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Effects of Some Pesticides on the Growth of ARF18 and Its Pathogenicity to *Heterodera glycines*[1]

D. G. Kim[2] and R. D. Riggs[3]

Abstract: The effects of 22 pesticides on the mycelial growth and pathogenicity of the biocontrol fungus ARF18 to *Heterodera glycines* were tested in vitro. The chemicals were added to agar at 10, 100, and 1,000 ppm a.i.; a block of agar containing the fungus was added to each test concentration; and fungal growth was measured. Subsequently, a block of the fungus on the pesticide-containing agar was used to determine the ability of the fungus to parasitize eggs of *H. glycines*. Aldicarb, bentazone, and chlorothalonil had little or no effect on fungal growth, whereas benomyl and thiophanate methyl completely inhibited growth of the fungus at 10 ppm. The relative insensitivity of ARF18 to certain pesticides would permit selected use of those pesticides with ARF18 in an integrated control program if the effects on the fungus in the field are similar to results from petri dish studies.

Key words: aldicarb, ARF18, benomyl, bentazone, biocontrol fungus, biological control, chemical pesticides, chlorothalonil, *Heterodera glycines*, nematophagous fungus, pesticide, soybean cyst nematode, thiophanate methyl.

During an investigation of possible biocontrol agents of *Heterodera glycines* Ichinohe, a sterile hyphomycete fungus designated ARF18 was isolated from eggs (Kim and Riggs, 1991). ARF18, when added to soil, significantly suppressed the number of viable *H. glycines* eggs on soybean roots compared to soil without the fungus (Kim and Riggs, 1995). This fungus has been isolated from cysts collected in several southern states, indicating the adaptability of ARF18 in natural soil (Kim et al., 1998).

Exploitation of this fungus as a biocontrol agent of nematodes demands that it be compatible with chemical pesticides that are applied to the soil or crops to control other pests and diseases. The effects of pesticides on nematode biocontrol fungi, such as *Cylindrocarpon destructans, Verticillium chlamydosporium* (Crump and Kerry, 1986), and *Verticillium lecanii* (Meyer et al., 1991), have been studied on agar. However, nothing is known of the effects of pesticides on ARF18. Additionally, in vitro growth studies may have more value if accompanied with pathogenicity data because growth inhibition does not always correlate with reductions in pathogenicity. Consequently, the objectives of this study were to determine how mycelial growth and pathogenicity of ARF18 to eggs of *H. glycines* were affected by: (i) selected chemical pesticides commonly used on soybean, and (ii) a number of different fungicides that might be used on soybean or other crops grown in rotation with soybean.

Materials and Methods

Mycelial growth: The fungus ARF18 originally was isolated from eggs of *H. glycines* from greenhouse cultures at Fayetteville, Arkansas, in 1987. It was maintained on cornmeal agar (CMA, Difco, Detroit, MI) at 24 °C and was transferred to new medium approximately every 30 days.

The 22 pesticides that were tested are used on soybean or on other crops that are rotated with soybean and included 9 fungicides, 5 insecticides, 2 acaricides, 5 herbicides, and 1 nematicide (Table 1). Each of the pesticides was tested at 10, 100, and 1,000 ppm a.i., even though they would not likely occur at 1,000 ppm a.i. in soils on which soybean is grown. Molten CMA was cooled to 40 to 45 °C and the required quantity of pesticide in 1 ml of distilled sterile water was added. The resulting mixture was thoroughly agitated and quickly poured into petri plates (60 × 15 mm). After the agar had gelled, a 4-mm-square plug was cut from a

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[2] Department of Food Crops, Kyeongbuk Province Rural Development Administration, Daegu, Seoul, Korea 720-320.

[3] Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

E-mail: rdriggs@comp.uark.edu

TABLE 1. Colony growth and percentage of parasitism of *Heterodera glycines* eggs by ARF18 on agar containing pesticides.

