Influences of Certain Fungicides on Parasitism of the Nematode *Criconemella xenoplax* by the Fungus *Hirsutella rhossiliensis*

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ABSTRACT

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Seven fungicides commonly used in South Carolina peach orchards were tested in the laboratory and greenhouse for their effects on *Hirsutella rhossiliensis*, a fungal parasite of *Criconemella xenoplax*. Benomyl at 1, 5, and 10 μg a.i./ml completely inhibited hyphal growth of the fungus. Chlorothalonil, dicloran, iprodione, and triforine significantly suppressed hyphal growth at 20 μg a.i./ml, but captan inhibited growth only at 40 μg a.i./ml. Sulfur had no effect at the concentrations studied. Conidial germination and germ tube elongation were more sensitive to benomyl than to iprodione or triforine. Captan and dicloran slightly inhibited spore germination and germ tube elongation, but chlorothalonil and sulfur had no effect. In the greenhouse, *H. rhossiliensis* suppressed populations of *C. xenoplax*, but fungicides did not affect nematode populations, reproduction, or development. The frequency of parasitism of *C. xenoplax* was not measurably affected by any fungicide. However, fewer conidia were observed on nematodes exposed to some fungicides, and nematodes infested with conidia in chlorothalonil-treated soil were more likely to be parasitized. Soil residues after foliar application of benomyl or chlorothalonil in peach orchards were low relative to the concentrations studied in the greenhouse. Routine applications of fungicides commonly used in South Carolina peach orchards should not suppress activity of *H. rhossiliensis* as a parasite of *C. xenoplax*.

The ring nematode, *Criconemella xenoplax* (Raski) Luc & Raski, is an important agent in the early mortality of peach trees (*Prunus persica* (L.) Batsch) (4,16,19). *Hirsutella rhossiliensis* Minter & Brady, a common fungal endoparasite of *C. xenoplax* in the sandy soils of South Carolina where these nematodes occur, was found in infected *C. xenoplax* in 10 of 23 orchards sampled in South Carolina and Georgia (10) and suppressed *C. xenoplax* in some tests (7).

Agricultural fungicides applied to peach foliage and fruits to control diseases might affect the interaction of *C. xenoplax* and *H. rhossiliensis*. Fungicides may enter the soil through a number of routes, including direct application or as runoff or drift after foliar application (12). The mobility and persistence of such fungicides depend upon many factors. Soil properties that most affect fungicide behavior include adsorption, leaching, pH, soil moisture, and soil temperature (12,15). Microorganisms capable of degrading or altering the fungicide also may be important in the fate of these chemicals (12).

Fungicides in the soil may affect nontarget organisms, including those that may suppress plant pathogens. Many organisms having potential for biological control are parasitic and do not compete well as saprophytes (1,17). Saprophytic soil organisms usually suffer only short-term effects from pesticide applications, but parasites normally require more time to return to original population densities. Application of formalin to soil reduced the effectiveness of *Nematophthora gynophila* Kerry & Crump for control of the cereal cyst nematode (13). Baien (2) found in the laboratory that benomyl and captan inhibited germination of *Trichoderma viride* Pers.:Fr. but that sulfur had no effect. Hyphal growth was arrested completely by benomyl, whereas thiophanate-methyl and captan suppressed growth by 40%.

Little is known about the biology and ecology of *H. rhossiliensis* in soils. Because fungicide use in peach orchards might affect growth and survival of *H. rhossiliensis* and its parasitism of *C. xenoplax*, we studied seven of the fungicides most commonly used in South Carolina peach orchards for effects on hyphal growth, conidial germination, germ tube elongation, and parasitism of *C. xenoplax* by the fungus.

