Adaptation of Mancozeb by *Bipolaris oryzae* and *B. sorokiniana*, the Causal Organisms of Brown Spot of Wild Rice

M. K. KARDIN, Former Research Assistant, and J. A. PERCICH, Assistant Professor, Department of Plant Pathology, University of Minnesota, St. Paul 55108

ABSTRACT

Kardin, M. K., and Percich, J. A. 1983. Adaptation of mancozeb by *Bipolaris oryzae* and *B. sorokiniana*, the causal organisms of brown spot of wild rice. Plant Disease 67:477-480.

Isolates of *Bipolaris oryzae* and *B. sorokiniana* from wild rice (*Zizania aquatica*) initially did not grow on potato-dextrose agar (PDA) amended with mancozeb higher than 100 μg/ml. Resistance of both species to mancozeb was obtained by transferring repeatedly to media containing increasing concentrations of the fungicide. After eight to nine passages on media amended with increasing concentrations of mancozeb, four isolates of *B. oryzae* and *B. sorokiniana* produced colonies that grew at 8,000 μg/ml of mancozeb. Growth and sporulation of mancozeb-resistant strains of both species on unamended PDA were reduced compared with the wild types. Resistance to mancozeb was lost after subculturing once on fungicide-free media. The similarity of sensitivity of a limited number of isolates of these fungi from fungicide-treated or untreated fields to mancozeb suggested that resistance to this fungicide did not occur in the field.

Brown spot of wild rice (*Zizania aquatica* L.) incited by *Bipolaris oryzae* (Breda de Haan) Shoemaker (teleomorph: *Cochliobolus miyabeanus* (Ito & Kuribayashi) Drechs. ex Dastur) and *B. sorokiniana* (Sacc.) Shoemaker (teleomorph: *C. sativus* (Ito & Kuribayashi) Drechs. ex Dastur) has caused widespread, recurring, and severe losses in cultivated fields of wild rice (3). Mancozeb (Dithane M-45), the only registered fungicide used in Minnesota to control fungal diseases of wild rice, has been used consecutively in many wild rice fields for 4 yr.

There is no clear evidence for fungal resistance to ethylene bisdithiocarbamate fungicides in the field. Lorbeer and Ellerbrock (4) suggested that failure of mancozeb to control Botrytis leaf blight (*Botrytis squamosa* Walker; teleomorph: *Sclerotinia squamosa* ((Viennob-Bouegin) Dennis) of onion (*Allium cepa* L.) was due to formation of mancozeb-resistant strains of the pathogen. However, they found no resistance of the pathogen to the fungicide in in vitro tests. Smiley (11) also reported a significant increase in severity of dollar spot and Fusarium patch on turf treated with mancozeb, but he did not mention whether it was caused by development of strains of the pathogen resistant to the fungicide or by other factors.

Fungal resistance to ethylene bisdithiocarbamate fungicides has been observed mainly in the laboratory by growing fungi on media amended with the chemical (1,2,6–10). Resistance to these compounds often was unstable and was lost after several passages of the fungus through fungicide-free media (1,2,6,8,9).

Sen Gupta and Das (10) obtained strains of *B. oryzae* resistant to mancozeb by repeated transfers of fungal conidia to increasingly higher fungicide suspensions. They reported that their mancozeb-resistant strains caused more infections than did mancozeb-sensitive strains when used to inoculate rice (*Oryza sativa* L.) seedlings treated with mancozeb. Similarly, Rana and Sen Gupta (7) obtained strains of *B. oryzae* that were resistant to mancozeb in both in vitro and in vivo tests.

The objectives of this study were to explore the possibility of developing strains of *B. oryzae* and *B. sorokiniana* resistant to mancozeb and to describe and compare the morphological and physiological characters of mancozeb-sensitive and -resistant strains of these fungi.

MATERIALS AND METHODS

Development of fungal resistance. Various concentrations of mancozeb-water suspension were mixed with sterilized Difco potato-dextrose agar (PDA) (1:19, v/v) to obtain the desired fungicide concentration. Single-conidial isolates of *B. oryzae* and *B. sorokiniana* isolated from non-fungicide-treated wild rice plants in 1972 were used in all experiments.