Pesticide	Pesticide concentration (ppm)	ARF18 colony diameter[a] (mm)	Percentage of AE, SE, and J2[b]		
			AE	SE	J2
No pesticide, no fungus	0	n.a.	93a	1f	6e
No pesticide, w/fungus	0	45a	3f	90ab	6e
		Fungicides			
Benomyl	10	0g	—	—	—
Carboxin	10	12ef	25e	61d	14c
	100	0g	—	—	—
Chlorothalonil	10	45a	14e	82ab	4ef
	100	43a	11f	86ab	3ef
	1,000	41ab	21ef	73bc	6e
Dicloran	10	24d	16ef	79bc	5ef
	100	17e	26de	67c	8e
	1,000	9g	23ef	76bc	1f
Formaldehyde	10	11f	6f	93a	0f
	100	0g	—	—	—
Mancozeb	10	41ab	11f	86ab	3ef
	100	12e	33de	63cd	4ef
	1,000	0g	—	—	—
Myclobutanil	10	11f	49cd	33e	18b
	100	0g	—	—	—
Quintozene	10	27cd	24ef	72bc	5ef
	100	12e	50cd	44de	6e
	1,000	9g	20ef	70bc	9de
Thiophanate methyl	10	0g	—	—	—
		Insecticides			
Carbaryl	10	22de	10f	82ab	8e
	100	0g	—	—	—
Diazinon	10	28cd	14ef	75bc	11cd
	100	16ef	53c	25e	22d
	1,000	0g	—	—	—
Potassium salt of fatty acid	10	44a	27de	64c	9de
	100	41ab	20ef	72bc	7de
	1,000	12ef	13ef	87ab	0f
Fenpropathrin	10	42a	69b	30e	2f
	100	27cd	28de	66cd	6e
	1,000	7fg	100a	0f	0f
Malathion	10	44a	27de	70bc	3ef
	100	34bc	36de	54d	11cd
	1,000	10fg	43cd	48d	9de
		Acaricides			
Cyhexatin	10	14e	14ef	83ab	3ef
	100	8fg	14ef	82ab	5ef
	1,000	0g	—	—	—
Dicofol	10	24d	26de	68bc	6e
	100	11f	31de	62d	7de
	1,000	0g	—	—	—
		Herbicides			
Bentazone	10	45a	12f	85ab	3ef
	100	44a	11f	78bc	11cd
	1,000	42a	4f	93a	3ef
Glyphosate-isopropyamine	10	40ab	16ef	80b	4ef
	100	26cd	20ef	71bc	9de
	1,000	13ef	24ef	67c	8de
Paraquat	10	23d	36de	62cd	3ef
	100	10fg	34de	56cd	10d
	1,000	0g	—	—	—

TABLE 1. *Continued*

Pesticide	Pesticide concentration (ppm)	ARF18 colony diameter[a] (mm)	Percentage of AE, SE, and J2[b]		
			AE	SE	J2
Sethoxydim	10	43a	15ef	80b	5ef
	100	21d	20ef	75bc	5ef
	1,000	9fg	38d	56cd	6e
Trifluralin	10	41ab	15ef	80b	5ef
	100	23d	27de	66cd	7de
	1,000	0g	—	—	—
		Nematicide			
Aldicarb	10	45a	11f	86ab	3ef
	100	45a	17ef	83ab	0f
	1,000	36b	42cd	58cd	0f
C.V.		62.3	126.9	41.3	129.3

[a] Colony diameter was measured after culturing ARF18 on control cornmeal agar (CMA) or on pesticide-amended CMA for 15 days at 23 °C; the test was repeated once. N = 10 per treatment.

[b] Parasitism was determined by placing five cysts of *H. glycines* on each of three agar plugs with ARF18, followed by incubation for 10 days at 23 °C. The test was repeated for a total of 30 cysts per treatment. Uninoculated cysts had 276 asymptomatic eggs, 3 symptomatic eggs, and 17 juveniles in an average of two tests. AE = asymptomatic eggs (eggs with no apparent infection or damage); SE = symptomatic eggs (eggs infected by the fungus or damaged in some other way); J2 = second-stage juveniles that had hatched from the eggs. – indicates that parasitism was not measured because the fungus did not grow.

pure culture of ARF18 on CMA and transferred aseptically to the center of each plate. The cultures were incubated at 23 °C in the dark for 15 days, at which time colony diameters were measured. Five replicate plates were used for each concentration of pesticide, and the experiment was repeated.

