Resistance of Bipolaris oryzae to Fenapanil

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ABSTRACT

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 susceptible strains were effectively controlled on fenapanil-treated plants in the greenhouse; however, the fenapanil-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 seeding 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also effective against B. oryzae (Breda de Haan) Shoem., the cause of brown spot of wild rice (Zizania aquatica L.) (unpublished).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 sterilized 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
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.

<table>
<thead>
<tr>
<th>Strain</th>
<th>First transfer (µg/ml)</th>
<th>Eighth transfer</th>
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<tbody>
<tr>
<td>8GE</td>
<td>100</td>
<td>800</td>
</tr>
<tr>
<td>11GU</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>10GT</td>
<td>100</td>
<td>400</td>
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<tr>
<td>67</td>
<td>100</td>
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<td>T92</td>
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*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 × 10^8 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*.

**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 192 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 192-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* (192-S100, 8GE-S200, and 192-4) grew as fast as the fenapanil-sensitive strains (192 and 8GE) on PDA without fenapanil (Table 3). Two of these fenapanil-resistant strains (192-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, 192-S600, and 192-S800) grew slower on PDA (Fig. 2D–F) and each other medium (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*...
germinated on a medium containing 200 μg/ml of fenapnil. 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).

Fenapnil-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 fenapnil-sensitive ones did. As the degree of resistance of the fungus to fenapnil 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 fenaramol-resistant strains of Aspergillus nidulans (Eidam) Wint. (18).

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

Therefore, it appears that although fenapnil-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 fenapnil.

LITERATURE CITED