Eight isolates of *B. oryzae* and five isolates of *B. sorokiniana* were tested on PDA amended with mancozeb. All isolates were cultured on PDA at 28 C for 10 days in the dark. Mycelial disks (4 mm diam.) from the periphery of the cultures were transferred onto unamended PDA and amended PDA with each fungicide at 1, 10, 100, and 1,000 μg/ml. The highest concentration on which the fungi grew after each transfer was observed at 3 wk. The colonies growing at the highest concentrations were transferred to media of the same fungicide concentration and to PDA containing higher concentrations of the fungicide. Fungal isolates growing at fungicide concentration of 100 but not at 1,000 μg fungicide per milliliter were transferred to PDA containing 100, 200, 400, 800, and 1,000 μg/ml. Isolates growing at 1,000 μg fungicide per milliliter were subsequently transferred to PDA containing 1,000, 2,000, 4,000, 6,000, 8,000, and 10,000 μg/ml, depending on the amount of available mycelia. Each fungal isolate involved in the adaptation procedure had eight to nine passages through a fungicide-amended medium.

Five isolates of *B. oryzae* and five isolates of *B. sorokiniana* were used to obtain strains resistant to mancozeb by selection. One milliliter of conidial suspension (10^5–10^6 conidia/ml) of each isolate was spread on the medium containing mancozeb at 100 and 1,000 μg/ml. The number of colonies that grew on each plate inoculated at 28 C in the dark were observed at 7-day intervals for 3 wk.

Retention of resistance to mancozeb. Mycelial disks (4 mm diam.) taken from mancozeb-resistant and -sensitive strains of *B. oryzae* and *B. sorokiniana* were placed in the centers of plates containing PDA and grown at 28 C in the dark for 3 wk. Each isolate was replicated three times. After 3 wk, mycelial disks were taken from the periphery of the colony and transferred to PDA containing mancozeb at 2,000 μg/ml and to PDA containing the same concentration of the fungicide on which the resistant strains had been cultured previously. Four

Paper No. 12,261, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

Accepted for publication 29 October 1982.

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.

mycelial disks were placed in each plate (three plates per isolate).

Growth rate and cultural characters of mancozeb-resistant strains on PDA. Mycelial disks of mancozeb-resistant and -sensitive strains of *B. oryzae* and *B. sorokiniana* were placed in the centers of plates containing PDA. Each isolate was replicated three times and the experiment was done twice. The growth rate of each isolate was observed 2 wk after transfer and the cultural characters were described and compared with the original isolates.

Monitoring for field resistance to mancozeb. Diseased leaves of wild rice were collected from nine growers' fields where mancozeb had been used consecutively for 1–4 yr, from four growers' stands that had never been treated, and from six natural stands. These leaves were cut into small sections (5 × 5 mm), surface-sterilized with a mixture of 95% ethanol and 5% NaOCl (1:1, v/v) for 15–20 sec, rinsed twice with sterile water, drained, and placed on PDA. Colonies of *B. oryzae* and *B. sorokiniana* were obtained by this technique and then single conidia were obtained for each isolate with a micromanipulator.

The sensitivity of each isolate of *B. oryzae* and *B. sorokiniana* to the fungicide was evaluated by a modified technique for simultaneous determination of fungicidal and fungistatic properties of a fungicide as described by Neely and Himelick (5). Fungicidal properties of mancozeb were evaluated at 2,500, 500, 100, 20, 5, and 1 μg/ml and its fungistatic properties were evaluated at 100, 20, 5, 1, 0.2, and 0.04 μg/ml.

Isolates of *B. oryzae* and *B. sorokiniana* were incubated on PDA for 2 wk at 28 C under near-ultraviolet light and a 12-hr day. The conidial suspension was adjusted to 15–30 conidia per field observation at ×100 and dropped onto cellophane disks with a 25-μl micropipet. The conidial suspension was prepared not more than 15 min before seeding the cellophane disks. The experiment was repeated twice and the fungicidal and fungistatic concentration of mancozeb for each isolate was determined from the mean value.

RESULTS

Resistance of *B. oryzae* and *B. sorokiniana* to mancozeb was obtained by successive transfers to media mixed with increasingly higher concentrations of the fungicide (Table 1). In the first transfer, all five isolates of *B. sorokiniana* grew at a mancozeb concentration of 100 μg/ml, but only five of seven isolates of *B. oryzae* grew at this concentration (Table 1). None of these isolates grew at 1,000 μg fungicide per milliliter. After eight to nine passages on medium amended with increasing concentrations of mancozeb, however, four isolates of *B. oryzae* and *B. sorokiniana* grew on medium containing the fungicide at 8,000 μg/ml. Neither *B. oryzae* nor *B. sorokiniana* produced mancozeb-resistant strains by selection on media containing mancozeb at 100 and 1,000 μg/ml except isolate Bs 36, which produced one colony at 100 μg/ml.

