μg a.i./ml, the growth of resistant strains was inhibited the most by dichloran and least by procymidone (Fig. 1).

Fruit-rotting capability and sporulation. All strains caused fruit rot in nontreated fruit; however, only one resistant strain, R8-2, caused a lesion as large as that caused by its fungicide-sensitive parent strain (Table 1). No sporulation was observed for seven resistant strains; furthermore, only two of 22 resistant strains produced more conidia than were produced by their sensitive parent strains (Table 1).

The percentage of resistant conidia detected on lesions resulting from the mixed inoculation of fruit with resistant and sensitive strains ranged from 0 to 36, depending on the strain combination (Table 2).

Growth and sporulation of resistant and sensitive strains in iprodione-treated peach fruit. In iprodione-treated fruit, resistant strains caused larger lesions than did sensitive strains (Table 3); however, all resistant strains produced smaller lesions in iprodione-treated fruit than in untreated fruit. With the exception of strain R10-1, all sensitive and resistant strains sporulated on untreated fruit, whereas no sporulation by sensitive or resistant strains was observed on iprodione treated fruit (Table 3). Lesion development in iprodione-treated fruit mixed inoculated with a sensitive and resistant strain was similar to lesion development in fruit inoculated only with the resistant strain (Table 3).

DISCUSSION

Strains of *M. fructicola* resistant to dichloran, iprodione, procymidone, and vinclozolin produced smaller lesions or sporulated less, or both, on nontreated fruit than did sensitive parental strains. In mixed inoculations with resistant and sensitive