MATERIALS AND METHODS

Cultures. Strain A3a of *H. rhossiliensis* was isolated in 1981 from *C. xenoplax* obtained from soil in a South Carolina peach orchard (10). Cultures were maintained on a medium developed for culturing *H. thompsonii* Fisher, a parasite of citrus rust mite (14), modified by the addition of 15 g of agar/L. The medium (HRGM) contained (g/L) dextrose, 20.0; yeast extract, 15.0; peptone, 0.5; $MgSO_4 7H_2O$, 0.01; KH_2PO_4, 0.1; $CaCl_2$, 0.01; and Bacto agar, 15.0. Cultures were transferred onto fresh HRGM approximately every 2 mo. Individuals of *C. xenoplax* were inoculated with conidia of *H. rhossiliensis* (10) to test infectivity and virulence and to initiate new cultures.

Growth suppression. The following fungicides were tested for suppression of *H. rhossiliensis*: benomyl 50WP, captan 50WP, chlorothalonil 500F, DCNA 75WP, iprodione 50WP, sulfur (96%), and triforine 1.6EC.

Concentrations of benomyl tested were 0, 1, 5, and 10 μg a.i/ml, but all other fungicides were tested at 0, 10, 20, and 40 μg a.i./ml. Stock solutions were prepared by dissolving commercial formulations of each fungicide in 1.5 ml of 70% ethanol, except that iprodione was dissolved in 1.5 ml of acetone (5). Benomyl was added to the medium before autoclaving, whereas others were added to autoclaved medium cooled to 50 C. Flasks were swirled gently before approximately 20 ml of medium was poured into plastic petri dishes with a diameter of 15 × 100 mm. After 24 hr, four 5-mm-diameter agar plugs containing *H. rhossiliensis* were cut from the edges of 3-wk-old HRGM colonies and inverted onto the medium of each dish to make direct mycelial contact. Dishes were enclosed in bags to minimize drying and incubated in the dark at 25 C for 2 wk. On days 7 and 14, the radial growth of each colony was measured. There were three dishes per treatment in the first two tests and four in the third.

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Conidial germination. Fungal cultures were maintained on HRGM. Fungicide-amended water agar (WA) was used to test germination on the lowest and highest fungicide concentrations used in the radial growth test. Two 5-mm-diameter plugs of the fungal culture were removed, inverted, and touched gently to the fungicide-amended agar surface. Later (24 and 48 hr), 100 conidia that had adhered to the agar surface were observed at ×250, and percent germination and germ tube length were recorded. Germ tubes at least as long as the conidium were considered to be germinated. The test was repeated with two replicates per fungicide concentration. Because results were similar for both tests, data were pooled and tested by analysis of variance, and the least significant difference among treatments and concentrations was calculated.

Effects on nematode parasitism. Cultures of *H. rhossiliensis* were grown on V-8 juice-vermiculite medium (3 g of $CaCO_3$ mixed in 800 ml of distilled water plus 200 ml of V-8 juice). Fifty cubic centimeters of vermiculite was placed in glass petri dishes with a diameter of 10 × 100 mm, and 35 ml of the V-8 juice medium was added. Dishes were autoclaved for 45 min at 100 C and 717 kPa. After cooling overnight, they were seeded with approximately 4 ml of a mycelium-conidial suspension prepared from 55 3-wk-old sporulating colonies of *H. rhossiliensis* blended in 500 ml of sterile distilled water for 30 sec in a sterilized blender (Osterizer Model #848-31B, Oster Corp., Milwaukee, WI). Cultures were incubated in the dark at 25 C for 4 wk.

Lakeland sand (89% sand, 5% silt, 6% clay) was collected from the Sandhill Research and Education Center, Elgin, SC, and treated with aerated steam for 35 min at 65 C. The contents of two petri dishes of the final V-8 juice-vermiculite culture described above were mixed with 3 L of soil to give a 3.0% (v/v) concentration. Controls were mixed with sterile V-8 juice-vermiculite medium in the same proportions. Seven-week-old seedlings of peach cultivar Nemaguard then were transplanted and watered with approximately 100 ml of tap water. One week after the seedlings were transplanted, the following fungicides were added to appropriate pots as a soil drench in 750 ml of tap water: benomyl 50WP, captan 50WP, chlorothalonil 500F, iprodione 50WP, and triforine 1.6EC.

