Resistance of *Bipolaris oryzae* to Fenapanil

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

ABSTRACT

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Fenapanil-resistant strains of *Bipolaris oryzae* were obtained by successive transfers on a series of fenapanil-amended potato-dextrose agar media containing increasing concentrations of the fungicide or by selection from a conidial suspension on a medium containing 100 μg/ml of fenapanil. The ability of the fungus to acquire resistance to fenapanil was accompanied by a reduction in its fitness for survival and virulence. Generally, strains with a greater degree of fenapanil resistance grew slower and produced fewer conidia than strains with less resistance to fenapanil. Most of the fenapanil-resistant strains of *B. oryzae* declined in their resistance to fenapanil after one passage on a fungicide-free medium. Both fenapanil-resistant and -sensitive strains were effectively controlled on fenapanil-treated plants in the greenhouse; however, the fanapanil-sensitive strain was more virulent on untreated plants.

Fenapanil is a new systemic fungicide developed by Rohm and Haas Co. (4). Fenapanil has been reported by Edgington et al (6) and Martin (8) to be systemic. In barley (*Hordeum vulgare* L.), the fungicide was translocated only apoplastically, but in soybean (*Glycine max* (L.) Merr.) and cucumber (*Cucumis sativus* L.), limited symplastic movement occurred (8).

Fenapanil is effective in controlling several diseases of barley such as seedling blight and spot blotch caused by *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., loose smut (*Ustilago nuda* (Jens.) Rostr.), and powdery mildew (*Erysiphe graminis* (DC.) Merat f. sp. *hordei* Marchal) (5,8,9). Two applications of fenapanil at a 7- to 20-day interval during the period from primary infection to early logarithmic increase also gave good control against stem rust on spring wheat (13). In the greenhouse, the fungicide was also effective against *B. oryzae* (Breda de Haan) Shoem., the cause of brown spot of wild rice (*Zizania aquatica* L.) (*unpublished*).

Fenapanil, imazalil (2,17), and triadimefon (1,3) belong to the azole group of fungicides. These fungicides and also triarimol (10–12,16) inhibit ergosterol biosynthesis in fungi. There is no known report of fungi with resistance to fenapanil. Resistance to other ergosterol biosynthesis inhibitor fungicides has been obtained, however, using mutagenic agents (7,18) or by spontaneous mutation (7,14,18). In this study, the development of resistance of *B. oryzae* to fenapanil in the laboratory and the morphological-physiological characteristics of fungicide-resistant strains is reported.

MATERIALS AND METHODS

Development of fenapanil resistance by successive transfers and selection. Various concentrations of fenapanil in sterile distilled water were mixed with sterilized Difco potato-dextrose agar (PDA) at 45 C (1:19, v/v) to obtain the desired fungicide concentration (μg/ml) in the medium. Five single-conidial cultures of *B. oryzae* were isolated from wild rice in 1972. Fungal cultures were

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Table 1. The highest tolerable concentration of fenapanil in amended potato-dextrose agar on which *Bipolaris oryzae* could grow at the first and eighth successive transfers[a]

Strain[b]	Greatest fenapanil conc. (μg/ml) permitting growth	
	First transfer[c]	Eighth transfer
8GE	100	800
11GU	100	400
10GT	100	400
67	100	600
T92	100	800

[a]The fungus was allowed to grow on potato-dextrose agar medium amended with fenapanil for 3 wk before being transferred to media with higher concentrations of fenapanil.
[b]Obtained from wild rice.
[c]Growth was limited (diam. 1.5–2 cm after 3 wk).

grown on PDA and incubated at 28 C under continuous darkness.

Mycelial disks (4 mm diam.) taken from the periphery of 10-day-old cultures of *B. oryzae* were transferred onto PDA media containing 0, 1, 10, 100, and 1,000 μg/ml of fenapanil. After 3 wk of growing on the amended medium containing the highest tolerable concentration of the fungicide, the strains were again subcultured on a medium containing the same fungicide concentration and on a series of media containing higher concentrations of the fungicide. The successive-transfers procedure was repeated up to eight serial transfers on a medium amended with fenapanil.