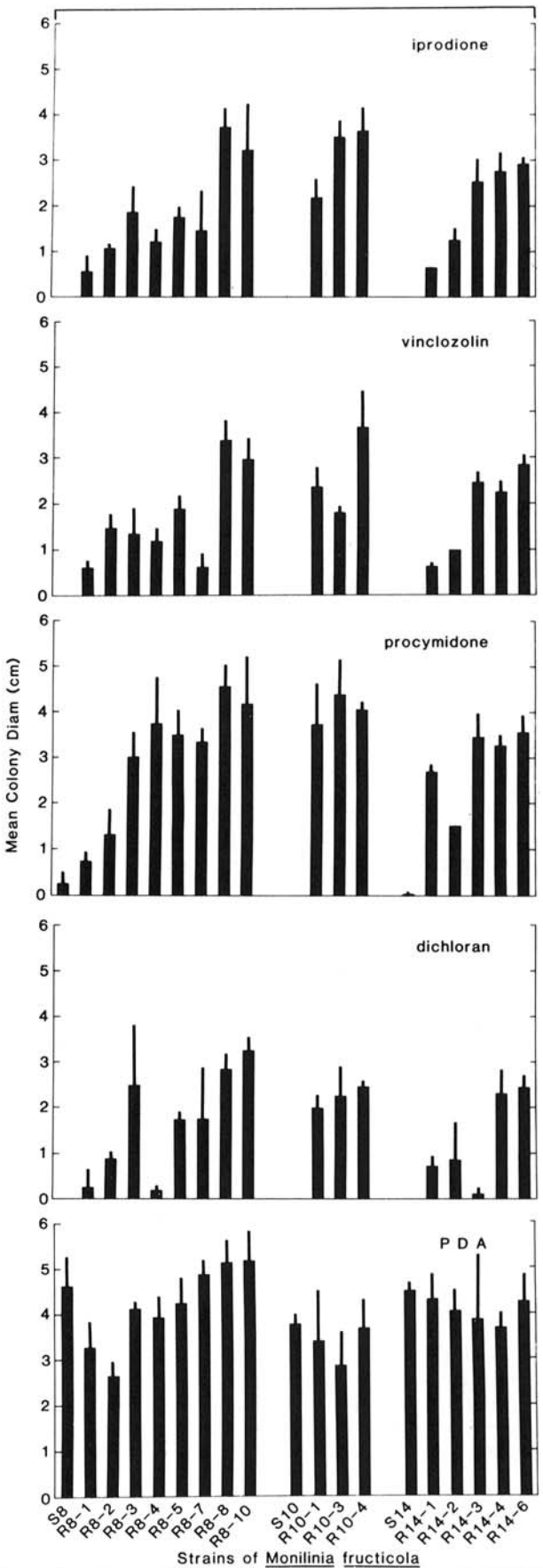

Fig. 1. Mycelial growth of three fungicide-sensitive (S) and 16 resistant (R) strains of *Monilinia fructicola* on nonamended potato-dextrose agar (PDA) and on PDA amended with 25 μg a.i. of fungicide per milliliter. Colony diameter was recorded after 1 wk incubation at 24 C in the dark. Results are the means of four replications per strain per medium ± the standard deviation.

TABLE 1. Peach fruit-rotting capability and sporulation of dicarboximide-sensitive (S) and -resistant (R) strains of *Monilinia fructicola* in fungicide-free fruit (cultivar Correll)

Strain	Lesion area[a] (cm^2)	Conidia per lesion[a] (no. × 1,000)
S8	12.3 ± 2.7	769 ± 591
R8-2	14.8 ± 2.9	3 ± 5
R8-3	3.8 ± 1.2	25 ± 27
R8-4	2.8 ± 0.5	+[b]
R8-5	3.5 ± 1.9	−
R8-6	4.3 ± 1.0	+
R8-7	4.6 ± 1.7	25 ± 27
R8-8	1.9 ± 0.1	+
R8-9	10.1 ± 1.5	16 ± 21
R8-10	2.7 ± 0.6	+
R8-11	2.8 ± 0.4	+
S10	9.2 ± 2.8	169 ± 85
R10-1	2.5 ± 0.2	6 ± 11
R10-2	3.2 ± 0.6	31 ± 54
R10-3	1.0 ± 0.1	−
R10-4	1.6 ± 0.5	−
S14	16.7 ± 4.5	+
R14-1	1.7 ± 0.2	−
R14-2	3.7 ± 0.6	−
R14-3	2.3 ± 0.7	3 ± 5
R14-4	1.7 ± 0.5	−
R14-5	6.0 ± 2.0	−
R14-6	3.5 ± 0.9	16 ± 21
S20	5.4 ± 1.6	109 ± 134
R20-1	1.8 ± 0.5	+
R20-2	2.0 ± 0.3	19 ± 33

[a] Means of four replications ± standard deviation.
[b] Symbols: + indicates conidia were observed on the lesion but were too few to detect with the assay, − indicates no conidia observed or detected with the assay. Conidia were counted 2 days after lesion measurements were recorded.

strains, the percent of resistant conidia produced by the resistant strain ranged from 0 to 36% of the total conidia produced on the lesion (Table 2). Fruit dipped in an iprodione suspension prior to inoculation with sensitive or resistant strains, or both, rotted only if resistant strains were used, but no sporulation was observed (Table 3). Rosenberger (13) also reported that some strains of *Penicillium expansum* Lk. ex Thom., resistant to iprodione and vinclozolin, were less pathogenic than wild-type isolates. Gullino and Garibaldi (5) reported that strains of *B. cinerea* resistant to vinclozolin produced fewer conidia and were less pathogenic to grapes than were vinclozolin-sensitive strains.

Strains resistant to one of the dicarboximide fungicides are cross-resistant to other members of this group (12,13,17). However, the data shown in Fig. 1 indicate differences in sensitivity to the four fungicides tested. Mycelial growth of the strains tested was more similar on PDA amended with iprodione and vinclozolin than with procymidone and dichloran. These observations may in part be explained by the fact that these fungicides have different molecular weights (dichloran MW = 207, iprodione MW = 340, procymidone MW = 278, and vinclozolin MW = 286), and the mycelial growth was compared at only one concentration (25 μg/ml). Rosenberger (13) reported that not all isolates of *P. expansum* resistant to vinclozolin were cross-resistant to iprodione. These fungicides apparently have at least one site of action in the fungus in common; however, there appear to be either multiple or secondary sites of action, which may be affected differently in different fungi or even fungi of the same species. Biochemical studies on mode(s) of action tend to support this hypothesis (10,11).

