Effect of Dichloran, Iprodione, Procymidone, and Vinclozolin on the Mycelial Growth, Sporulation, and Isolation of Resistant Strains of *Monilinia fructicola*

DAVID F. RITCHIE, Assistant Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27650

ABSTRACT

Ritchie, D. F. 1982. Effect of dichloran, iprodione, procymidone, and vinclozolin on the mycelial growth, sporulation, and isolation of resistant strains of *Monilinia fructicola*. Plant Disease 66: 484-486.

Incubation of *Monilinia fructicola* mycelial plugs on potato-dextrose agar (PDA) amended with dichloran, iprodione, procymidone, or vinclozolin (25 μg a.i./ml) reduced but did not always prevent mycelial growth. Incubation for 7 days on amended PDA followed by transfer to and incubation on nonamended PDA resulted in an initial increase of sporulation compared with the cultures transferred from nonamended PDA. Isolation of fungicide-resistant strains increased from 8.1% after 4 days to 42% after 21 days of incubation on amended PDA. No resistant strains were detected in cultures incubated on nonamended PDA. Strains resistant to one of the dichloronitroaniline fungicides were cross-resistant to the other dichloronitroaniline fungicides but not to benomyl or CGA-64251 (Vangard). The resistant strains produced a black mycelium; the wild-type strains produced brown mycelium.

Additional key words: aromatic hydrocarbon fungicides, brown rot, dicarboximides, stone fruits

Brown rot, caused by *Monilinia fructicola* (Wint.) Honey, is a destructive disease of stone fruits. Benomyl became the primary fungicide for brown rot control in the 1970s. However, the search for new fungicides was intensified in the mid-1970s after benomyl-resistant strains of *M. fructicola* were detected (5,12,16). The dicarboximide fungicides iprodione, procymidone, and vinclozolin are effective against *M. fructicola* and, like dichloran, are aromatic hydrocarbons or dichloronitroanilines. Also, iprodione was reported to control benomyl-resistant strains (12).

In 1978, Sztejnberg and Jones (13) reported strains of *M. fructicola* resistant to the dichloronitroaniline fungicides and that resistance to one resulted in cross-resistance to the others. Most mechanism(s) of action of these fungicides and characterization of resistant strains have been studied with *Rhizopus stolonifer* (14), *Botrytis cinerea* (6,9,15), or *Alternaria alternata* (7).

When mycelial disks of fungicide-sensitive isolates (wild type) of *M. fructicola* were incubated on fungicide-amended PDA, many isolates remained viable (11). Also, the frequency of isolation of fungicide-resistant strains increased as the incubation time in the presence of the fungicides increased.

The objective of this study was to examine the effects of dichloran, iprodione, procymidone, and vinclozolin on the mycelial growth, sporulation, and isolation of resistant strains of *M. fructicola*.

Paper 6955 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27650.

Use of trade names implies neither endorsement of the products by the North Carolina Agricultural Research Service nor criticism of similar ones not mentioned.

Accepted for publication 14 August 1981.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/82/06048403/$03.00/0

MATERIALS AND METHODS

Fungicides. The following fungicides were used: dichloran (Botran 75W, Upjohn Co., Kalamazoo, MI 49001), iprodione (Rovral 50W, Rhone-Poulenc Inc., Monmouth Junction, NJ 08852), procymidone (DPX-4424 50W, E. I. du Pont de Nemours & Co., Wilmington, DE 19898), and vinclozolin (Ronilan 50W, BASF Wyandotte Corp., Parsippany, NJ 07054). Two additional fungicides having different mechanisms of action were included for comparison: benomyl (Benlate 50W, E. I. du Pont de Nemours & Co., Wilmington, DE 19898) and an ergosterol biosynthesis inhibitor, CGA-64251 (Vangard 10W, Ciba-Geigy Corp., Greensboro, NC 27409).

Fresh fungicide suspensions were prepared in sterile distilled water and appropriate dilutions added to autoclaved, warm (45–50 C) potato-dextrose agar (PDA) immediately before pouring into petri dishes. Rates are given as active ingredient. PDA from the same lot number (654423, Difco Laboratories, Detroit, MI 48232) was used because fungal growth and sporulation may vary with the source (6).

Isolates. *M. fructicola* isolates 8-1, 10-1, 14-1, 20-1, and 22-1 were obtained from infected peaches from an orchard that had not been treated with fungicides. Isolations were made by washing conidia from sporulating lesions with distilled water and spreading 0.2-ml aliquots over the PDA surface. After 18-hr incubation at 24 C, germinating, single spores were selected.