One week after fungicides had been applied, *C. xenoplax* was extracted from greenhouse soil in which Nemaguard peach served as the host. Nematodes were extracted by hand sieving (18) followed by sugar flotation-centrifugation (11) and collected on a 500-mesh wire screen (38-μm-mesh opening). Five hundred individuals (juveniles and adults) were pipetted into each pot. Treatments were *C. xenoplax* only, *C. xenoplax* + *H. rhossiliensis*, fungicide + *C. xenoplax*, and fungicide + *C. xenoplax* + *H. rhossiliensis*. Fungicide concentrations were 10 μg a.i. for benomyl and 40 μg a.i./ml for chlorothalonil, captan, iprodione, and triforine.

Seedlings were watered daily by an automated drip irrigation system (Stuppy, Inc., Greenhouse Supply Division, North Kansas City, MO) to maintain the soil at or near field capacity. Fertilizer in 100 ml of water per pot was added every 2 wk alternately as 45 g of $CaNO_3$ + 15 g of $MgSO_4$, or 35 g of 15-0-14 (NPK) + 12 g of 0-46-0 (super phosphate) + 2 ml of Stoller's Super Crop Mix (Stoller Chemicals, Houston, TX) diluted in 19 L of tap water. The first test was terminated after 3 mo, and the last two were terminated after 4 mo. Soil temperatures approached 32 C in the last two repetitions but did not exceed 28 C in the first.

At termination, the soil was mixed by hand, and a 500-cm^3 sample was extracted by a combination of semiautomatic elutriation (3) followed by sugar flotation-centrifugation (11). Gravid and nongravid adults, juveniles, number of *C. xenoplax* containing hyphae, number with conidia adhering to the cuticle, and total number of conidia observed were counted with the aid of a dissecting microscope. Nematodes not inoculated with *H. rhossiliensis* were examined periodically for parasitism or conidial attachment as controls. Periodically, colonized nematodes were surface disinfested for 15 sec in 1 drop of 15% NaOCl, rinsed in sterile distilled water, then plated onto HRGM to isolate *H. rhossiliensis*.

These tests were conducted as 2 × 2 factorial experiments arranged in randomized complete block designs, with eight replicates in the first test and five replicates in the second and third tests. Data were subjected to analyses of variance, and mean separation was performed by least significant difference tests when treatment differences were significant ($P = 0.05$).

Field application of fungicides and assays. Two applications (7 days apart) of chlorothalonil 500F, each at 2,345 g a.i./ha, were applied with an air-blast sprayer on peach trees on Lakeland sand at the Clemson University Sandhill Research and Education Center, Elgin, SC. Two applications of benomyl 50WP, each at 560 g a.i./ha, also were applied there and on peach trees growing on Cecil sandy loam (65% sand, 17% silt, and 18% clay) at the Simpson Experiment Station near Pendleton, SC. Fungicides were sprayed on three adjacent rows of trees, and samples were taken from soil beneath three trees in the middle row 12 hr after the second application. A single slice of soil was removed with a transplanting shovel from each quadrant beneath the drip line to a depth of approximately 20 cm. Subsamples from each tree were combined and stored in an ice chest until frozen (−8 C) before analysis. Soil collected under untreated trees in the same orchards served as a control, and additional samples were collected from nearby areas with no known history of fungicide application. Known amounts of analytical grade fungicide were added to these samples to determine the efficiency of residue recovery for each procedure.

Benomyl extraction. Samples were air dried for 48 hr at room temperature. Percent moisture at sampling and after air drying was determined with an Ohaus moisture determination balance (Ohaus Scale Corp., Union, NJ) set at 30.5 C for 8 min. The technique used to extract and quantitate methyl-2-benzimidazole-carbamate (MBC) and 2-aminobenzimidazole (2-AB) was obtained from Jim Prince, E.I. Du Pont de Nemours & Co., Inc., White Plains, NY (*personal communication*). Analytical standards of MBC (99.8%) and 2-AB (99.8%) were supplied by E.I. Du Pont de Nemours, Wilmington, DE. All solvents were pesticide grade.

