Failure of Metalaxyl to Control Pythium Blight on Turfgrass in Pennsylvania

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ABSTRACT

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Sixty to 75% of the propagules of *Pythium aphanidermatum* recovered from a site where metalaxyl failed to control Pythium blight on turfgrass grew without inhibition on a medium containing 50 and 100 ppm of metalaxyl. Representative isolates were pathogenic on pot-grown bentgrass, producing symptoms typical of Pythium blight. These isolates were more aggressive than a pool of wild-type *P. aphanidermatum* and were not controlled by the commercial rate of metalaxyl on pot-grown bentgrass. They were adequately controlled in vivo by propamocarb and fosetyl Al.

The acylalanine fungicide metalaxyl (Subdue 2E, CGA-48988) is a systemic fungicide with selective activity against fungi of the order Peronosporales, including the Pythiaceae. The fungicide has been reported to control damping-off (Pythium spp.) on soybean (7) and Pythium blight (Pythium aphanidermatum) on turfgrasses (9).

Pythium blight (cottony blight, grease spot, and spot blight) caused by *P. aphanidermatum* is a severe and devastating disease of turfgrasses during hot, wet weather. The fungus is most aggressive (aggressiveness = rate at which virulence is expressed) under conditions of high humidity at air temperatures of 30-35 C (12). Ryegrasses and bentgrasses are especially susceptible. Metalaxyl provides excellent control of this disease and is widely used on golf courses for this purpose.

The mode of action of this fungicide appears to be highly specific, apparently involving a site in the synthesis of RNA

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(5). Metalaxyl-resistant isolates have been induced experimentally in *Phytophthora megasperma* f. sp. *medicaginis* (3), *Phytophthora capsici* (1), and *Pythium ultimum* (1) and selected in *Albugo candida* (white rust) on radish (11). Commercial control failures resulting from fungicide resistance in target populations outside the United States have been reported in *Phytophthora infestans* on potato (4), *Pseudoperonospora cubensis* on cucumber (6,8), *Peronospora tabacina* on tobacco (10), and *Plasmopara viticola* on grape (10).

This study describes metalaxyl sensitivity in a population of *P. aphanidermatum* on turfgrass where the fungicide had failed to control Pythium blight.

MATERIALS AND METHODS

In July 1983, six turfgrass samples were received from a golf course in eastern Pennsylvania. The samples were taken from four bentgrass fairways where metalaxyl failed to control Pythium blight. Metalaxyl had been used to control the disease for 3 yr at this site.

Grass foliage was removed from the six samples and thatch and soil from individual samples to a depth of 2 cm were homogenized (70 g/700 ml of glass-distilled water) in a Waring Blendor and serially diluted (1:10 and 1:100), then the *P. aphanidermatum* population was assayed on a modification of a medium described by Burr and Stanghellini (2).

Our modification contained 1,000 ml of glass-distilled water, 17 g of Difco cornmeal agar, 100 mg of rose bengal, 25 mg of pimaricin, 300 mg of streptomycin sulfate, and 1 mg a.i. of benomyl (Tersan 1991, 50W). To partition P. aphanidermatum populations into metalaxylsensitive and metalaxyl-resistant proportions, the medium was used unamended as described and amended with 50 and 100 mg a.i. metalaxyl 2E per 1,000 ml of medium. One milliliter each of homogenates, 1:10, and 1:100 dilutions were dispersed separately onto 20 ml of sterile solidified assay medium in petri plates, and the plates were incubated at 36 C for 48 hr. After incubation, the surfaces of the substrate were thoroughly washed with cool tap water to remove soil and plant debris, and colonies with morphology typical of P. aphanidermatum were counted to determine total number of P. aphanidermatum propagules present and the proportion of the recovered P. aphanidermatum population that showed decreased sensitivity to metalaxvl.

Eleven of the recovered metalaxylresistant isolates were selected at random for identification and greenhouse testing. These isolates were transferred to potatodextrose agar and water agar. Growth habit and oospore and sporangial morphology were observed. Species identification of the isolates were confirmed by A. F. Schmitthenner (Ohio Agricultural Research and Development Center, Wooster).