Thus, the dicarboximide-resistant strains of *M. fructicola* used in this study were less parasitically fit than were the sensitive parent strains. At present, no field experiments testing the fitness of dicarboximide-resistant strains of *M. fructicola* have been reported. Based on current data, it seems likely that if resistant strains of *M. fructicola* appeared, they would most likely not rapidly increase to a dominant level in the population. Also, since

TABLE 2. Percent of resistant conidia produced on fungicide-free peach fruits (cultivar Clayton) inoculated with dicarboximide-sensitive (S) and -resistant (R) strains of *Monilinia fructicola* or mixed inoculum of two strains

Strain or strain combination	Germination[a] (%)		Conidia resistant (%)
	PDA[b]	I-PDA	
S8	90 ± 4.1	0	0
R8-2	N[c]		−
R8-3	85 ± 7.5	85 ± 5.4	100
S8 + R8-2	96 ± 0.9	0	0
S8 + R8-3	95 ± 1.3	18 ± 2.5	19
S10	93 ± 2.9	0	0
R10-1	90 ± 7.1	90 ± 6.3	100
R10-2	N		
S10 + R10-1	89 ± 5.1	32 ± 2.6	36
S10 + R10-2	92 ± 0	0	0
S20	90 ± 2.1	0	0
R20-2	89 ± 3.9	75 ± 12.3	84
S20 + R20-2	90 ± 2.1	4 ± 1.3	4

[a] Means of three replications ± standard deviation.
[b] Conidia were plated on potato-dextrose agar (PDA) and PDA amended with 25 μg a.i. of iprodione per milliliter (I-PDA).
[c] N = no sporulation.

TABLE 3. Peach fruit-rotting capability and sporulation of dicarboximide-sensitive (S) and -resistant (R) strains of *Monilinia fructicola* in iprodione-treated fruits (cultivar Clayton)

Strain or strain combination	Iprodione-treated[a]		Untreated	
	Lesion area[b] (cm^2)	Sporulation[b] (0–4)[c]	Lesion area[b] (cm^2)	Sporulation[b] (0–4)[c]
Noninoculated	0.6 ± 0.1	0	0.7 ± 0.1	0
S8	0.6 ± 0.3	0	44.1 ± 29.8	2.3 ± 1.5
R8-4	4.9 ± 2.6	0	10.7 ± 0.9	0.7 ± 0.6
R8-5	3.9 ± 5.9	0	12.8 ± 1.7	1.0 ± 1.0
S8 + R8-4	2.0 ± 2.5	0	49.9 ± 25.1	3.0 ± 1.0
S8 + R8-5	2.4 ± 1.7	0	40.2 + 28.9	1.7 + 0.6
S10	0.4 ± 0.2	0	78.9 ± 21.0	1.7 ± 1.5
R10-1	11.7 ± 10.6	0	28.8 ± 21.9	0
S10 + R10-1	9.2 ± 2.5	0	44.4 ± 14.7	1.3 ± 0.6
S20	1.0 ± 0.7	0	14.1 ± 5.8	0.3 ± 0.6
R20-2	4.6 ± 3.1	0	13.2 ± 10.9	1.0 ± 1.0
S20 + R20-2	3.0 ± 1.8	0	34.0 ± 15.1	1.0 ± 1.0

[a] Fruit were dipped in a suspension of iprodione (0.469 a.i./L) for 30 sec prior to inoculation.
[b] Means of three replications ± standard deviation.
[c] Sporulation rating: 0 = no conidia observed and 4 = lesion surface covered with conidia.

sporulation of resistant strains was reduced in the absence of the fungicides, the resistant population should be rapidly diluted. Although some strains were eliminated in one sporulation cycle, it is not known whether the other resistant strains would also eventually be eliminated. However, resistant strains can be easily isolated in the laboratory (13); if they readily develop in the field then the selection pressure may be toward improved parasitic fitness.

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