Liquid chromatography was performed with a Varian Vista 5060 liquid chromatograph (Varian Associates Inc., Sunnyvale, CA) equipped with a Varian UV-100 ultraviolet detector (280 nm) and Varian MicroPak reverse phase C-18 column. Twenty-microliter samples were delivered with a Varian Model 8055 autosampler; a Varian Vista 402 data system was used to calculate the concentrations of MBC and 2-AB detected. Chromatographic conditions were as follows: mobile phase, 50% methanol, 49% water, 1% acetic acid, and 0.5 g of sodium acetate/L; flow-rate, 1.0 ml/min; inlet pressure, 237 kPa; and ultraviolet detector, 280 nm. Two injections of 20 μl were made for each sample, and 20 μl of a 50% acetonitrile:water blank was run between each sample.

Analytical standard solutions of 2-AB and MBC were prepared by dissolving 1 mg of test chemical in 50% acetonitrile:H_3PO_4 (high-performance liquid chromatography grade). Dilutions of 1 or 5 μg/ml were used to calibrate the chromatograph each day and compared with standard response curves. One milliliter each of 40 μg/ml of MBC and 2-AB standard solutions was pipetted into 50 g of air-dried soil (48 hr at room temperature), mixed thoroughly, placed in a freezer overnight, then extracted and chromatographed by the procedure and conditions reported below.

A 50-g soil sample was placed in a 1-L screw-cap flask, and 100 ml of ethyl acetate (EtOAc) and 2 ml of 6.5N NaOH were added. The flask was agitated and placed on a gyrorotatory shaker for 1 hr; samples were vacuum filtered through Whatman No. 1 filter paper into a 1-L flask. Contents were transferred to a 250-ml round-bottom flask, 25 ml of 1 N HCl was added, and the flask was swirled gently for 60 sec. EtOAc was removed with a rotoevaporator set at 50 C, and the remaining aqueous phase was transferred to a 250-ml separatory funnel. The round-bottom flask was rinsed twice with hot, deionized distilled water, and the rinsate was added to the aqueous phase. The aqueous phase was washed three times with 50 ml of hexane and once with 30 ml of EtOAc.

Fifteen milliliters of 6.5 N NaOH was added to the extract, mixed, and left to stand for 2 min, then extracted four times with 75 ml of EtOAc. The EtOAc was saved each time in a 1-L beaker and dried with 30 g of $NaSO_4$ as follows. A glass funnel was plugged with glass wool, and the $NaSO_4$ was placed on top of the plug. EtOAc was carefully poured down the side of a glass pipette into the funnel and collected in two 250-ml round-bottom flasks.

EtOAc was concentrated to 15–20 ml in a rotoevaporator at 50 C and transferred to a 50-ml beaker, and 1 ml of 0.1 N H_3PO_4 was added. The beaker was placed in a warm-water bath, and the extract was concentrated to 1 ml with a stream of nitrogen and transferred to a 15-ml graduated centrifuge tube. Volume was increased to 4 ml with a 50:50 solution of acetonitrile:0.2 N H_2PO_3; sides of the beaker were rinsed with the solution during this procedure. Then the entire extract was filtered through 0.2-μm nylon Acrodiscs (Gelman Sciences Inc., Ann Arbor, MI) into a 15-ml concentration tube. Samples not chromatographed immediately were stored in a freezer (−8 C) until analyzed.

Chlorothalonil extraction. Chlorothalonil residues were determined by gas-liquid chromatography by a technique obtained from B. D. Ripley, Provincial Pesticide Residue Testing Laboratory, Ontario Ministry of Agriculture and Food, University of Guelph, Guelph, Ont., Canada (*personal communication*). Analytical grade chlorothalonil (98.6%) was obtained from SDS Biotech Corp., Painesville, OH.

