The Effect of Chemical Pesticides on the Infection of Sclerotia of *Sclerotinia minor* by the Biocontrol Agent *Sporidesmium sclerotivorum*

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ABSTRACT

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Pesticides were evaluated in vitro and in soil for toxicity to *Sporidesmium sclerotivorum*. Of the 46 pesticides evaluated in vitro, five fungicides (benomyl, captafol, chlorothalonil, thiabendazole, and thiophanate-methyl) and one herbicide (naptalam+dinoseb) were highly toxic to the biocontrol agent at 1 μg/ml in poison agar assays. Only three fungicides (anilazine, pentachloronitrobenzene, and thiram) were moderately toxic at 10 μg/ml. The remaining 37 pesticides were either slightly toxic (100 μg/ml) or less. In soil, chlorothalonil was the most toxic pesticide. This fungicide prevented the infection of sclerotia of *Sclerotinia minor* in soil by *S. sclerotivorum* at 10 μg/g of soil. Five fungicides were further evaluated in soil columns. None of the fungicides were toxic to the biocontrol agent in soil at concentrations likely to be encountered in the field. Apparently, pesticides can be used in conjunction with *S. sclerotivorum* in an integrated approach to disease control.

Biological control of plant diseases is a promising alternative to chemical control, but biocontrol agents will not replace all fungicides in the foreseeable future. Thus, biocontrol agents must be compatible with the pesticides used on a particular crop. For this reason, fungicide resistance has been developed in several potential biocontrol agents but has not been evaluated in soil (8,12). A realistic approach was taken when Fravel et al (11) evaluated a potato seed-piece applied biocontrol agent for sensitivity to pesticides normally applied to potato seed-pieces before planting. Their tolerance to fungicides in vitro was not consistent with field compatibility.

Sporidesmium sclerotivorum Uecker et al may have potential as a practical biocontrol agent for disease caused by *Sclerotinia* species (5,6). Effects of three soybean pesticides on *S. sclerotivorum* in vitro and in soil were studied, and captan at the label rate completely prevented germination of macroconidia in vitro. However, 100 times the label rate was required to prevent infection of sclerotia of *Sclerotinia minor* Jagger in soil. The present study was conducted to determine the effect of a range of pesticides on *S. sclerotivorum* in vitro and in soil.

MATERIALS AND METHODS

The 23 fungicides evaluated in this study for toxicity to *S. sclerotivorum* were anilazine, benomyl, captafol, captan, chloroneb, chlorothalonil, 1,3-dichloropropene and related chlorinated C_3 hydrocarbons, dodine, 5-ethoxy-3-trichloromethyl-1,2,4-thiadizole, fenaminosulf, ferbam, hexachlorophene, iprodione, maneb, maneb-zinc, nabam, pentachloronitrobenzene, procymidone, thiabendazole, thiophanate-methyl, thiram, vinclozoline, and zineb.

The 13 herbicides tested were alachlor, *N,N*-diallyl-2,2-chloroacetamide, 2-chloroallyl diethyldithiocarbamate, chloramben, dimethyl tetrachloroterephythalate, dinoseb, diuron, *S*-ethyl dipropylthiocarbamate, naptalam, naptalam+dinoseb, nitrofen, paraquot, and propachlor.

The 10 insecticides evaluated were carbaryl, chlordane, diazinon, dimethoate, ethion, ethoprop, fonofos, malathion, methyl demeton, and methomyl.

In vitro toxicity. Pesticides were tested for toxicity to *S. sclerotivorum* (isolate CS-4) in a poison agar test. The medium used in the assays was SM-4 (10). Pesticides were added to the molten agar medium after sterilization and before the medium was poured into 9-cm-diameter plastic petri dishes. Fifteen milliliters of the pesticide-SM 4 agar medium was poured into each petri dish. Each pesticide was evaluated at 1, 10, 100, and 1,000

μg of active ingredient (a.i.) per milliliter of medium. After the medium had solidified, the plates were seeded with a 7-mm agar plug taken from the margin of a colony of *S. sclerotivorum* grown on SM-4. After 21 days of incubation at 25 C, the diameter of the colony was determined on each plate. The pesticide was considered to be toxic to *S. sclerotivorum* at a particular concentration when growth of the fungus did not occur or was negligible. There were three replications of the treatments, and the experiments were repeated.

