A Benzimidazole Resistant Strain of Erysiphe graminis

J. M. Vargas, Jr.

Assistant Professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48823.
The author wishes to express his thanks to Ronald Detweiler for his technical assistance.
Contribution from the Michigan Agricultural Experiment Station, East Lansing, Michigan 48823, Paper No. 6257.
Partially supported by grants from the Michigan Turfgrass Foundation.
Accepted for publication 28 April 1973.

ABSTRACT

A strain of *Erysiphe graminis* suspected of being resistant to benomyl was removed from a benomyl-treated field plot of *Poa pratensis* 'Merion'. This strain was compared with a common (benomyl-susceptible) strain of *E. graminis* for sensitivity to 4 different systemic fungicides, thiabendazole, thiophanate-methyl, triarimol and benomyl. The fungicides were applied as drenches in one or two 50 ml applications/replicate of potted *P. pratensis* Merion at concentrations of 50, 100, 500, and 1,000 μg/ml (active ingredient). The plants were then inoculated either with the suspected benzimidazole resistant strain (BRS), or with a common benzimidazole susceptible strain (BSS). The BRS of *E. graminis* proved resistant to benomyl, thiabendazole and thiophanate-methyl through one application of the 1,000 μg/ml rate, but was sensitive to two applications of benomyl and thiophanate-methyl at 1,000 μg/ml rate, a rate that was phytotoxic to the plants. The BSS, on the other hand, was eliminated by benomyl, thiabendazole and thiophanate-methyl after one application of 100 μg/ml. Triarimol controlled both the BRS and BSS with one application of 50 μg/ml.

Phytopathology 63:1366-1368

Powdery mildew, caused by *Erysiphe graminis* DC. is especially serious on Kentucky bluegrass (*Poa pratensis* L. cv. 'Merion') grown under conditions of low light intensity. Control of powdery mildew with recommended non-systemic fungicides is largely impractical because of the numerous applications required. The advent of systemic fungicides offered hope for more practical control of powdery mildews. Leath & Berg (5) obtained 9 and 12-month protection against *E. graminis* on Orchard grass (*Dactylis glomerata* L.) under greenhouse conditions with 30 mg and 120 mg active ingredients of benomyl, respectively, applied as a soil drench to 450 ml of soil. Vargas & Detweiler (9) obtained 4 to 6 weeks control of powdery mildew of Merion, under field conditions with 121 and 243 gms (formulation) of benomyl, thiophanate-methyl or triarimol/100 m^2. After a season and a half, powdery mildew began to appear in the benomyl and thiophanate-methyl treated plots while those treated with triarimol remained free of infection. Schroeder and Provvidenti (7) observed field resistance to benomyl in *Sphaerotheca fuliginea* (Schlecht.) Poll, the fungus causing powdery mildew of cucurbits. Bartels-Schooley & MacNeill (1) and Hastie & Georgopoulas (4) have induced resistance to benomyl, other benzimidazole fungicides and a benomyl derivative, benzimidazole carbamic acid, methyl ester (BCM) in irradiated mutants of *Fusarium oxysporium* f. sp. *melonis* and *Aspergillus nidulans*, respectively.

The objectives of this study were: (i) to determine whether strains of *E. graminis* existed which were resistant to benomyl and (ii) to determine whether these benomyl resistant strains of *E. graminis* were also resistant to other benzimidazole systemic fungicides, a thiophanate systemic fungicide, and a pyrimidine systemic fungicide.

MATERIALS AND METHODS.—*P. pratensis* (cultivar Merion) plants were grown in a greenhouse in 15-cm diam clay pots in a sand:soil:peat (1:1:1, v/v) mixture. The plants were fumigated weekly with sulfur to protect against infection by common strains of *E. graminis*.

Plugs of Merion bluegrass infected with the suspected benzimidazole-resistant strain (BRS) of *E. graminis* were removed from benomyl-treated plots, placed in 15-cm diam clay pots, and transferred to a growth chamber along with similarly prepared pots of healthy grass plants. All plants received two 50-ml drench applications of 100 μg benomyl/ml active ingredient to determine whether the BRS isolate of *E. graminis* could increase. Fifteen centimeter pots of grass infected with a common or benzimidazole-susceptible strain (BSS) of *E. graminis* were placed in a second growth chamber without treatment and the disease allowed to increase. The chambers were maintained with a light intensity of between 20,000-30,000 lux at a temperature of 21 $\pm$ 1 C for the duration of the experiment.

Benomyl and thiabendazole [2-(4-thiazolyl)-benzimidazole], two benzimidazole systemic fungicides, as well as thiophanate-methyl [1, 2-bis (3-methoxycarbonyl-2-thiouredido)-benzene], a thiophanate systemic fungicide, and triarimol [α-(2, 4-dichlorophenyl)-α-phenyl-*t*-pyrimidinemethanol], a pyrimidine systemic fungicide, were used in this study at concentrations of 50, 100, 500, and 1,000 μg/ml active ingredients. Fifty ml of each concentration of benomyl, thiabendazole, thiophanate-methyl, and triarimol were applied as a drench to twelve pots of grass. Six pots of each treatment were then exposed to the BRS strain of *E. graminis* in one chamber, and six other pots were exposed to the BSS strain in the other. After two weeks, one half the pots for each treatment received a second 50 ml of fungicide. Control plants received only water and were included in both chambers.