Gas-liquid chromatography was performed on a Varian 3700 gas chromatograph (Varian Associates) connected to a CDS 111 chromatography data system and recorder. The chromatograph was equipped with a 2-m glass column, with an inside diameter of 2 mm, packed with 10% OV-101 on Chromsorb W-HP (80/100 mesh) and a ^{63}Ni electron capture detector. Chromatographic conditions were as follows: injector, 250 C; oven, 200 C; detector, 250 C; and nitrogen carrier gas, 55 ml/min. Sample size injected was 1 μl, and 1-μl blanks of pesticide grade EtOAc were run between each sample.

Standard solutions were prepared by dissolving 0.1 g of analytical standard chlorothalonil in 100 ml of EtOAc (1 mg/ml), followed by dilutions of 1,000, 100, 20, 10, 7.5, 5, and 1 ng/ml to determine response. Soil containing no chlorothalonil was amended with 1 ml of 1 mg/ml analytical grade chlorothalonil dissolved in EtOAc, frozen overnight, extracted, and chromatographed. The amount of chlorothalonil detected in two amended samples was averaged and plotted on the standard curve to obtain a mean recovery.

A 100-g soil sample was shaken for 2 hr in 300 ml of a 2:1 acetonitrile:water solution, then vacuum filtered through Whatman No. 1 filter paper. Acetonitrile was evaporated with a rotoevaporator set at 35 C. The aqueous phase was transferred to a 250-ml separatory funnel, and chlorothalonil was extracted with two 75-ml portions of methylene chloride ($MeCl_2$). The $MeCl_2$ was evaporated to dryness, and the residue was redissolved in 10 ml of EtOAc for gas-liquid chromatography analysis.

To determine the amount of chlorothalonil residue, the peak area of each sample was compared with that of standards. This value was multiplied by 10 (volume of extract used), divided by 100 (number of grams per soil sample), and multiplied by the percent recovery (59.6) to give nanograms of chlorothalonil per gram of soil.

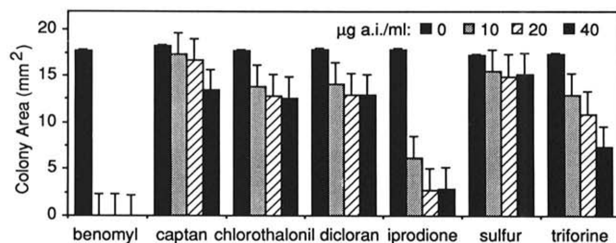

Fig. 1. Suppression of hyphal growth of *Hirsutella rhossiliensis* 14 days after transfer to HGRM agar amended with certain fungicides. Fungicide concentrations shown apply to all except benomyl, which was tested at 0, 1, 5, and 10 μg a.i./ml. Colony area measurements represent the mean of 16 observations per treatment. Vertical lines represent the standard errors.

RESULTS

In vitro sensitivity. Benomyl prevented growth of *H. rhossiliensis* at all concentrations tested (Fig. 1). Chlorothalonil, dicloran, iprodione, and triforine significantly suppressed ($P = 0.05$) fungal growth at 10, 20, and 40 μg/ml. Except for sulfur, which was not suppressive, fungal growth was inversely proportional to fungicide concentration. Captan significantly suppressed growth only at 40 μg/ml ($P = 0.05$). Trends were similar after 7 and 14 days for all three tests; therefore, colony size at 14 days for one test only is shown in Figure 1.

Conidia of *H. rhossiliensis* did not germinate on benomyl-amended water agar at 1 or 10 μg/ml (Table 1). Iprodione and triforine, at both 10 and 40 μg/ml concentrations, suppressed but did not prevent conidial germination or germ tube elongation as compared with the control. Captan and dicloran slightly suppressed germination and elongation, whereas chlorothalonil and

TABLE 1. Percent germination and germ tube length of conidia of *Hirsutella rhossiliensis* after 48 hr on 1.5% water agar amended with fungicides[a]

Fungicide	Concentration (μg/ml)	Germination (%)	Germ tube length[b]
None	0	67	8
Benomyl	1	0	0
	10	0	0
Captan	10	55	6
	40	53	5
Chlorothalonil	10	63	8
	40	53	6
Dichloran	10	59	5
	40	33	3
Iprodione	10	46	5
	40	33	3
Sulfur	10	66	7
	40	65	7
Triforine	10	41	5
	40	30	4
Least significant difference ($P = 0.05$)		23	3

[a] Test was performed twice and results were not significantly different between tests ($P = 0.05$). Therefore, the data were pooled.