Toxicity in soil. Rumford loamy sand was infested with 1,000 macroconidia of *S. sclerotivorum* per gram of soil. Pesticides were mixed into 100-g aliquots of the soil in 250-ml beakers at the rates of 1, 10, and 100 μg a.i./g of soil (wt/wt). Beakers were covered with plastic film and incubated at room conditions (21–24 C). After 4 wk, 1 g of sclerotia of *S. minor* (SS-13) was mixed into each of the beakers and incubated at room temperature. Sclerotia were obtained from 4- to 6-wk-old cultures grown on a sand-corn meal medium (7). After 4 wk, sclerotia were retrieved from the soil (1), and 50 sclerotia were placed on moist filter paper in 9-cm-diameter petri dishes (25 sclerotia per dish). After 2 wk of incubation at room temperature, the number of sclerotia that exhibited growth of *S. sclerotivorum* were counted. These sclerotia were considered infected by *S. sclerotivorum*, and the percentage of infected sclerotia was determined (4). A pesticide was considered to be toxic to *S. sclerotivorum* at a particular concentration when no sclerotia exhibited growth of the mycoparasite. There were three replications (beakers) of the treatments, and the experiments were repeated with statistically the same results.

Because most fungicides are applied to the foliage or to the soil surface, experiments were conducted in which benomyl, chlorothalonil, iprodione, procymidone, or vinclozoline were applied to the soil surface, and the activity of *S. sclerotivorum* at various depths was determined. Norfolk sandy loam infested with *S. sclerotivorum* was mixed with soil infested with *S. minor*, and 90 g of the mixture was placed into transparent plastic soil columns (3.1 cm diameter × 10 cm). The fungicides were applied to the soil surface at 0, 100, and 1,000 μg per column (0, 1.32, and 13.2 kg/ha). Four hours later, 15 ml of water was applied to the columns to simulate a rainfall. Approximately twice each week, 10 ml of water was applied to the columns to keep the soil moist. The amount of water applied was sufficient to moisten all the soil in the columns. At 4 and 6 wk, soil was removed from the the 0–2, 2–4, and 4–6 cm depths of the columns. Sclerotia from these soil samples were recovered as previously described (1) and placed onto moist filter paper. After 2 wk of incubation at room temperature, sclerotia were observed for colonization by *S. sclerotivorum*, and the percentage of infected sclerotia was determined. *S. sclerotivorum* does not infect dead sclerotia (9), so viability of the sclerotia retrieved from the soil was determined for 50 sclerotia retrieved from these soil samples. These sclerotia were surface-sterilized and plated onto an agar medium to assess viability (7). There were three replications, and the experiments were repeated.

Several applications of a fungicide are commonly applied to a crop to obtain disease control. For this reason, another series of experiments was conducted in which three applications of iprodione, procymidone, and vinclozoline were applied to the soil columns at 7- to 10-day intervals. Soils in the columns were assayed 4 and 6 wk after the first application of the chemical for viability of sclerotia of *S. minor* and for degree of infection of sclerotia by the mycoparasite. Each treatment was replicated with four columns, and the experiments were repeated at least once and analyzed separately. Duncan's multiple range test was used to analyze the data of each experiment.

RESULTS

Of the 46 pesticides evaluated in vitro, five fungicides (benomyl, captafol, chlorothalonil, thiabendazole, and thiophanate-methyl) and one herbicide (naptalam+dinoseb) were highly toxic (1 μg/ml), and only three fungicides (anilazine, pentachloronitrobenzene, and thiram) were moderately toxic (10 μg/ml) in the poison agar test (Table 1). The remaining 37 pesticides were either slightly toxic (100 μg/ml) or less in the in vitro experiments. Only chlorothalonil was found to be moderately toxic (10 μg/ml) to the mycoparasite in soil. The one highly toxic herbicide in vitro (naptalam+dinoseb) showed little or no toxicity in soil.

In the soil column studies in which one application of fungicides was applied, varying results occurred. Viability of the sclerotia of *S. minor* was not affected by the fungicides (approximately 80% in fungicide treated and untreated soil). When benomyl or chlorothalonil was applied to the columns at 1,000 μg, complete inhibition of infection of the sclerotia by *S. sclerotivorum* at all soil depths occurred. At 100 μg, there was a reduction of infection of sclerotia by benomyl at the 0–2 cm depth but not at lower depths. Effects of chlorothalonil were similar to that of benomyl at 4 wk but not at 6 wk (Table 2).