All mancozeb-resistant strains of *B. oryzae* and *B. sorokiniana* that grew at concentrations >2,000 μg/ml of the fungicide changed in their cultural characters. Mycelial growth was very limited and few or no conidia were

Table 1. The highest concentrations of mancozeb that *Bipolaris oryzae* and *B. sorokiniana* could grow on at the beginning and at the end of the adaptation period[a]

Isolate	Fungicide concentration (μg/ml)		
	1st Transfer (lowest concentration)	9th Transfer (highest concentration)	10th Transfer (highest concentration)
B. oryzae			
8 GE	100	...	6,000
11 GU	100	...	6,000
10 GT	100	...	8,000
67	100	...	6,000
T92	100	...	8,000
64	10	...	...[b]
6 GF	10	...	...
B. sorokiniana			
36	100	8,000	...
58	100	8,000	...
66	100	4,000	...
66-1	100	4,000	...
1 GM	100	...	...

[a]Cultures were grown for 3 wk at 28 C in the dark on mancozeb-amended potato-dextrose agar before being transferred to a higher concentration of the fungicide.
[b]Failed to grow and was lost during the course of the experiment.

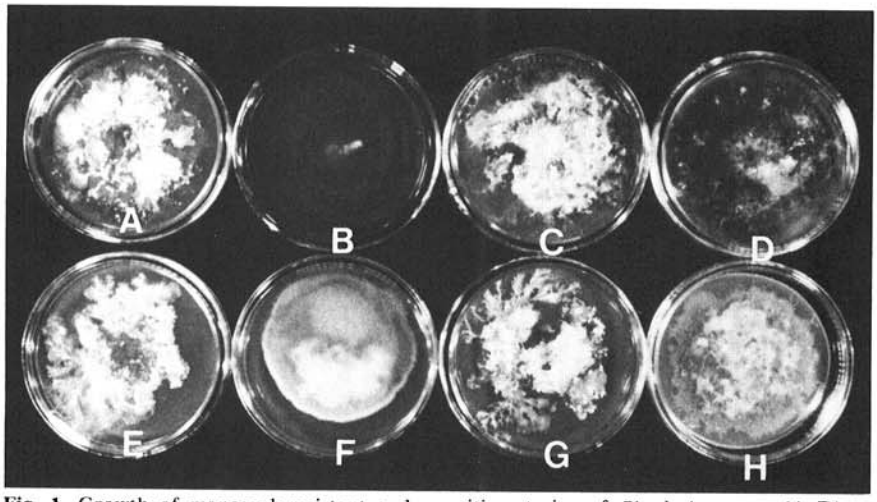

Fig. 1. Growth of mancozeb-resistant and -sensitive strains of *Bipolaris oryzae* **(A–D)** on mancozeb-free potato-dextrose agar (PDA) and **(E–H)** on PDA containing mancozeb at 100 μg/ml: **(A and E)** strain that grew at 8,000 μg/ml of mancozeb, **(B and F)** strain that grew at 6,000 μg/ml of mancozeb, **(C and G)** strain that grew at 2,000 μg/ml of mancozeb, and **(D and H)** mancozeb-sensitive wild-type strain.

Table 2. Mycelial growth and sporulation of mancozeb-resistant and -sensitive strains of *Bipolaris oryzae* and *B. sorokiniana* on mancozeb-free potato-dextrose agar

Isolate	Sporulation	Diameter (cm) of fungal colonies[a]
B. oryzae		
37-D8000 III (R)[b]	+[c]	3.6
8GE-D6000 II (R)	+	7.9
T92-D4000 III (R)	+	7.5
T92-D2000 I (R)	+	5.1
T92 (S)[d]	++[e]	8.3
37 (S)	++	8.8
10GT (S)	++	7.8
B. sorokiniana		
36-D8000 II (R)	+	5.4
36-D8000 I (R)	+	5.6
58-D4000 II (R)	+	5.6
36-D2000 I (R)	+	5.0
36 select I (R)	++	7.3
36 (S)	++	7.1
58 (S)	++	8.0

[a]Average from two experiments each with three replicates grown at 28 C in the dark for 2 wk after transfer.
[b]R = Mancozeb-resistant strain.
[c]+ = Very poor or no sporulation.
[d]S = Mancozeb-sensitive strain.
[e]++ = Abundant sporulation.

produced. Most of mancozeb-resistant strains of *B. oryzae* and *B. sorokiniana* grew slower than the wild types when they were transferred back to fungicide-free medium (Table 2). On PDA unamended and PDA amended to contain mancozeb at 100 μg/ml, nearly all mancozeb-resistant strains produced white colonies and sporulated poorly. Only one mancozeb-resistant strain of *B. sorokiniana* obtained by selection still retained the cultural characters of its original parent strain on both unamended and amended PDA (Figs. 1A,E and 2B,E).