[b] Germ tube length expressed in multiples of conidium length after 48 hr. Values represent means of 400 conidia measured in two replicates of this test.

TABLE 2. Numbers of *Criconemella xenoplax* on greenhouse-grown seedlings of peach cultivar Nemaguard 3–4 mo after treatment, as related to inoculation with *Hirsutella rhossiliensis* and fungicide drenches

	No. *C. xenoplax* in 100 cm^{-3} of soil[a]					
	Fungus present			Fungus absent		
Fungicide drench	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
None	27	68	153	44	134	316
Benomyl	37	130	288	48	155	468
Chlorothalonil	31	103	469	52	181	526
Triforine	56	96	164	49	150	514
Captan	39	90	281	48	104	461
Iprodione	29	68	167	36	107	427
Least significant difference ($P = 0.05$)	22	42	177	NS	NS	NS

[a] Nematode numbers in the absence of *H. rhossiliensis* were not significantly (NS) affected ($P = 0.05$) by the fungicides as compared with the controls. Values represent means of eight observations in the first test and five in the last two tests.

TABLE 3. Percentage of *Criconemella xenoplax* with conidia of *Hirsutella rhossiliensis* attached to the cuticle (S), percent colonized (C), and total number of conidia observed on infested nematodes (T) as related to fungicide drenches[a]

	Test 1		Test 2			Test 3		
Fungicide drench	S	C	S	C	T	S	C	T
None	8	4	10	0.5	22	6	0.2	21
Benomyl	5	6	3	0.1	2	2	0.2	12
Chlorothalonil	8	6	2	2	6	1	0.2	10
Triforine	5	7	3	0.9	10	4	0.1	14
Captan	7	5	5	2	10	2	0.04	14
Iprodione	10	4	9	0.7	19	5	0.2	19
Least significant difference ($P = 0.05$)	NS	NS	3	NS	11	2	NS	NS

[a] Data were obtained from *C. xenoplax* grown 3–4 mo on seedlings of peach cultivar Nemaguard in soil infested with *H. rhossiliensis* in the greenhouse. Values represent means of eight observations in the first test and five in the last two tests.

TABLE 4. Percent infection of *Criconemella xenoplax* contaminated with conidia of *Hirsutella rhossiliensis* as influenced by fungicide drenches

Fungicide	Mean percent infection of *C. xenoplax*[a]		
drench	Test 1	Test 2	Test 3
Chlorothalonil	41	18	45
Triforine	56	4	21
Captan	48	1	21
Iprodione	27	4	7
Benomyl	40	7	3
None	27	3	5
Least significant difference ($P = 0.05$)	NS	NS	25

[a] Percent infection was calculated by the formula: colonized *C. xenoplax*/colonized *C. xenoplax* + *C. xenoplax* with conidia attached. Nematodes were allowed to feed on seedlings of peach cultivar Nemaguard grown in the greenhouse for 3–4 mo after infestation of the soil with *H. rhossiliensis*. Values represent means of eight observations in the first test and five in the last two tests.

sulfur did not affect germination or germ tube elongation. Average germination for the controls was 67% after 48 hr (Table 1).

When conidia from colonies grown on fungicide-amended HRGM were transferred to unamended WA, germination and growth resumed. Because no spores were produced on the medium containing benomyl, its effects on germination and growth were not determined.