To obtain fenapanil-resistant strains by selection, 1 ml of conidial suspension ($4–8 \times 10^5$ conidia/ml) of each isolate of *B. oryzae* was dropped and spread on the surface of a PDA plate containing 100 and 200 μg/ml of fenapanil (three plates per isolate/fungicide conc.). The number of colonies that grew on each plate were recorded at weekly intervals for 3 wk.

Retention of resistance to fenapanil. Mycelial disks from fenapanil-resistant strains of *B. oryzae* were subcultured on PDA (one disk per plate, three plates per isolate). After 3 wk, mycelial disks were taken from the periphery of the fungal colonies and transferred to PDA containing 100 μg/ml of fenapanil and to PDA containing the same concentration of the fungicide on which the fenapanil-resistant strains had been cultured previously (four disks per plate, three plates per isolate). Checks were mycelial disks taken from the fenapanil-resistant strains that were continuously cultured on medium amended with fenapanil. Results were observed weekly for 3 wk.

Growth rate and virulence of fenapanil-resistant and -sensitive strains of *B. oryzae*. The growth rate of fenapanil-resistant and -sensitive strains of *B. oryzae* was recorded on unamended PDA and on PDA amended with 100 μg/ml of fungicide. Mycelial disks were removed from fenapanil-resistant and -sensitive strains and placed in the centers of plates containing the test medium. After 2 wk, colony diameters were measured and the cultural characters of the fenapanil-resistant and -sensitive strains of *B. oryzae* were determined on both unamended and amended PDA containing 100 μg/ml of fungicide. The virulence of two fungicide-resistant strains of *B. oryzae* was determined on fungicide-treated and untreated wild rice plants in the greenhouse. One original fenapanil-sensitive isolate of the fungus served as a control. Each treatment consisted of five replicates and the experiment was done twice.

The fenapanil-resistant and -sensitive strains of *B. oryzae* were cultured for 3 wk on PDA containing 100 μg/ml of fenapanil and on unamended PDA, respectively. Plants of the Netum cultivar at early boot stage (three plants per pot, five pots per treatment) were sprayed with 20 ml of fenapanil suspension (1.2 ml/L) per pot. The fungicide-treated and untreated plants were then inoculated with a conidial suspension of *B. oryzae* (about 20,000 conidia/ml, 20 ml of conidial suspension per pot). All the inoculated plants were placed in a moist chamber at 100% RH and 30 ± 2 C in the greenhouse for 7 days.

An average disease index (DI) on a scale of 0–9 with increasing severity, where 0 = no leaf lesions, 1 = <1%, 3 = 1–4%, 5 = 5–24%, 7 = 25–50%, and 9 = >50% leaf area infected, was used to evaluate the virulence of *B. oryzae*.

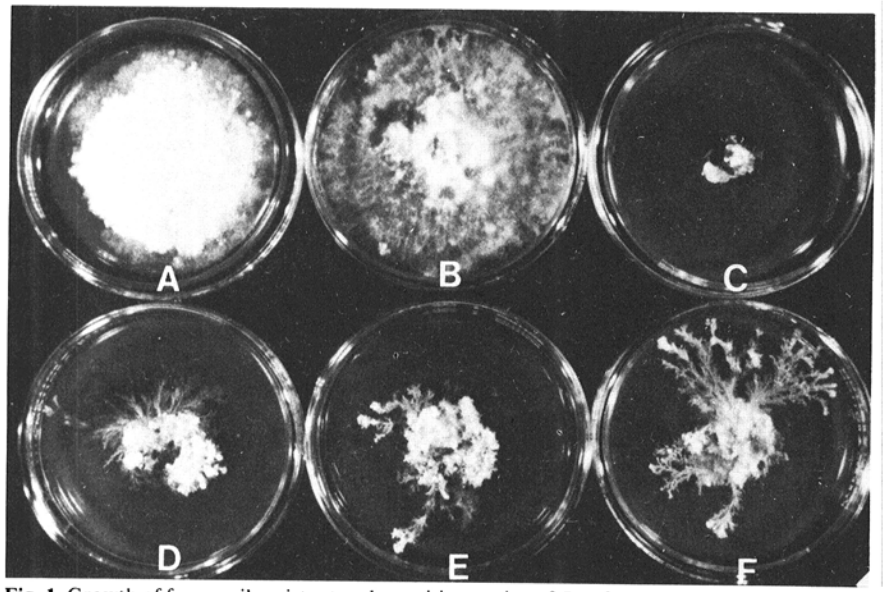

Fig. 1. Growth of fenapanil-resistant and -sensitive strains of *Bipolaris oryzae* on potato-dextrose agar medium containing 100 μg/ml of fenapanil. **(C)** Sensitive wild type strain. Resistant strains grew at **(B)** 100, **(A)** 200, **(F)** 400, **(E)** 600, and **(D)** 800 μg/ml of fenapanil.