Iprodione, procymidone, and vinclozoline did not alter infection of sclerotia by the mycoparasite in soil at various depths. Vinclozoline at 1,000 μg at the 0–2 or 4–6 cm depth caused a moderate reduction of infection when compared to the appropriate control treatments at 4 wk but not at 6 wk (Table 3). Compared to the appropriate control treatments, iprodione, and procymidone had

TABLE 1. Toxicity of pesticides to *Sporidesmium sclerotivorum* in vitro and in soil

Chemical	In vitro toxicity (μg a.i./ml medium)	In soil toxicity (μg a.i./g soil)
Fungicides		
Anilazine	10	100
Benomyl	1	100
Captafol	1	100
Captan	100	100
Chloroneb	1,000	ND
Chlorothalonil	1	10
1,3-Dichloropropene and related chlorinated C_3 hydrocarbons	1,000	
2,6-Dichloro-4-nitroaniline	1,000	1,000
Dodine	100	1,000
5-Ethoxy-3-trichloromethyl-1,2,4-thiadazole	1,000	ND[z]
Fenaminosulf	100	1,000
Ferbam	100	100
Hexachlorophene	100	1,000
Maneb	100	100
Maneb-zinc	100	100
Pentachloronitrobenzine	10	1,000
Thiabendizole	1	100
Thiophanate-methyl	1	100
Thiram	10	100
Zineb	100	100
Herbicides		
Alachlor	100	ND
N,N-Diallyl-2,2-chloroacetamide	100	ND
2-Chloroallyl diethyl-dithiocarbamate	100	ND
Chloramben	1,000	ND
Dinoseb	100	ND
Dimethyl tetrachloroterephthalate	1,000	ND
Diuron	100	ND
S-Ethyl dipropylthiocarbamate	1,000	ND
Naptalam+dinoseb	1	1,000
Nitrofen	1,000	ND
Paraquot	1,000	ND
Trifluralin	1,000	ND
Insecticides		
Carbaryl	100	ND
Chlordane	100	ND
Diazinon	1,000	ND
Dimethoate	1,000	ND
Ethion	1,000	ND
Ethoprop	100	ND
Methomyl	1,000	ND
Methyl demeton	1,000	ND

[z] Not determined.

TABLE 2. The effect of benomyl and chlorothalonil on the infection of sclerotia of *Sclerotinia minor* by *Sporidesmium sclerotivorum* in soil columns at various depths

Concentration (μg)	Soil depth (cm)	Percentage of infection of sclerotia[z]			
		Benomyl		Chlorothalonil	
		4 wk	6 wk	4 wk	6 wk
0	0–2	58 a	80 a	84 a	66 ab
	2–4	72 a	88 a	86 a	90 a
	4–6	70 a	88 a	90 a	84 a
100	0–2	16 b	34 b	45 b	45 b
	2–4	48 a	85 a	82 a	70 ab
	4–6	59 a	98 a	78 a	83 a
1,000	0–2	0	0	0	0
	2–4	0	0	0	0
	4–6	0	0	0	0
$P =$		.01	.01	.01	.05

[z]Values followed by the same letter are not statistically different at the indicated level of probability according to Duncan's multiple range test.

TABLE 3. The effect of vinclozoline, iprodione, and procymidone on the infection of sclerotia of *Sclerotinia minor* by *Sporidesmium sclerotivorum* in soil columns at various depths

Concentration (μg)	Soil depth (cm)	Percentage of infected sclerotia[y]					
		Vinclozoline		Iprodione		Procymidone	
		4 wk	6 wk	4 wk	6 wk	4 wk	6 wk
0	0–2	76 ab	86 ab	49	72	85 abc	75
	2–4	74 ab	90 ab	36	75	83 abc	90
	4–6	78 a	92 a	34	66	94 ab	88
100	0–2	84 a	78 b	65	86	84 abc	87
	2–4	79 a	92 a	58	82	88 abc	88
	4–6	83 a	90 ab	65	86	99 a	88
1,000	0–2	46 c	80 ab	41	75	78 bc	74
	2–4	61 abc	84 ab	36	72	72 c	88
	4–6	51 bc	84 ab	38	66	92 abc	90
$P =$		.05	.01	ns[z]	ns	.01	ns

[y]Values in a column followed by the same letter are not statistically different at the indicated level of probability according to Duncan's multiple range test.
[z]Not significant.

no adverse effect on the infection of sclerotia of *S. minor* by *S. sclerotivorum*.

When iprodione or vinclozoline were applied to the columns three times at 7- to 10-day intervals, vinclozoline had no effect on infection of the sclerotia. Iprodione, however, caused a slight reduction of infection of the sclerotia at the 2–4 and 4–6 cm depths at both 4 and 6 wk in one experiment and only at the 4–6 cm depth at the 6-wk assay in a second experiment (Table 4).

Viability of sclerotia was also found not to be affected by iprodione, procymidone, or vinclozoline.

DISCUSSION

The purpose of this study was to determine if pesticides applied to a crop would adversely affect the activity of *S. sclerotivorum* in soil. Based on the in vitro data (Table 1), if any one of the moderately or highly toxic pesticides (eight fungicides and one herbicide) were sprayed onto a crop in a tank mix together with *S. sclerotivorum* the mycoparasite would probably be killed or at least inactivated. With *S. sclerotivorum*, the use of a mycoparasite-pesticide mix is not likely to occur in practice.