Effects on nematode parasitism. In each test with *C. xenoplax* on peach in the greenhouse, *H. rhossiliensis* significantly ($P = 0.01$) suppressed population increase. Fungicides alone did not affect final nematode populations or reproduction and development, but in each of the three tests at least one fungicide significantly affected the suppressive activity of *H. rhossiliensis* (Table 2). In the first experiment, there was less suppression with triforine, but in the second and third experiments, benomyl and chlorothalonil, respectively, had similar effects.

Fungicides and *H. rhossiliensis* together did not affect reproduction or developmental stages of *C. xenoplax*. The percentage of *C. xenoplax* colonized by *H. rhossiliensis* was not significantly affected ($P = 0.05$) (Table 3), but the percentage of *C. xenoplax* with conidia attached was significantly lower in the second and third tests ($P = 0.0001$) with all fungicides except iprodione. Fungicides also affected the total number of spores observed (Table 3), but the difference was significant ($P = 0.05$) only in the second test.

The percent infection of *C. xenoplax* after conidial attachment was relatively high in the chlorothalonil treatment in the second and third tests but was significantly greater ($P = 0.02$) only in the third test (Table 4). The other fungicides did not affect infection relative to the control.

Residue analysis. When samples of analytical grade chlorothalonil were chromatographed, the retention time of chlorothalonil was 3.5–3.6 min (Fig. 2A). Percent recovery of chlorothalonil residues was 59.6%, and the lower limit of detection was approximately 0.02 μg/ml. The background level of

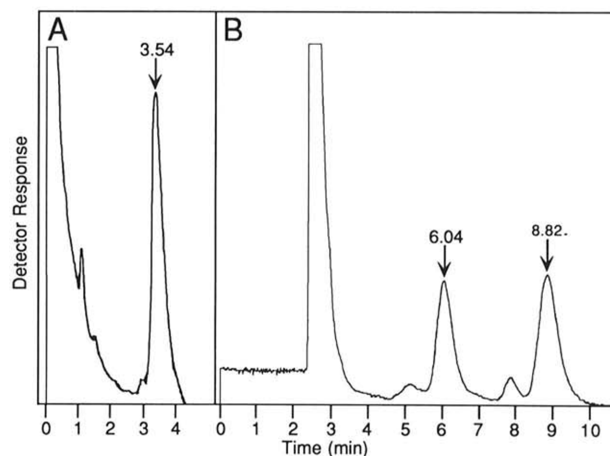

Fig. 2. Retention times for standard solutions of: **A,** chlorothalonil, and **B,** 2-aminobenzimidazole (left) and methyl-2-benzimidazole carbamate (right) when analyzed by liquid or gas-liquid chromatography. Retention times in minutes are shown above the respective peaks.

chlorothalonil detected in Lakeland sand was approximately 0.04 μg/g of soil, but in the test site the concentration from the three samples averaged 0.97 μg/g of soil (Table 5).

The procedure used to extract MBC and 2-AB from the soils affected the conversion of benomyl to MBC, and both MBC and 2-AB were extracted by organic solvent partitioning. Quantitative detection of residues was accomplished by reverse-phase high-performance liquid chromatography, and retention times are shown (Fig. 2B). Mean recovery (Table 5) of MBC and 2-AB from the Lakeland sand was higher than from the Cecil sandy loam. Background levels of MBC and 2-AB detected in the Lakeland sand were 0.05 μg/g of soil and none, respectively; no background residues were detected in the Cecil sandy loam. After two benomyl applications, levels of 2-AB and MBC detected in the soil rose to levels only slightly above the limits of detection (Table 5).

DISCUSSION

Among the fungicides tested, all (except sulfur) at high residue levels might have potential to affect growth and development of *H. rhossiliensis* in field soil. Inhibition could in turn affect the parasitic ability of this fungus against *C. xenoplax*. Benomyl may be of particular concern because growth, germination, and germ tube elongation of *H. rhossiliensis* were completely inhibited by the fungicide at relatively low concentrations. Triforine and iprodione also were suppressive, but suppression decreased within 7 days, which might have resulted from degradation of the fungicides under conditions of this experiment. As stated on the pesticide label, chemical breakdown of iprodione occurs in water, and triforine also is hydrolyzed (6).