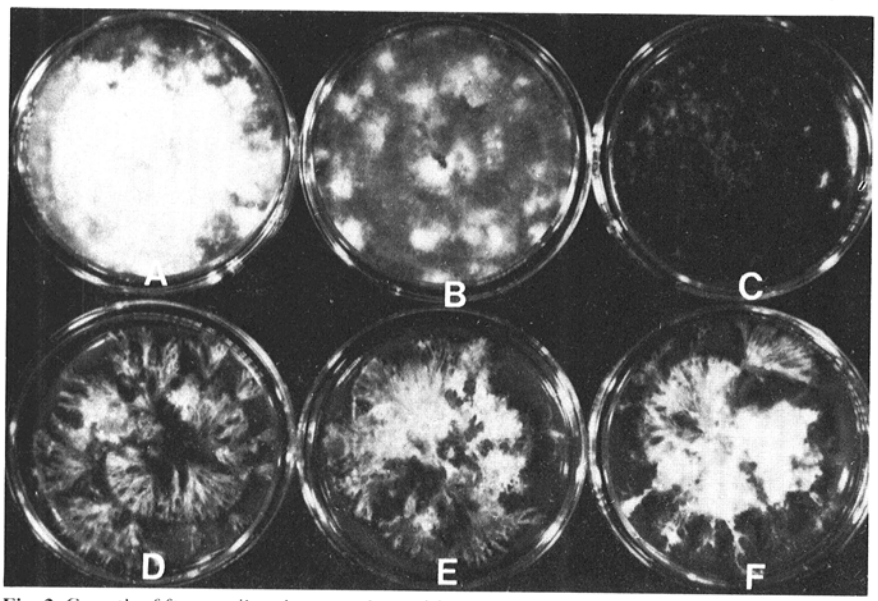

Fig. 2. Growth of fenapanil-resistant and -sensitive strains of *Bipolaris oryzae* on potato-dextrose agar. **(C)** Sensitive wild type strain. Resistant strains grew at **(B)** 100, **(A)** 200, **(F)** 400, **(E)** 600, and **(D)** 800 μg/ml of fenapanil.

RESULTS

Fenapanil-resistant strains obtained by successive transfers. In the first successive

transfer, all strains of *B. oryzae* still grew at 100 μg/ml of fenapanil, but growth was poor (Table 1). In the second transfer, mycelial disks from strain T92 of *B. oryzae* grown on PDA medium containing 10 μg/ml of fenapanil produced colonies that grew rapidly at 100 μg/ml. After seven successive passages through medium mixed with fenapanil, two of five strains of *B. oryzae* produced colonies on a medium containing 800 μg/ml of fenapanil (Table 1). All fenapanil-resistant strains of *B. oryzae* changed in their cultural characters and were mostly white with reduced sporulation (Fig. 1A,B,D–F and Fig. 2A,B,D–F). All strains of *B. oryzae* that grew at ≥600 μg/ml of fenapanil produced calluslike colonies.

Resistant strains obtained by selection. No *B. oryzae* strains could produce colonies on a medium containing 200 μg/ml of fenapanil. At this concentration, the conidia had 100% germination but the germ tubes became distorted and stopped growing after reaching about three times the length of the conidium. At 100 μg/ml of fenapanil, all strains of *B. oryzae* produced white colonies that sporulated poorly (Fig. 3D,E).

Retention of resistance, growth rate, and cultural characteristics of fenapanil-resistant strains. When resistant cultures were grown on PDA without fenapanil, all except strain T92-S100 of *B. oryzae* lost part or all of their resistance to fenapanil after only one passage through fungicide-free medium. Their growth was inferior compared with those that were continuously cultured on a medium containing fenapanil (Table 2).