Egg parasitism: Following colony measurement, a 2- to 4-mm-diam. plug was cut from the periphery of a colony that was actively growing on pesticide-containing agar and was transferred to another petri plate containing 1.5% water agar. Five cysts, freshly collected from soybean roots, were surface-disinfested for 10 minutes in 0.5% NaOCl, rinsed in sterile water (Kim and Riggs, 1994), and placed upon the fungus plug. Plates with fungus and cysts were stored in a plastic bag in an incubator at 23 °C for 10 days. Each concentration of pesticide was replicated three times, and the test was repeated (total of 30 cysts per concentration per pesticide). Two kinds of controls were prepared: (i) 30 cysts placed on CMA plugs with fungus but no pesticide, and (ii) 30 cysts placed on sterile water agar with no fungus and no pesticide.

After 10 days, each cyst was placed in a drop of lactoglycerol solution (lactic acid [85%]:glycerol [99.5%]:distilled water = 2:2:1) on a glass slide (Kim and Riggs, 1994) and broken to release the eggs within. In the lactoglycerol solution symptomatic eggs became clear in about a minute, whereas the asymptomatic eggs remained unchanged for more than 5 minutes. The numbers of asymptomatic eggs, symptomatic eggs, and second-stage juveniles (J2) in each cyst were identified and counted at ×30. The results were expressed as percentages in relation to the number of nematodes in the control cysts on water agar without pesticides. Data from growth and pathogenicity experiments were subjected to analyses with a GLM procedure (SAS Institute, Cary, NC) and the Waller mean separation test.

RESULTS AND DISCUSSION

Two systemic fungicides, benomyl and thiophanate methyl, were the most toxic of the pesticides (Table 1). These two fungicides completely inhibited mycelial growth at 10 ppm. Benomyl was similarly reported as potentially the best inhibitor of *Cylindrocarpon destructans* (Crump and Kerry, 1986). When the effects of seven fungicides, including benomyl and chlorothalonil, were tested on the fungus *Hirsutella rhossiliensis,* a para-

site of *Criconemella xenoplax,* only benomyl completely inhibited growth of hyphae and germination of conidia at 1 and 10 µg/ml (Pullen et al., 1990). Benomyl also inhibited hyphal growth and germination of conidia of *H. rhossiliensis* at rates as low as 1 µg a.i./ml (Pullen et al., 1990).

Other pesticides exhibiting toxicity were carbaryl, carboxin, formaldehyde, and myclobutanil, which completely inhibited mycelial growth of ARF18 at 100 ppm, and mancozeb, which inhibited growth at 1,000 ppm. These pesticides, along with benomyl and thiophanate methyl, probably should not be used in fields where biocontrol agents such as ARF18 have been or will be used in an integrated pest management program. Even when applied to the leaves of crop plants, pesticides can reach the soil, either directly or by washing from the leaf surfaces. For example, when benomyl was applied to peach trees, the residue measured in the soil was 0 to 0.18 µg/gram of soil (Pullen et al., 1990).

It should be noted, however, that pesticides generally are much less effective in soil than in petri plates. For example, benomyl, iprodione, and vinclozoline in agar at 1 ppm were all highly toxic to *Sporidesmium sclerotivorum* (Adams and Wong, 1991). However, when applied to the surface of a soil column, only benomyl at 100 ppm markedly reduced the activity of the mycoparasite, and the reduction was observed only in the top 2 cm of soil. Below this depth, the fungicide had no adverse effect on the biocontrol agent. Foliar acaricides such as dicofol, when properly applied over a well-developed canopy, would never infiltrate the soil unless rain or irrigation water washed the acaricide from the plant into the soil.

