Degrees of Sensitivity to Metalaxyl Within the *Pythium* spp. Pathogenic to Wheat in the Pacific Northwest

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ABSTRACT

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A minimum of 20–25%, and in some cases more than 80%, of *Pythium* colonies selected at random from soil-dilution plates of a *Pythium*-selective medium grew when transferred to the same medium amended with metalaxyl at 1 ppm. The other colonies made no visible growth within 72 hr. Isolates of *P. ultimum* var. *ultimum*, *P. ultimum* var. *sporangiiferum*, *P. aristosporum*, *P. heterothallicum* (both male and female isolates), an unidentified heterothallic species (both male and female isolates), and an unidentified homothallic *Pythium* sp., all from wheat roots, failed to grow within 72 hr on the selective medium amended with metalaxyl at 1 ppm. In contrast, isolates of *P. torulosum*, *P. irregulare*, and a second unidentified homothallic *Pythium* sp., also from wheat roots, grew on the metalaxyl-amended *Pythium*-selective medium at 15–50% of their respective normal growth rates on the same medium without metalaxyl. On cornmeal agar with metalaxyl at 1 ppm, the less sensitive isolates grew at 30–80% of normal growth on the same medium without metalaxyl. With 0.1 ppm of metalaxyl added to cornmeal agar, most isolates of the more sensitive species grew at 70–80% and those of the less sensitive species grew at 95–100% of normal growth. The failure of metalaxyl to inhibit growth of some species of *Pythium* involved in root rot of Pacific Northwest wheat may account for observed failures of this fungicide to control this disease in the field.

Additional key words: *Triticum aestivum*

Pythium spp. have become more important as pathogens of winter wheat (*Triticum aestivum*) in the Pacific Northwest states of the United States as growers have changed their management from common use of summer fallow, rotation, and conventional tillage to more recropping (wheat after wheat) and less tillage (6). Several species or subspecies may be involved, including *P. torulosum* Coker & Patterson, *P. aristosporum* Vanderpool, *P. volutum* Vanderpool & Truscott, *P. ultimum* Trow var. *ultimum* Drechs., *P. ultimum* var. *sporangiiferum* Drechs., *P. irregulare* Buisman, and *P. heterothallicum* Campbell & Hendrix (3,4). Damage may occur either as seedling blight or as a decay of the roots, rootlets, and root hairs, resulting in poor stands or stunted, poorly tillered plants. Yields in some fields have been 20–25% greater in plots with soil treated to eliminate or inhibit *Pythium* species (6). Trends toward consecutive cereal crops and minimum tillage are likely to continue in the region, and thus a method for the control of *Pythium* is needed.

In an earlier experiment (7), metalaxyl at about 2 kg/ha in the seed furrow as a 5G (5% a.i. granular) formulation was equivalent to fumigation with methyl bromide for control of *Pythium* damage to direct-drilled (no-till) wheat. Metalaxyl was then tested in various formulations (wettable powder, emulsifiable concentrate, and granular) with various rates and application methods (to seed, in the seed furrow, and postplant) in an effort to find the best method and rate of application for wheat. Yields were up to 20% greater in some trials, but there was no response in other trials despite high populations of *Pythium* in the fields (6). Moreover, *Pythium* oospores were found in abundance in roots and rootlets of metalaxyl-treated and untreated plants alike.

Use of metalaxyl to control members of the Peronosporales has been shown to select for resistant strains in an otherwise sensitive species, including *Phytophthora infestans* (10), *Pseudoperonospora cubensis* (11), and *Pythium aphanidermatum* (13). In addition, resistance to metalaxyl has been induced to *Phytophthora megasperma* f. sp. *medicaginis* (9) and *Pythium ultimum* (2). However, the wheat fields where metalaxyl was ineffective had no prior history of metalaxyl use, ruling out any possibility that a resistant strain had been favored. In preliminary work (8), a portion of the *Pythium* population in Pacific Northwest wheat fields was relatively insensitive to metalaxyl. Of the four *Pythium* spp. tested, *P. torulosum* showed the least sensitivity. At least 10 species or subspecies are involved in the Pythium root rot complex of Pacific Northwest wheat (4); this has prompted studies of sensitivity of the six species not included in our preliminary work (8). New isolates of the original four species were also included. Coffey et al (5) and Shew (14) have reported that strains as well as species of *Phytophthora* vary in ED_{50} values when exposed to metalaxyl.