Three of the fenapanil-resistant strains of *B. oryzae* (T92-S100, 8GE-S200, and T92-4) grew as fast as the fenapanil-sensitive strains (T92 and 8GE) on PDA without fenapanil (Table 3). Two of these fenapanil-resistant strains (T92-S100 and 8GE-S200) grew better than the other fenapanil-resistant and -sensitive strains on PDA containing 100 μg/ml of fenapanil (Fig. 1A,B and Fig. 3A,B). The resistant strains that survived at high concentrations of fenapanil (8GE-S400, T92-S600, and T92-S800) grew slower on PDA (Fig. 2D–F) and PDA containing 100 μg/ml of fenapanil (Fig. 1D–F) than those that could only grow on the lower concentrations of fenapanil.

Virulence of fenapanil-resistant strains on wild rice. There was little difference between fenapanil-resistant and -sensitive strains of *B. oryzae* in their abilities to infect wild rice plants treated with fenapanil (Table 4). All strains of *B. oryzae* were effectively controlled by the fungicide (DI = 1–1.4). On untreated plants, however, the sensitive strain produced more serious leaf damage (avg. DI = 6.7) than the fenapanil-resistant strains of *B. oryzae*.

DISCUSSION

Up to 100% of the conidia of *B. oryzae*

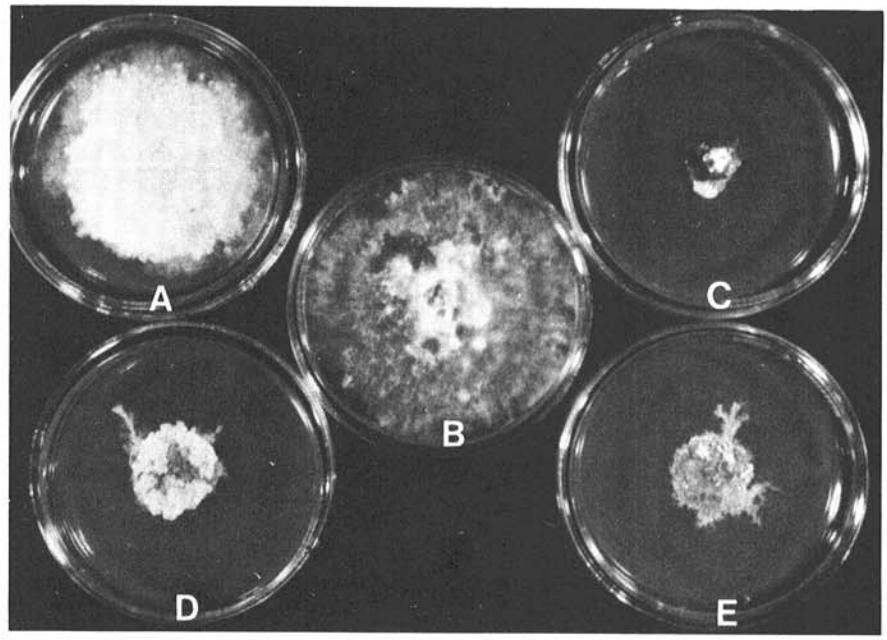

Fig. 3. Growth of fenapanil-resistant and -sensitive strains of *Bipolaris oryzae* on potato-dextrose agar medium containing 100 μg/ml of fenapanil. Fenapanil-resistant strains grew at **(A)** 200 and **(B)** 100 μg/ml of fenapanil obtained by successive transfers. **(C)** Sensitive wild type. **(D and E)** Fenapanil-resistant strains obtained by selection on potato-dextrose agar media containing 100 μg/ml of fenapanil.

Table 2. Retention of resistance of *Bipolaris oryzae* to fenapanil

Strain[a]	Conc. of fenapanil (μg/ml) in PDA on which fungus was previously cultured	Growth on indicated conc. of fenapanil (μg/ml)[b]			
		100	200	400	600
T92-S100 (R)	0	++	–	...	...
T92-S100 (R)	100	++	–	...	...
8GE-S200 (R)	0	+	+	–	...
8GE-S200 (R)	200	...	++	–	...
8GE-S400 (R)	0	+	...	+	–
8GE-S400 (R)	400	...	...	++	–
T92-S600 (R)	0	+	–	...	–
T92-S600 (R)	600	...	...	...	++
10GT-3 (R)	0	+	–	...	...
10GT-3 (R)	100	++	–	...	...
T92-4 (R)	0	+	–	...	...
T92-4(R)	100	++	–	...	...
T92 (S)	0	+	–	...	...
8GE (S)	0	+	–	...	...