All mancozeb-resistant strains of *B. oryzae* and *B. sorokiniana* lost their resistance to mancozeb after one passage through the fungicide-free medium, whereas the mancozeb-resistant strain 36 S of *B. sorokiniana* obtained by selection still retained its resistance to mancozeb after at least one passage through the fungicide-free medium (Table 3).

Twenty isolates of *B. oryzae* and six of *B. sorokiniana* were obtained from diseased tissue taken from nine growers' fields that had been sprayed regularly with mancozeb and two isolates of *B. oryzae* and six of *B. sorokiniana* from growers' fields or natural stands not sprayed with mancozeb. Concentrations of the fungicide fungistatic or fungicidal to both groups of isolates were 5–20 and >2,500 μg/ml, respectively. These concentrations were not essentially different from comparable values for selected check cultures that had never been selected for resistance to mancozeb.

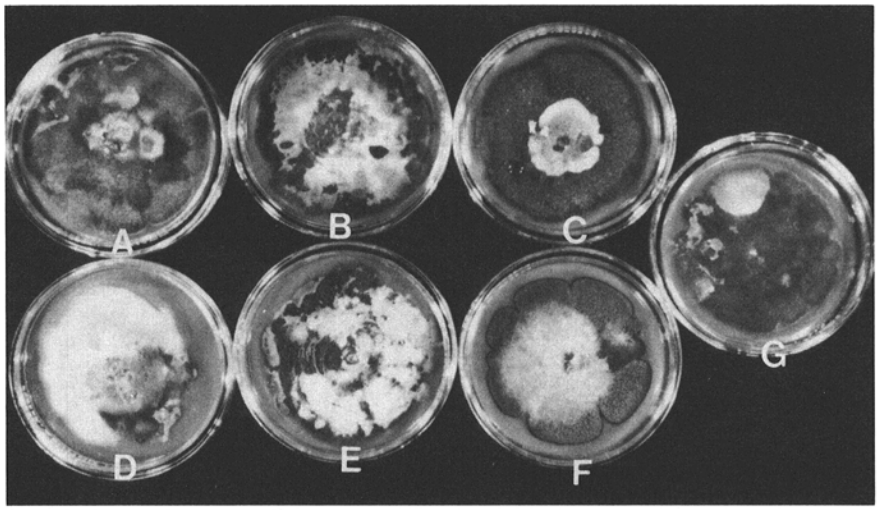

Fig. 2. Growth of mancozeb-resistant and -sensitive strains of *Bipolaris sorokiniana* on **(A–C)** mancozeb-free potato-dextrose agar (PDA) and **(D–F)** on PDA containing mancozeb at 100 μg/ml: **(A and D)** strain that grew at 8,000 μg/ml of mancozeb, **(B and E)** strain that grew at 4,000 μg/ml of mancozeb, **(C and F)** strain that grew at 2,000 μg/ml of mancozeb, and **(G)** mancozeb-sensitive wild-type strain on fungicide-free PDA.

Table 3. Retention (+) or loss (−) of resistance of *Bipolaris oryzae* and *B. sorokiniana* to mancozeb

Isolates	Conc. of mancozeb (μg/ml) on which fungi were previously cultured	Growth on potato-dextrose agar amended with increasing conc. of mancozeb (μg/ml)				
		100	2,000	4,000	6,000	8,000
B. oryzae						
37-D8000 I (R)[a]	0	+	−[b]	...	...	−
37-D8000 I (R)	8,000	+	...	...	...	+
8GE-D6000 III (R)	0	+	−	...	−	...
8GE-D6000 III (R)	6,000	+	...	...	+	...
T92-D4000 III (R)	0	+	−	−	...	...
T92-D4000 III (R)	4,000	+	...	+	...	...
T92-D2000 I (R)	0	+	−	...	...	...
T92-D2000 I (R)	2,000	+	+	...	...	...
T92 (S)	0	+	−	...	...	...
37 (S)	0	+	−	...	...	...
10GT (S)	0	+	−	...	...	...
B. sorokiniana						
36-D8000 II (R)	0	+	−	...	...	−
36-D8000 II (R)	8,000	+	...	...	...	+
36-D8000 I (R)	0	+	−	...	...	−
36-D8000 I (R)	8,000	+	...	...	...	+
58-D4000 II (R)	0	+	−	−	...	...
58-D4000 II (R)	4,000	+	...	+	...	...
58-D2000 I (R)	0	+	−	...	...	...
58-D2000 I (R)	2,000	+	+	...	...	...
36 select I (R)	0	+	...	...	...	...
36 select I (R)	100	+	...	...	...	...
36 (S)	0	+	−	...	...	...