Mycelial growth. Mycelial growth was determined by inoculating fungicide-amended or nonamended PDA with 5-mm-diameter mycelial-agar disks cut from the margin of 7- to 10-day-old cultures. Four disks per plate were used. The plates were incubated at 24 C without light for 2 wk and colony diameters measured. Fungicide suspensions were added before or after autoclaving, to test the effect of autoclaving on fungicidal activity.

Sporulation. Effect of the fungicides on sporulation after incubation of mycelial-agar disks on fungicide-amended substrates and nonamended PDA was studied. Two 5-mm-diameter mycelial-agar disks were transferred to PDA amended with dichloran, iprodione, procymidone, or vinclozolin and to nonamended PDA. After 1-wk incubation at 24 C without light, two pieces (approximately 2 mm^3) from each mycelial-agar disk were transferred to nonamended PDA and incubated at 24 C for 5 days without light. Four 5-mm-diameter disks per colony were cut from the area with the greatest visible sporulation, placed in 1 ml of distilled water plus 1% Tween 80 and vigorously agitated, and the number of conidia was counted by using a hemacytometer. One section (approximately 2 mm^3) from each colony was cut from the mycelium adjacent to the section that previously had been transferred to nonamended PDA, incubated for 5 days, and assayed for conidia. A third transfer and count also was done.

Isolation of resistant strains. Strains resistant to the dichloronitroaniline fungicides could be readily isolated after incubation of wild-type isolates on fungicide-amended PDA. The following experiment was done to determine whether the length of incubation time on fungicide-amended PDA would affect the frequency of isolation of resistant strains. Wild-type isolates 8-1, 10-1, 14-1, 20-1, and 22-1 were used. Four 5-mm-diameter mycelial-agar disks of each

isolate were transferred to amended PDA (25 μg/ml). After 4, 7, 14, and 21 days of incubation at 24 C without light, two 2-mm^3 sections from each mycelial-agar disk were transferred to nonamended PDA. After incubation on nonamended PDA for 1 wk, the transfers were observed for mycelial growth and black pigmented sectors of mycelium. Sections of mycelium were transferred to iprodione-amended PDA (25 μg/ml) to test for resistance.

RESULTS

Mycelial growth. Incubation of mycelial plugs of three wild-type isolates on fungicide-amended PDA for 2 wk reduced but did not prevent mycelial growth of 8-1, but vinclozolin and iprodione prevented growth of 10-1 and 14-1 (Table 1). Although mycelial growth did not always occur on fungicide-amended PDA, mycelial growth did occur after transfer to and incubation on nonamended PDA. No growth was observed after isolates were initially incubated on benomyl (10 μg/ml) or CGA-64251 (10 μg/ml), then transferred to and incubated on nonamended PDA. Growth of fungal colonies on dichloronitroaniline-amended PDA was raised and leathery, with sparse or no sporulation, compared with the flat, sporulating growth on nonamended PDA (Fig. 1). Autoclaving reduced the effectiveness of iprodione and vinclozolin (Table 1).

Sporulation. Whenever sections of mycelial-agar plugs that had been incubated on dichloronitroaniline-amended PDA were transferred to nonamended PDA, sporulation initially increased compared with cultures that had been incubated on nonamended PDA (Fig. 2). When two subsequent transfers were made to nonamended PDA, the level of sporulation was reduced to the level similar to that of the strains grown on nonamended PDA (Fig. 2). Although variability was large, a similar trend was observed with each isolate.

Isolation of resistant strains. When isolations were made from mycelium incubated on dichloronitroaniline-amended PDA 4, 7, 14, and 21 days after inoculation, 8.1, 11.3, 19.7, and 42.0% of the isolates, respectively, were resistant. No resistant strains were recovered from cultures on nonamended PDA.

The relationship between a particular fungicide and the percentage of strains resistant varied (Table 2). Resistant strains were more readily obtained from isolates 8-1, 14-1, and 22-1 than from isolates 10-1 and 20-1 (Table 2). Of 20 strains resistant to one of the dichloronitroaniline fungicides, all were cross-resistant to the other dichloronitroaniline fungicides but not cross-resistant to benomyl or CGA-64251. Resistant strains produced black mycelium compared with the brown mycelium of wild-type strains (Fig. 3). Resistant strains have remained resistant 1.5 yr.