Seedling pot-grown Penncross creeping bentgrass was treated with metalaxyl 2E at 29.6 ml/93 m² as a foliar spray and a soil drench. The foliar spray was applied at a dilution rate equivalent to 11.5 L/93 m² and the soil drench was applied in 60 ml of water per 10-cm pot. Grass was inoculated 3 days after fungicide application with the 11 test isolates and a wild-type isolate pool of *P. aphanidermatum*. Disease ratings were made after 3 days of incubation under plastic at

27 C on a shaded greenhouse bench. A visual rating scale of 0-10 was employed, where 0 = no disease, 1 = 10%, 2 = 20%, and 10 = 100% blighting of foliage in pot.

Recovered metalaxyl-resistant isolates were tested for resistance to propamocarb (Banol) and fosetyl Al (Aliette) in the greenhouse. Pot-grown, seedling Pennfine perennial ryegrass was sprayed with propamocarb 6S at 89 ml/93 m² and fosetyl Al 80W at 227 g/93 m² in water equivalent to 11.5 L/93 m². Untreated checks were included for comparison. Grass was inoculated 24 hr after chemical treatment with nine pathogenic, metalaxyl-resistant isolates and a wild-type pool of *P. aphanidermatum*. Disease ratings were made after 5 days of incubation as described previously.

RESULTS AND DISCUSSION

All isolates recovered from homogenates of the six samples from the control-failure location resembled *P. aphanidermatum* in growth habit, colony morphology, and oospore and sporangial morphology. They were subsequently identified by A. F. Schmitthenner as *P. aphanidermatum*. All samples yielded *P. aphanidermatum* propagules at a density of 160-2,800/g. Of the propagules recovered by assay, 60-75% were able to grow without inhibition on a medium containing 50 and 100 mg of metalaxyl per 1,000 ml.

Results of the greenhouse test for pathogenicity and in vivo response to metalaxyl are shown in Table 1. All but two of the test isolates were extremely virulent on creeping bentgrass, producing 80–100% foliar blighting after 3 days of incubation on both treated and untreated grass. The grass that had been inoculated with the wild-type *P. aphanidermatum* pool showed no disease after 3 days of incubation. After 5 days of incubation, the untreated grass inoculated with the wild-type pool showed 97% blighting. The metalaxyl-treated grass inoculated with the wild-type pool was 90–100%

Table 1. Pathogenicity and response to metalaxyl of 11 isolates from a metalaxyl control failure location and a wild-type isolate pool of *Pythium aphanidermatum* inoculated onto pot-grown seedling Penncross bentgrass

Metalaxyl treatment	Foliar blight after 3 days of incubation ^a											
	1 ^b	2	3	4	5	6	7	8	9	10	11	12
Spray (29.6 ml/93 m ²)	10.0°	10.0	9.3	10.0	9.7	8.0	1.0	10.0	10.0	0.3	10.0	0 (0) ^d
Drench (29.6 ml/93 m ²)	10.0	10.0	10.0	10.0	9.3	8.3	0.3	10.0	10.0	1.3	10.0	0 (1.0) ^d
No treatment	10.0	10.0	9.3	10.0	10.0	8.3	1.3	10.0	10.0	1.0	10.0	0 (9.7) ^d

^a Mean of three replicates.

protected against blight.

Metalaxyl-resistant isolates showed no resistance to propamocarb or fosetyl Al but were adequately controlled by both chemicals in the greenhouse. These findings concur with those of Davidse (4), who tested metalaxyl-resistant *Phytophthora infestans* from control-failure locations for sensitivity to propamocarb and fosetyl Al.

We conclude from these findings that the reported failure of metalaxyl to control Pythium blight at the eastern Pennsylvania site was due to the high proportion of pathogenic metalaxylresistant individuals in the *P. aphanidermatum* population.

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^b Isolate numbers: 1-11 = isolates from control failure site, 12 = pool of six wild-type isolates of P.

[°]Visual rating scale of 0-10, where 0 = no disease, 1 = 10%, 2 = 20%, and 10 = 100% of foliage blighted.

^dFoliar blight rating after 5 days of incubation.