Use of *S. sclerotivorum* as an economical biocontrol agent will require application to a diseased crop after harvest by disking into the soil (3,6). The mycoparasite will then parasitize sclerotia of *Sclerotinia* species at a soil depth of 2 cm or greater (2). All of the fungicides tested in this study except 2,6-dichloro-4-nitroaniline and pentachloronitrobenzene are applied as a spray to crop foliage or the soil surface. The most toxic fungicide to

TABLE 4. The effect of iprodione on infection of sclerotia of *Sclerotinia minor* by *Sporidesmium sclerotivorum* when applied three times at 7- to 10-day intervals to soil tubes, washed into the soil by simulated rain and assayed 4 and 6 wk after the first application of the fungicide

Concentration (μg/tube)	Soil depth (cm)	Percentage of infected sclerotia[y]			
		Exp. 1		Exp. 2	
		4 wk	6 wk	4 wk	6 wk
0	0–2	26 c	56 abc	78	80 abc
	2–4	77 a	74 a	78	89 ab
	4–6	76 a	74 a	80	94 a
100	0–2	31 c	54 abc	80	78 bc
	2–4	58 b	62 ab	86	88 ab
	4–6	92 a	72 a	72	94 a
1,000	0–2	27 c	28 c	50	84 abc
	2–4	15 c	30 c	50	76 bc
	4–6	13 c	31 bc	51	69 c
$P =$		0.01	0.05	ns[z]	0.01

[y]Values in a column not followed by the same letter are different at the indicated level of confidence.
[z]Not significant.

S. sclerotivorum in soil was chlorothalonil. This fungicide prevented macroconidia of the mycoparasite from germinating and infecting sclerotia of *S. minor* in soil at a concentration of 10 μg/g of soil. If chlorothalonil was uniformly incorporated into the soil at the label rate (2.52 kg/ha) to a depth of 2 cm, the concentration of the chemical would be 8.5 μg/g of soil. If incorporated to greater depths, the concentration would be diminished. Chlorothalonil is highly toxic to *S. sclerotivorum* in vitro but probably would not suppress the mycoparasite in soil. The other fungicides that were highly toxic in vitro were even less toxic than chlorothalonil to *S. sclerotivorum* in soil.

The experiments in which selected fungicides were applied to the surface of soil columns also indicate that fungicides applied in the usual manner will probably not adversely affect *S. sclerotivorum* in soil. Benomyl, iprodione, and vinclozoline are all highly toxic to the mycoparasite in vitro (P. B. Adams and E. Banks, *unpublished results*). However, when applied to the surface of soil columns, only benomyl at 100 μg markedly reduced the activity of *S. sclerotivorum*, and this effect was only in the top 2 cm of soil. Below this depth, the fungicide had no adverse effect on the biocontrol agent. At 1,000 μg, benomyl completely prevented the sclerotia from becoming infected at all soil depths. The sclerotia of *S. minor* though were not killed by the fungicide treatment. The other fungicides were much less toxic when applied to the soil columns and thus, would be expected to have little adverse effect on mycoparasitism when applied at label rates in the field.

Data on the viability of sclerotia of *S. minor* in soil was important because *S. sclerotivorum* infects only live sclerotia of host fungi (9). The viability of the sclerotia usually was 80% or greater. In most treatments, 80–90% of these sclerotia were commonly infected by *S. sclerotivorum*. None of the fungicides tested in soil in this study were toxic to the sclerotia of *S. minor* in soil when viability data were compared to the controls.

In the in vitro experiments, a number of pesticides were highly toxic to *S. sclerotivorum* but were 10 to 100 times less toxic in soil (Table 1). Thus, these chemicals apparently were rapidly degraded or were chemically bound to soil particles or organic matter and rendered biologically inactive. In the soil column experiments, benomyl applied at 100 μg to the soil surface penetrated only 2 cm at concentrations sufficient to adversely affect *S. sclerotivorum* (Table 2). The 100 μg rate is slightly higher than the label rate for benomyl.

Effects of surface-applied pesticides on the activity of fungal biocontrol agents that are active in the soil appears to be negligible. Effects of pesticides in general on the activity of fungal biocontrol agents in the soil may be minor.

This study suggests that an approach to control of diseases caused by *S. minor* based on integration of *S. sclerotivorum* and a fungicide is both practical and prudent. Chemical methods may

be useful for disease control until activity of the biocontrol agent is sufficient to suppress the pathogen. This is particularly important in the first season of the use of the biocontrol agent. Subsequent seasons should not need supplementary chemical control.

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