DISCUSSION

The four dichloronitroaniline fungicides were considered fungistatic to mycelium

Table 1. Mycelial growth of three wild-type *Monilinia fructicola* isolates after 2-wk incubation on potato-dextrose agar (PDA) alone or amended with 25 μg/ml of autoclaved or nonautoclaved fungicide

	Mean colony diameter (cm)[a] of isolate					
	8-1		10-1		14-1	
PDA amendment	Autoclaved[b]	Not autoclaved	Autoclaved	Not autoclaved	Autoclaved	Not autoclaved
PDA alone	4.7 ± 0.2		5.0 ± 1.1		4.9 ± 1.2	
Procymidone	0.6 ± 1.3	0.8 ± 0.2	0.2 ± 0.4	1.4 ± 0.1	0.6 ± 1.0	1.3 ± 0.4
Dichloran	0.8 ± 1.0	1.4 ± 0.3	0.9 ± 1.2	1.4 ± 0.9	0.6 ± 0.4	1.1 ± 0.9
Vinclozolin	1.6 ± 0.8	0.2 ± 0.3	0.4 ± 0.1	0	0.7 ± 0.4	0
Iprodione	1.4 ± 0.3	0.3 ± 0.2	1.1 ± 0.1	0	1.7 ± 0.5	0

[a] Mycelial growth reported as the mean diameter of four colonies ± the standard deviation.
[b] Autoclaved fungicides were added to PDA before autoclaving; nonautoclaved fungicides were added to PDA after cooling to 40–50 C before pouring into petri plates.

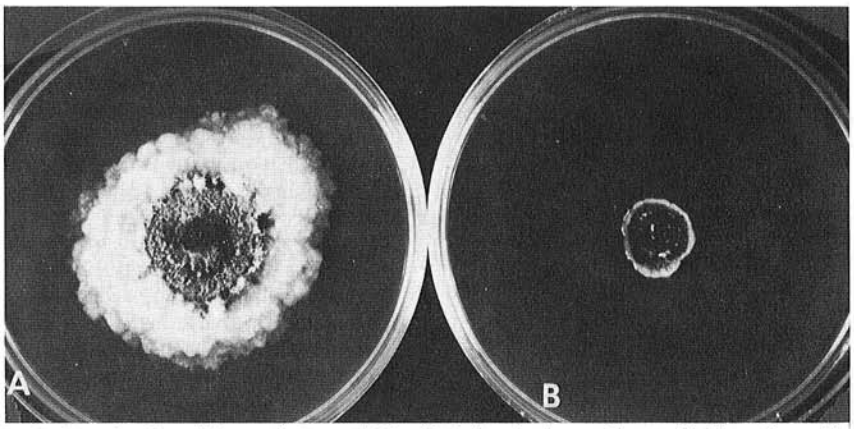

Fig. 1. Growth of a wild-type isolate of *Monilinia fructicola* sensitive to dichloronitroaniline fungicides on potato-dextrose agar **(A)** not amended and **(B)** amended with procymidone (25 μg/ml) after 10 days at 24 C without light.

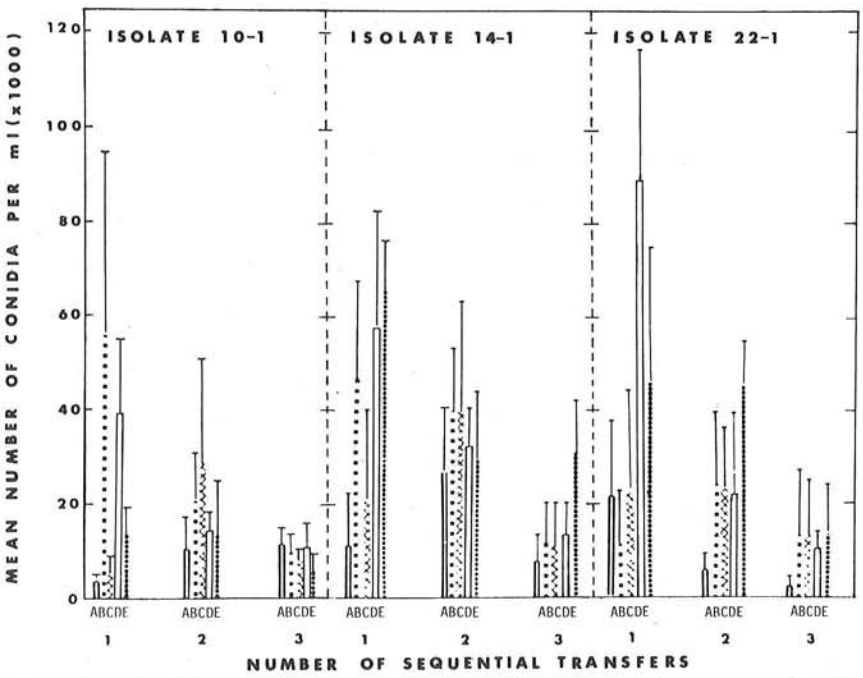

Fig. 2. Sporulation of three *Monilinia fructicola* isolates after 1-wk incubation on potato-dextrose agar: **(A)** not amended; amended with 25 μg/ml of **(B)** procymidone, **(C)** dichloran, **(D)** vinclozolin, or **(E)** iprodione, after three sequential transfers to nonamended potato-dextrose agar. Bars indicate standard deviation.