Benomyl and chlorothalonil did not accumulate to high residue

TABLE 5. Mean residue concentrations of chlorothalonil (CTL) and benomyl (2-AB and MBC)[a] recovered from either Lakeland sand[b] or Cecil sandy loam[c] after two foliar applications of each fungicide on peach trees (benomyl 50WP at 560 g a.i./ha and chlorothalonil 500F at 2,345 g a.i./ha) 7 days apart

Soil type	Residue type	Retention time (min)	Limit of detection (μg/ml)	Percent recovery	Background residues[d] (μg/g)	Orchard residues[e] (μg/g)
Lakeland sand	CTL	3.5–3.6	0.02	59.6	0.04	0.97
	2-AB	5.9–6.1	0.08	26	none	0.08
	MBC	8.7–8.9	0.06	73	0.05	0.18
Cecil sandy loam	2-AB	5.9–6.1	0.08	10	none	none
	MBC	8.7–8.9	0.06	65	none	0.12

[a] 2-AB = 2-aminobenzimidazole; MBC = methyl-2-benzimidazole-carbamate.
[b] Lakeland sand: 89% sand, 6% silt, 5% clay.
[c] Cecil sandy loam: 65% sand, 17% silt, 18% clay.
[d] Average of two samples.
[e] Average of three samples.

levels in soil after application to peach foliage. Because each fungicide residue was assayed only once, the low residue levels found may have resulted either from low accumulation (most of the residue remaining on the trees), rapid biological or photochemical degradation, or both. Increased degradation after repeated applications has been reported. After six or seven applications of chlorothalonil in peanut fields (0.8–1.2 kg/ha) at 14-day intervals, residues of only 0.2–0.5 μg/g were detected (8), and degradation of benomyl was faster in soils previously treated with the fungicide (9). However, the amount of either fungicide applied for disease control on peaches is relatively small, and levels less than 1 μg/g of soil might be expected even if little fungicide degradation had occurred.

Despite the sensitivity of *H. rhossiliensis* to benomyl in vitro, a soil drench of benomyl at 10 μg/ml did not strongly influence the parasitic activity of the fungus on the nematode. Although population suppression by the fungus was reduced slightly and numbers of conidia attached to nematodes were fewer, parasitism continued. Dilution of the fungicide concentration in the soil solution, chemical degradation, adsorption to soil particles, and uneven distribution of the fungicide in the soil all may be partial explanations for the lack of a more dramatic effect on the host-parasite interaction. However, we suggest that even large fungicide doses to soil probably would exert only a temporary influence on nematode suppression by the fungus. With these observations, plus the finding that routine foliar sprays of benomyl resulted in only minute detectable residues in the soil, we conclude that commercial use of this fungicide in peach orchards probably would not strongly influence the parasitic activity of *H. rhossiliensis*.

More captan and sulfur than benomyl are used in peach orchards in the southeastern United States. *H. rhossiliensis* was less sensitive to these fungicides and, in fact, was not inhibited at all by sulfur at the rates tested. It appears, therefore, that neither of these fungicides would appreciably interfere with the parasitic activity of *H. rhossiliensis* on *C. xenoplax*. Iprodione had little visible effect on parasitism or fungus activity in the greenhouse experiments. No effect in the field is expected. The comparatively small amounts of dicloran, triforine, and chlorothalonil used in peach orchards would not be expected to affect significantly the nematode-fungus interaction, given the small effects observed in the greenhouse. However, the possible interactive effects from combination or sequential applications of these fungicides were not examined.

Chlorothalonil seemed to increase parasitism in the fraction of the nematode population infested with conidia, but we have not investigated adequately the potential of this fungicide or others to enhance parasitic activity. Perhaps, further study of this fungicide applied in low doses over time to enhance nematode parasitism would be profitable. We know nothing about the effects of these fungicides as stressing agents for development or reproduction of *C. xenoplax* at the concentrations used in our tests.

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