Cyhexatin, diazinon, dicofol, paraquat, and trifluralin were comparatively less toxic to ARF18, slowing mycelial growth at 100 ppm but not eliminating pathogenicity of ARF18 to *H. glycines* eggs (Table 1). The fungus colonized 82%, 25%, 62%, 56%, and 66% of the eggs when exposed to the respective pesticides. The effects of the above pesticides on ARF18 would be minimal if there were no direct contact with the fungus upon application.

Aldicarb, bentazone, cholorothalonil, dicloran, fenpropathrin, glyphosate-isopropylamine, malathion, potassium salt of a fatty acid, quintozene, and sethoxydim allowed mycelial growth at concentrations of 1,000 ppm and could be used before, after, or in simultaneous application with the fungus. Aldicarb, bentazone, and chlorothalonil were the most innocuous to ARF18, inhibiting growth or pathogenicity very little even at the highest concentration (Table 1). Crump and Kerry (1986) also reported that aldicarb was a compatible pesticide with the nematode biocontrol agents *V. chlamydosporium* and *C. destructans.* Synergistic and(or) additive interactions between insecticides and an entomopathogenic fungus, *Beauveria bassiana,* have been observed (Anderson et al., 1989) for the control of Colorado potato beetle, although other data contradicted these results (Moorehouse et al., 1992). Therefore, aldicarb and ARF18 should be tested in an integrated control program to reduce initial nematode damage on crops in fields where the fungus is not established. The combined application of ARF18 and aldicarb would be particularly important in the first season of use of the biocontrol agent.

Mycelial growth was more sensitive to pesticides than was pathogenicity. Although the linear growth was greatly inhibited on plates amended with carbaryl (10 ppm), cyhexatin (10, 100 ppm), formaldehyde (10 ppm), and potassium salt of a fatty acid (1,000 ppm), the fungus still colonized as many eggs as were colonized in the control with nematodes and fungus but no pesticide (Table 1). The effect of some of the pesticides on ARF18 may be fungistatic rather than fungicidal. Similar observations were made in a test of 23 pesticides against *Verticillium* (Olmert and Kenneth, 1974).

Heterodera glycines eggs that were placed on CMA plugs hatched during the experimental period, and most plates had active J2, particularly on plates amended with carboxin (10 ppm), diazinon (10, 100 ppm),

malathion (100, 1,000 ppm), myclobutanil (10 ppm), bentazone (100 ppm), and paraquat (100 ppm) (Table 1). The J2 are more susceptible to pesticides than eggs, and eggs in plates with active J2 must be considered viable. In contrast, no J2 were observed in agar treated with aldicarb (100 and 1,000 ppm), potassium salt of a fatty acid (1,000 ppm), fenpropathrin (1,000 ppm), and formaldehyde (10 ppm); therefore, those pesticides are nematicidal or inhibit hatch of eggs of *H. glycines*. Other pesticides are known to affect nematode eggs. For example, the fungicide carbendazim prevented cleavage of eggs of *Criconemella xenoplax* at 1 mg a.i./liter (Jaffee and McInnis, 1990).

This study suggests that different pesticides have different effects on the growth and pathogenicity of the biocontrol fungus ARF18. A test in petri dishes cannot be related to what might happen under field conditions. Field studies will be needed to determine whether pesticides such as chlorothalonil, bentazone, and aldicarb, which appear to be more compatible with ARF18, could be used in an integrated pest management program for the control of *H. glycines* and other pests and pathogens of soybean. In contrast, benomyl, thiophanate methyl, carbaryl, carboxin, and myclobutanil strongly inhibit ARF18 and apparently could not be used in an integrated pest management program with ARF18, but these, too, may act differently under field conditions. Cyhexatin, diazinon, difocol, and paraquat are less inhibitory but not totally compatible with ARF18 and also should be tested under field conditions before being considered for an integrated pest management program with ARF18.

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