Table 2. Frequency of isolation of resistant strains of *Monilinia fructicola* after 3-wk incubation on potato-dextrose agar (PDA) amended with dichloronitroaniline fungicide (25 μg a.i./ml)

Fungicide	Percent of resistant strains[a]				
	8-1	10-1	14-1	20-1	22-1
PDA alone	0	0	0	0	0
Procymidone	6	0	0	38	38
Dichloran	13	0	31	0	50
Vinclozolin	13	0	13	0	44
Iprodione	38	0	31	0	31

[a]Sixteen samples per isolate-fungicide combination.

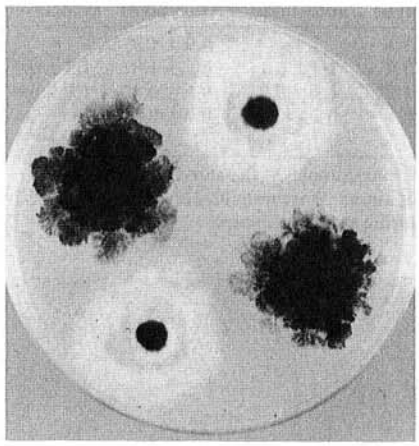

Fig. 3. Mycelial growth of a dichloronitroaniline-sensitive (wild-type) strain of *Monilinia fructicola* (light brown) and of a resistant strain (black growth) isolated from the sensitive strain. Cultures were incubated 8 days at 24 C.

of *M. fructicola* because sensitive strains of the fungus could be isolated after 2-wk incubation on fungicide-amended PDA. Vinclozolin was reported to be fungicidal in liquid medium but fungistatic on solid medium (2).

The cause of the raised, leathery growth produced after extended incubation on dichloronitroaniline-amended PDA is not known. McPhee suggested that iprodione may inhibit normal chitin metabolism in *A. alternata*, resulting in the lack of a rigid cell wall and abnormal spore germination (7). A similar phenomenon may occur in *M. fructicola* mycelium.

The initial increase in sporulation, after incubation on fungicide-amended PDA and subsequent transfer to nonamended PDA, was consistently observed. The mechanism for this increased sporulation is not known. It is not a permanent effect since sporulation returned to levels similar to the cultures not incubated on the amended PDA. Whether this would occur in the orchard is unknown.

Species of *Monilinia*, including *M. fructicola*, are heterokaryotic, often having 40 or more nuclei per hyphal cell (1). Continued exposure to the dichloronitroaniline fungicides combined with the ability of many isolates to survive in the presence of these fungicides could allow for the selection of resistant hyphae. A similar selection process to dichloran for resistant strains of *R. stolonifer* (14) and *B. cinerea* (15) has been reported.

Until the 1970s, fungicide resistance was rare except in members of the aromatic hydrocarbon group where resistance is the rule rather than the exception (4). Resistant strains of *M. fructicola* were readily observed as black sectors of mycelium occurring after incubation of wild-type isolates on amended PDA and subsequent transfer to and incubation on nonamended PDA. Using a diploid strain of *Aspergillus nidulans*, Georgopoulos et al (4) showed that fungicides having a site of action in the cell nucleus caused increased sectoring and that aromatic hydrocarbon fungicides were very effective in inducing such sectoring. Based on this, it could be speculated that, although other cellular sites are affected, the dichloronitroaniline fungicides may have a nuclear site of action.

Cross-resistance among the dichloronitroaniline fungicides presents a potential problem where dichloran is used for postharvest control of Rhizopus rot. Preharvest field sprays with the dichloronitroaniline fungicides to control *M. fructicola* could result in the selection of resistant strains that may remain undetected. Subsequent postharvest dichloran dips could then create an environment conducive for the increase in populations of resistant strains of *M. fructicola* or *Rhizopus* spp.

None of the four fungicides was more selective for resistant strains than any of the other three. A greater percentage of resistant strains was obtained from isolates 8-1, 14-1, and 22-1 than from isolates 10-1 or 20-1 (Table 2). This suggests that the frequency of isolation of resistant strains of *M. fructicola* depends more on a particular isolate of the fungus than on a particular dichloronitroaniline fungicide.