RESULTS.—Six weeks after the initial treatments were applied, benomyl, thiabendazole, and thiophanate-methyl inhibited the development of the BSS of *E. graminis* when applied as a drench starting at 100 μg/ml. While triarimol inhibited the development of the BSS of *E. graminis* starting at 50 μg/ml. The water-treated control plants had an estimated 99% of their leaf blade surface infected with *E. graminis*. Conversely, the benomyl, thiabendazole, and thiophanate-methyl-treated plants exposed to the BRS of *E. graminis* were comparable to the water-treated controls with an estimated 99% infection at all rates up to the two 50-ml applications of 1,000 μg/ml. At the two 50-ml applications of the 1,000 μg/ml rate, the benomyl and thiophanate-methyl-treated plants showed only 50% and 17% infection, respectively. However, at these rates benomyl and thiophanate-methyl were mildly phytotoxic, resulting in dieback from the leaf blade tips. Thiabendazole was severely phytotoxic starting at the 1,000 μg/ml rate, resulting in the complete destruction of the top growth.

Triarimol effectively controlled the BRS of *E. graminis* at the same 50 μg/ml rate at which it was effective against the BSS. Triarimol also was mildly phytotoxic at the 1,000 μg/ml rate, but the phytotoxicity was expressed as black spots on the leaf blades, not unlike Helminthosporium leaf spot symptoms.

DISCUSSION.—These results, which showed that the BRS of *E. graminis* was resistant to the two benzimidazole systemic fungicides, are similar to those of Hastie & Georgopoulas (4) who found that mutants of *A. nidulans* which were resistant to benomyl also were resistant to thiabendazole and (BCM). These results agree in part with those of Bartels-Schooley & MacNeill (1), who found that a mutant of *F. oxysporium* f. sp. *melonis* which was tolerant to benomyl was also tolerant to thiabendazole and furidazole [2-(furyl)-benzimidazole], and that the same was true for mutants tolerant to thiabendazole. However, mutants tolerant of furidazole were not tolerant of either benomyl or thiabendazole. They postulated that, while there is a mode of action similar to all three, benomyl and thiabendazole have an additional mode of action. Thiophanate-methyl was not included in either of these studies.

In a study with two *Penicillium* species in which thiophanate-methyl was included, Bollen (2) concluded that resistance to benomyl coincided with resistance to thiophanate-methyl and to a lesser extent with resistance to thiabendazole and furidazole. Cross-resistance to triarimol was not checked in any of the previously cited studies.

This study showed that the two benzimidazole and the thiophanate systemic fungicides had similar cross-resistance to the BRS of *E. graminis* at concentrations up through the single 50-ml application of the 1,000 μg/ml rate. It is impossible to draw any definite conclusions concerning treatments exceeding this rate because of the phytotoxicity encountered. This cross-resistance to thiophanate-methyl by the BRS strain of *E. graminis* is not surprising, since it has been shown to convert to 2-methoxycarbonylamino-benzimidazole in plant tissue (6) and to 2-benzimidazole carbamic acid methyl ester (BCM) in aqueous solution (8). Clemons & Sisler (3) have already shown that benomyl is rapidly converted to (BCM) in aqueous solution. The fact that there was no cross-resistance to triarimol suggests that it has a mode of action which differs from that of the benzimidazole and thiophanate systemic fungicides.

A benzimidazole-resistant strain of *E. graminis* in this study appeared to develop rapidly (one and one-half seasons). This phenomenon has occurred with other fungi; i.e., *Sclerotinia homeocarpa* (J. M. Vargas, *unpublished*). To protect against the development of resistance to the benzimidazole and thiophanate systemic fungicides by other important pathogens, the fungicides should not be used on an exclusive basis, but rather used in conjunction with nonsystemic or chemically different systemic fungicides such as triarimol. While there are many different benzimidazole fungicides and two thiophanate systemic fungicides under either full or experimental label, resistance to one of these fungicides by a fungal pathogen probably means resistance to others, with the possible exception of furidazole (1).

LITERATURE CITED

1. BARTELS-SCHOOLEY, J., & B. H. MAC NEILL. 1971. A

comparison of the modes of action of three benzimidazoles. Phytopathology 61:816-819.
2. BOLLEN, G. J. 1971. Resistance to benomyl and some chemically related compounds of Penicillium species. Neth. J. Plant Pathol. 77:187-193.
3. CLEMONS, G. F., & H. D. SISLER. 1969. Formation of a fungitoxic derivative from benlate. Phytopathology 59:705-706.
4. HASTIE, A. C., & S. G. GEORGOPOULAS. 1971. Mutational resistance to fungitoxic benzimidazole derivatives in Aspergillus nidulans. J. Gen. Microbiol. 67:371-373.
5. LEATH, K. T., & C. C. BERG. 1972. Longterm control of powdery mildew of orchard grass with benomyl. Plant Dis. Reptr. 56:75-76.
6. NOGUCHI, T., K. OHKUMA, & S. KASAKA. 1971. Chemistry and metabolism of thiophanate fungicides. 2nd Int. Congr. Pestic. Chem., Tel Aviv (Abstr.).
7. SCHROEDER, W. T., & R. PROVVIDENTI. 1969. Resistance to benomyl in powdery mildew of cucurbits. Plant Dis. Reptr. 53:271-275.
8. SELLING, H. A., J. W. VONK, & A. KAARS SIJPESTEIJN. 1970. Transformation of the systemic fungicide methyl-thiophanate into 2-benzimidazole carbamic acid methyl ester. Chem Ind. Lond. 1970:1625.
9. VARGAS, J. M., JR., & R. DETWEILER. 1972. Control of powdery mildew on Merion Kentucky bluegrass. Proc. Mich. Turfgrass Conference 1:13-14.