MATERIALS AND METHODS

Tests of *Pythium* spp. for sensitivity to metalaxyl. Cultures of *P. torulosum*, *P. aristosporum*, *P. ultimum* vars. *ultimum* and *sporangiiferum*, *P. irregulare*, *P. heterothallicum* (both male and female), and three unidentified *Pythium* spp. (one heterothallic, both male and female) were grown on the selective medium of Mircetich (12) and also on cornmeal agar (Difco) (plus 10 g of additional agar), each amended with metalaxyl at 1 ppm. Each culture was isolated originally from roots of wheat taken directly from fields. Tests were also conducted with metalaxyl at 0.01, 0.1, 1, 2, and 5 ppm added to cornmeal agar (plus 10 g of agar). The metalaxyl was added to the molten medium as a water dilution of the 2EC formulation. The same media without metalaxyl were used as controls. Colony growth rates at 20 C were measured after 48 or 72 hr.

Tests of randomly selected *Pythium* colonies for sensitivity to metalaxyl. The proportion of the population of *Pythium* spp. sensitive to metalaxyl was assessed by first preparing soil dilution plates using the *Pythium*-selective medium of Mircetich (12) without metalaxyl, then

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transferring randomly selected colonies to the same medium containing metalaxyl at 1 ppm. Soil dilutions of about 1:50 and 1:100 were used. Transfers to the medium with metalaxyl were made 48 hr after the dilution plates were seeded, by transferring blocks of agar containing mycelium from each colony selected. Five blocks representing five colonies were placed equidistantly around the margins of each petri dish of metalaxyl-amended medium. At least 50 colonies were transferred for each soil dilution plated. The presence or absence of growth from the respective disks was recorded after incubation for 48 hr at 20 C.

RESULTS

Growth of *Pythium* spp. on metalaxyl-amended media. All isolates of *P. aristosporum, P. ultimum* vars. *ultimum* and *sporangiiferum, P. heterothallicum* (both male and female), the unidentified heterothallic species (both male and female), and one of the two unidentified homothallic species failed to grow within 72 hr on either the selective medium (12) or cornmeal agar amended with metalaxyl at 1 ppm (Tables 1 and 2). In contrast, growth of the isolates of *P. torulosum, P. irregulare*, and the other unidentified (homothallic) *Pythium* spp. on media amended with metalaxyl at 1 ppm were 15–50% of the controls on the selective medium (12) (Table 1) and 30–80% of the controls on cornmeal agar (Table 2). Growth rates of *P. irregulare* within 72 hr were 10–20% of the control at 2 ppm and were completely prevented or limited to a few short hyphae at 5 ppm. The more sensitive species grew at 70–80% and the less sensitive isolates grew at 95–100% of their normal growth rates, respectively, on cornmeal agar with metalaxyl at 0.1 ppm (Table 3). None of the isolates tested had been previously exposed to metalaxyl.

Ability of isolates of *Pythium* from wheat field soils to grow on metalaxyl-amended media. With each wheat field soil tested, as few as 22%, but as many as 86%, of the colonies selected at random from dilution plates of the selective medium grew when transferred to the same medium amended with metalaxyl at 1 ppm (Table 4).

DISCUSSION

As reported for *Phytophthora* spp. (5,14), considerable variability in sensitivity to metalaxyl exists among and even within species and strains of *Pythium* spp. Unfortunately, it appears that the less sensitive species make up 25–75% and possibly more of the total population of *Pythium* in wheat field soils of the Pacific Northwest. This could possibly explain, at least in part, why metalaxyl has failed to protect wheat roots and give a yield response in fields with a known problem of Pythium root rot.

Of the several *Pythium* taxa thus far isolated from roots of wheat in the Pacific Northwest, *P. torulosum, P. irregulare*, and one of unidentified homothallic species were the least sensitive in vitro to metalaxyl. The ED_{50} values for the less sensitive species are estimated at 0.6–0.7 or greater. In contrast, isolates of *P. aristosporum, P. ultimum* vars. *ultimum* and *sporangiiferum, P. heterothallicum*, and two unidentified species (one heterothallic) showed the degree of sensitivity to metalaxyl commonly reported for members of the Peronosporales (1,15), with ED_{50} values estimated at 0.3 ppm or less. The 1-ppm concentration added to the medium serves mainly to separate the more sensitive from the less sensitive species.