Mycelium of *M. fructicola* often becomes darkly pigmented in sections as it matures (1). This pigmentation is attributed to melanin or melaninlike substances (17). Strains resistant to the dichloronitroaniline fungicides were black after 5–7 days of incubation on nonamended PDA. McPhee reported a similar darkening of resistant strains of *A. alternata* (7). Rich and Horsfall reported that *M. fructicola* develops a dark color when exposed to sublethal doses of certain polyphenols (10). The dichloronitroaniline fungicides may stimulate a similar response.

Culture of *M. fructicola* mycelium on dichloronitroaniline-amended PDA caused the fungus to exhibit changes in mycelial growth, sporulation, and frequency of isolation of resistant strains. It remains to be proved whether similar changes also occur in the field.

ACKNOWLEDGMENT

I wish to thank Mitchell Bennett for technical assistance.

LITERATURE CITED

1. Byrde, R. J. W., and Willetts, H. J. 1977. The brown rot fungi of fruit—Their biology and control. Pergamon Press, New York. 171 pp.
2. Eichhorn, K. W., and Lorenz, D. H. 1978. Investigations on the action of vinclozolin against *Botrytis cinerea* in vitro. Z. Pflanzenkr. Pflanzenschutz. 85:449-460.
3. Georgopoulos, S. G., Kappas, A., and Hastie, A. C. 1976. Induced sectoring in diploid *Aspergillus nidulans* as a criterion of fungitoxicity by interference with hereditary processes. Phytopathology 66:217-220.
4. Georgopoulos, S. G., and Zaracovitis, C. 1967. Tolerance of fungi to organic fungicides. Annu. Rev. Phytopathol. 5:109-130.
5. Jones, A. L., and Ehret, G. R. 1976. Isolation and characterization of benomyl-tolerant strains of *Monilinia fructicola*. Plant Dis. Rep. 60:765-769.
6. Leroux, P., Fritz, R., and Gredt, M. 1977. Etudes en laboratoire de souches de *Botrytis cinerea* Pers., resistantes à la dichlozoline, au dichloran, au quintozene, à la vinchlozoline et au 26019 RP (ou Glycophene). Phytopathol. Z. 89:347-358.
7. McPhee, W. J. 1980. Some characteristics of *Alternaria alternata* strains resistant to iprodione. Plant Dis. 64:847-849.
8. Ogawa, J. M., Gilpatrick, J. D., Uyemoto, J. K., and Abawi, G. S. 1978. Variations in fungal growth on various preparations of potato-dextrose agar media. Plant Dis. Rep. 62:437-441.
9. Pappas, A. C., and Fisher, D. J. 1979. A comparison of the mechanisms of actions of vinclozolin, procymidone, iprodione and prochloraz against *Botrytis cinerea*. Pestic. Sci. 10:239-246.
10. Rich, S., and Horsfall, J. G. 1954. Relation of polyphenol oxidases to fungitoxicity. Proc. Natl. Acad. Sci. 40:139-145.
11. Ritchie, D. F. 1981. Effect of dichloran, iprodione, procymidone and vinclozolin on the growth and sporulation of *Monilinia fructicola*. (Abstr.) Phytopathology 71:252.
12. Szkolnik, M., and Gilpatrick, J. D. 1977. Tolerance of *Monilinia fructicola* to benomyl in western New York state orchards. Plant Dis. Rep. 61:654-657.
13. Sztejnberg, A., and Jones, A. L. 1978. Tolerance of the brown rot fungus *Monilinia fructicola* to iprodione, vinclozolin and procymidone fungicides. Phytopathology News 12:187.
14. Webster, R. K., Ogawa, J. M., and Moore, C. J. 1968. The occurrence and behavior of variants of *Rhizopus stolonifer* tolerant to 2,6-dichloro-4-nitroaniline. Phytopathology 58:997-1003.
15. Webster, R. K., Ogawa, J. M., and Bose, E. 1970. Tolerance of *Botrytis cinerea* to 2,6-dichloro-4-nitroaniline. Phytopathology 60:1489-1492.
16. Whan, J. H. 1976. Tolerance of *Sclerotinia fructicola* to benomyl. Plant Dis. Rep. 60:200-201.
17. Willetts, H. J. 1968. Factors influencing the production of stromata and microconidia by *Sclerotinia fructicola* (Wint.) Rehm. Ann. Bot. 32:219-223.