[a] R = Fenapanil-resistant; S = fenapanil-sensitive.
[b] ++ = Growth typical for strains continuously cultured on medium amended with fenapil, + = growth, but inferior to strains continuously cultured on medium amended with fenapanil, and – = no growth.

Table 3. Growth rate and cultural characteristics of fenapanil-resistant and -sensitive strains of *Bipolaris oryzae* isolates on potato-dextrose agar and amended potato-dextrose agar containing 100 μg/ml of fenapanil

Strain[a]	Diam. (cm) of colonies after 2 wk[b]		Sporulation[c]	
	PDA	PDA + 100 μg/ml fenapanil	PDA	PDA + 100 μg/ml fenapanil
T92-S100 (R)	9.0	7.3	+	+
8GE-S200 (R)	8.6	4.4	–	–
8GE-S400 (R)	5.2	1.9	+	+
8GE-S400 (R)	4.9	2.0	+	+
T92-S800 (R)	4.3	1.6	+	+
10GT-3 (R)	4.6	1.4	+	+
T92-4 (R)	8.0	4.0	+	+
T92 (S)[d]	8.5	2.2	++	+
8GE (S)	8.5	1.5	++	+

[a] R = Fenapanil-resistant; S = fenapanil-sensitive.
[b] Average three replicates.
[c] Sporulation: – = none, + = poor, and ++ = abundant.

Table 4. Virulence of fenapanil-resistant and -sensitive strains of *Bipolaris oryzae* on wild rice plants inoculated in the greenhouse

Strain[a]	Disease severity[b]			
	Plants treated with fenapanil		Control	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
T92-S100 (R)	1.0	1.4	3.9	3.8
T92-4 (R)	1.0	1.2	1.3	1.9
T92 (S)	1.1	1.0	6.9	6.6

[a] R = Fenapanil-resistant; S = fenapanil-sensitive.

[b] Disease severity rated on a scale 0–9, where 0 = no lesions, 1 = <1%, 3 = 1–4%, 5 = 5–24%, 7 = 25–50%, and 9 = >50% leaf area infected.

germinated on a medium containing 200 μg/ml of fenapanil. The germ tubes grew to three times the conidial length but were distorted and then stopped growing. The abnormal growth of germ tubes caused by the ergosterol biosynthesis inhibitor fungicide has also been reported by Siegel et al (15).

Fenapanil-resistant strains of *B. oryzae* could be obtained by selection or serial passages through medium containing increasing concentrations of the fungicide. The resistant strains produced fewer conidia and grew more slowly on unamended PDA than the fenapanil-sensitive ones did. As the degree of resistance of the fungus to fenapanil increased, its ability to grow and sporulate decreased. This phenomenon has also been observed on triforine-resistant strains of *Cladosporium cucumerinum* Ell. & Anth. (7) and fenarimol-resistant strains of *Aspergillus nidullans* (Eidam) Wint. (18).

Most of fenapanil-resistant strains lost their resistance to fenapanil after only one passage through fungicide-free medium. The type of resistance in these strains may be a phenotypic adaptation. This type of resistance is unstable and is very often encountered when the resistant strains of a fungus are obtained by successive transfers on media amended with a fungicide.

All fenapanil-resistant and -sensitive strains of *B. oryzae* were effectively controlled on plants treated with fenapanil. This indicates that the resistance of strains of *B. oryzae* to fenapanil in vitro is not associated with fenapanil-resistance in vivo. The virulence of fenapanil-resistant strains was reduced when compared with the original fenapanil-sensitive strain.

Therefore, it appears that although fenapanil-resistant strains of *B. oryzae* can be selected in the laboratory, it is unlikely that wild rice plants would be seriously damaged if such strains appeared in the field. The reduction in fitness and virulence of fungi resistant to ergosterol biosynthesis inhibitor fungicides has also been reported (7,18). For these reasons, we, as Fuchs et al (7), question the possible economic and biological importance of the resistance of fungal pathogens to ergosterol biosynthesis inhibitor fungicides such as fenapanil.

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