Although the prospects for control of *Pythium* with metalaxyl are not encouraging, the potential for use of this fungicide should not be ruled out. Conceivably, different crop or soil management, practiced consistently in the different fields for many years, might

Table 1. Sensitivities of different *Pythium* spp. to metalaxyl added to a *Pythium*-selective medium[a]

Pythium sp. (no. of isolates)	Colony diameters (mm)			
	Control		1 ppm metalaxyl	
	2 Days	3 Days	2 Days	3 Days
P. aristosporum (2)	Tr[b]	Tr–15	0	0
P. torulosum (1)	19.5	30	10.5	16
P. ultimum				
var. *sporangiiferum* (2)	55.5–58.5	76–<85	0	0
var. *ultimum* (1)	53.5	80	0	0
P. ultimum (3)	55–63.5	<85	0	0
P. irregulare (8)	48–58	<85	9–26	21–45
Unknown A (homothallic)	51	<85	22	36
Unknown B (homothallic)	18.5	29.5	0	0
P. heterothallicum (female)	42	71.5	0	0
P. heterothallicum (male)	39	60	0	0
Unknown (male)	19.5	29.5	0	0
Unknown (female)	21.5	35	0	0

[a] Mircetich's (12) selective medium unamended or amended with 1 ppm of metalaxyl.
[b] Tr = trace, i.e., a few hyphae were observed to have grown a very short distance from the agar block into the *Pythium*-selective medium.

Table 2. Sensitivities of different *Pythium* spp. to metalaxyl added to cornmeal agar

Pythium sp. (no. of isolates)	Colony diameters (mm)		
	Metalaxyl (ppm)		Percent of control
	0	1	
P. ultimum			
var. *ultimum* (3)	<85	0–Tr[a]	0
var. *sporangiiferum* (4)	<85	0–Tr	0
P. torulosum (4)	32–40	18–23	30–66
P. aristosporum (3)	28–37	0–Tr	0
P. irregulare (5)	67–<85	38–<85	45–<85
Unknown (male)	72	0	0
Unknown (female)	<85	0	0
P. heterothallicum (male)	<85	Tr	0
P. heterothallicum (female)	<85	Tr	0

[a] Tr = trace, i.e., a few hyphae were observed to have grown a very short distance from the agar block into the metalaxyl-amended medium.

Table 3. Sensitivities of different *Pythium* spp. to concentrations of metalaxyl added to cornmeal agar

Pythium spp. (no. of isolates)	Growth at percent of no-metalaxyl control[a] at indicated metalaxyl concentration (ppm)		
	0.01	0.1	1
P. ultimum			
var. *sporangiiferum* (2)	101	71	0
var. *ultimum* (1)	100	100	14
P. ultimum (3)	99–106	68–88	0
P. irregulare (8)	93–100	79–102	14–33
Unknown A (homothallic)	99	95	32
Unknown B (homothallic)	102	79	0
P. heterothallicum (male)	96	75	0
P. heterothallicum (female)	99	75	0
Unknown (heterothallic)	99	80	0

[a] Measured after 48 hr at 20 C.

Table 4. Total population of *Pythium* in field soils and percentage of *Pythium* colonies from soil dilution plates that grew when transferred to a medium amended with metalaxyl at 1 ppm

Field	Total population (prop./g)	Growth on metalaxyl medium[a] (%)
1	346	70
2	380	79
3	515	22
4	758	86
5	628	86
6	430	51

[a] Mircetich's (12) selective medium amended 1 ppm of metalaxyl. Growth based on presence of mycelium growing into the agar 48 hr after transfer of an agar block from a colony formed on the same medium without metalaxyl. At least 50 random colonies were tested from the dilution plates for each field sampled.

lead to different mixtures of the species in the fields. Different populations could account for response of wheat to metalaxyl in some fields but not in others. Metalaxyl might even be used as a tool to characterize the population of *Pythium* spp. in fields. The greater yields obtained in some cases provide encouragement that metalaxyl and related compounds could in some way contribute to the total management for control of *Pythium*.

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