[a] R = mancozeb-resistant strains and S = mancozeb-sensitive strains.
[b] − = No growth on this concentration of mancozeb and + = growth on all concentrations up to this one.

DISCUSSION

Isolates of *B. oryzae* and *B. sorokiniana* can develop resistance to mancozeb after being transferred and grown on a succession of media containing increasing concentrations of the fungicide. The resistance to the fungicide was accompanied by loss of fitness, mainly reduction in sporulation.

Resistance of *B. oryzae* and *B. sorokiniana* to mancozeb was not retained even after only one passage through fungicide-free medium. The inability of fungi to retain resistance to ethylene bisdithiocarbamate fungicides has also been reported by other workers, but in those studies, the resistance to the fungicide was lost after several passages through fungicide-free media (1,2,6,8). Anilkumar (1) reported that the resistance of *Sclerotium rolfsii* Sacc. to mancozeb was reduced after five serial transfers on fungicide-free medium. Rana and Sen Gupta (6) also reported that *B. oryzae* lost its resistance to zineb (Dithane Z-78) after six transfers in fungicide-free medium. The rapid loss of resistance in this study was probably due to the higher concentrations of mancozeb used in our experiments. Concentrations of fungicides used by other workers were always below 1,000 μg/ml (1,2,6,8).

The comparison of sensitivity to mancozeb between isolates of *B. oryzae* or *B. sorokiniana* from fields consecutively treated with the fungicide and from untreated fields and natural stands indicates that resistance to this fungicide did not occur in the treated fields. However, failure to demonstrate differences among these fungal isolates because of the limited numbers tested and the insensitivity of the technique used should not be excluded.

LITERATURE CITED

1. Anilkumar, T. B. 1976. Adaptation of *Sclerotium rolfsii* Sacc. to fungicides. Mysore J. Agric. Sci. 10:77-82.
2. Anilkumar, T. B., and Sastry, M. N. L. 1979. Development of tolerance to fungicides in *Rhizoctonia bataticola*. Phytopathol. Z. 94:121-136.
3. Bean, G. A., and Schwartz, R. 1961. A severe epidemic of *Helminthosporium* brown spot disease on cultivated wild rice in northern Minnesota. Plant Dis. Rep. 45:901.
4. Lorbeer, J. W., and Ellerbrock, L. A. 1976. Failure of ethylene bisdithiocarbamates to control Botrytis leaf blight of onion. Proc. Am.

Phytopathol. Soc. 3:75-84.
5. Neely, D., and Himelick, E. B. 1966. Simultaneous determination of fungistatic and fungicidal properties of chemicals. Phytopathology 56:203-209.
6. Rana, J. P., and Sen Gupta, P. K. 1977. Adaptation of two pathogenic fungi to some fungicides. Z. Pflanzenkr. Pflanzenschutz. 84:738-742.
7. Rana, J. P., and Sen Gupta, P. K. 1979. In vivo fungicide tolerance and pathogenicity of some fungicide adapted isolates of *Helminthosporium oryzae*. Proc. Ind. Acad. Sci. 88:25-28.
8. Raymundo, S. A., and Exconde, O. R. 1974. Tolerance of three isolates of *Helminthosporium maydis* Nisikado and Miyake to four fungicides. Philipp. Agric. 58:1-16.
9. Sankhla, B., Sankhla, H. C., and Dalela, G. G. 1970. Adaptations of *Alternaria triticina* Prasada and Prabhu to fungicides. Rajasthan J. Agric. Sci. 1:116-119.
10. Sen Gupta, P. K., and Das, S. N. 1971. Induced tolerance of spores of *Helminthosporium oryzae* Breda de Haan to fungicides. Curr. Sci. 40:168-169.
11. Smiley, R. W. 1981. Nontarget effects of pesticides on turf grasses. Plant Dis 65:17-23.