Effect of Environmental Factors and Pesticides on Mycoparasitism of *Sclerotinia minor* by *Coniothyrium minitans*

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ABSTRACT

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The effects of soil temperature and moisture, and nine pesticides commonly used in peanut production, on the mycoparasitic activity of *Coniothyrium minitans* on sclerotia of *Sclerotinia minor* were evaluated. In vitro mycelial growth and conidia germination of *C. minitans* were sensitive to azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and diclosulam. *C. minitans* survived and infected sclerotia of *S. minor* in the presence of azoxystrobin, chlorothalonil, diclosulam, fluazinam, flumioxazin, S-metolachlor, pendimethalin, pyraclostrobin, and tebuconazole. Mycoparasitic activity was reduced by all pesticides except S-metolachlor compared with the nontreated control. Optimum conditions for infection of sclerotia were temperatures from 14 to 22°C and soil moisture from -0.33 to $-1 \text{ kPa} \times 10^2$. Mycoparasitic activity of *C. minitans* remained high (98% sclerotia infected) at temperatures ranging from 14 to 22°C, but decreased at temperatures above 28°C. Viability of sclerotia was inversely related to the proportion infected by *C. minitans* ($r = -0.9963$, $P = 0.001$). Mycoparasitic activity also declined when soil moisture was greater than $-1 \text{ kPa} \times 10^2$ or less than $-0.10 \text{ kPa} \times 10^2$. These results indicate that *C. minitans* should not be applied when temperatures exceed 28°C, during extremes in soil moisture, or when there is a high risk of contact with pesticides before it becomes established in the soil.

Coniothyrium minitans Campbell is a fungal mycoparasite of sclerotia formed by *Sclerotinia* spp. and has been shown to be an effective biological control agent in several crops (6,11,17,18,26,28). Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by the soilborne fungus *Sclerotinia minor* (Jaggar) Kohn (14), is an important disease in the peanut-growing regions in Virginia and North Carolina and also is found in Texas, Oklahoma, and New Mexico (5,25). Recent field studies have shown that *C. minitans* can colonize sclerotia of *S. minor* and reduce disease in peanut when active in the soil for three consecutive years (19). However, various environmental conditions and agrichemicals used in peanut production may affect the mycoparasitic activity of *C. minitans* (8,15).

The environment influences the mycoparasitic activity of *C. minitans*. The optimum temperature for *C. minitans* spore germination, infection, and destruction of sclerotia of *S. sclerotiorum* is 20°C (23). Temperatures $<7^\circ\text{C}$ are not favorable for

the growth of *C. minitans* and reduce the rate of spore germination and infection (23). Optimum relative humidity for the mycoparasitism of *S. sclerotiorum* sclerotia is $>95\%$ (23). In vitro studies found that 80% spore germination occurred after 7 days at temperatures ranging from 7 to 20°C, and spore germination occurred at temperatures as low as 4°C and as high as 30°C (23).

In all, 23 fungicides, 12 insecticides, and 20 herbicides are recommended for use on peanut in North Carolina (13). Previous studies have examined the interaction of *C. minitans* with pesticides used in lettuce production, including the fungicides iprodione and thiram, which were found to reduce mycelial growth and spore germination, but not mycoparasitism of sclerotia of *S. sclerotiorum* (8). *C. minitans* growth and spore germination are also highly sensitive to benomyl and vinclozolin (50% effective concentration [EC_{50}] $< 1 \mu\text{g a.i. ml}^{-1}$) (15). Among the most widely used pesticides in peanut production are the fungicides azoxystrobin, pyraclostrobin, chlorothalonil, fluazinam, and tebuconazole and the herbicides diclosulam, flumioxazin, S-metolachlor, and pendimethalin (22). These pesticides potentially could interact with *C. minitans* in the field during the growing season.

For *C. minitans* to become widely accepted as a management tool, it must be successfully integrated with current pro-

duction practices and persist and infect sclerotia of *S. minor* under field conditions. The objectives of this study were to determine the optimum temperature and moisture requirements for *C. minitans* infection of sclerotia of *S. minor*, and to evaluate the sensitivity of *C. minitans* to nine pesticides commonly used in peanut production to determine whether they could interfere with mycoparasitism on sclerotia of *S. minor*. Knowledge of these factors will allow us to better predict the potential for use of *C. minitans* in controlling Sclerotinia blight of peanut.

MATERIALS AND METHODS

Isolate information and production of sclerotia. An isolate of *C. minitans* (CON/M/91-08), obtained by plating out the commercial formulation Contans WG (Propyta Biologischer Pflanzenschutz GmbH, Malchow/Poel, Germany), was grown on potato dextrose agar (PDA) in an incubator at 18°C for 7 days. A spore suspension of *C. minitans* was made by flooding the culture dishes with sterilized water and gently scraping the colony surfaces to free conidia from the pycnidia. The spore suspension was adjusted to 1×10^6 conidia ml^{-1} with the aid of a hemacytometer.

An isolate of *S. minor* (no. 20, obtained from Dr. Barbara Shew, North Carolina State University; 10), originally isolated from a diseased peanut plant in Gatesville, NC, was used in this study. Sclerotia were produced at 18°C on sterilized oat kernels infested with *S. minor* (1). After 5 weeks, oat kernels were dried for 2 weeks at room temperature (about 22°C) and then stored in a refrigerator at 5°C until used in experiments.

Pesticides used. Commercial formulations of all pesticides were used in this study. Fungicides used and the maximum labeled rate for peanuts were azoxystrobin (Abound Flowable; Syngenta Crop Protection, Greensboro, NC) at 450 g a.i. ha^{-1} , chlorothalonil (Bravo Weather Stik; Syngenta Crop Protection) at 1.26 kg a.i. ha^{-1} , fluazinam (Omega 500F; Syngenta Crop Protection) at 875 g a.i. ha^{-1} , pyraclostrobin (Headline; BASF Corp., Research Triangle Park, NC) at 275 g a.i. ha^{-1} , and tebuconazole (Folicur 3.6 F; Bayer Crop Science, Research Triangle Park, NC) at 229 g a.i. ha^{-1} . Herbicides and their corresponding maximum use rates were diclosulam (Strongarm; Dow AgroSciences LLC, Indianapolis, IN) at 27 g a.i. ha^{-1} ,

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flumioxazin (Valor; Valent U.S.A. Corp., Walnut Creek, CA) at 107 g a.i. ha⁻¹, S-metolachlor (Dual Magnum; Syngenta Crop Protection) at 2.14 kg a.i. ha⁻¹, and pendimethalin (Prowl H2O; BASF Corp.) at 1.07 kg a.i. ha⁻¹. These herbicides are soil applied pre-plant incorporated (PPI) or pre-emergence (PRE) and, therefore, are most likely to interact with *C. minitans* in the soil early in the season.

Mycelial growth and conidia germination. Stock suspensions of each of the nine pesticides were prepared using sterile distilled water. The stock suspension of each pesticide then was adjusted to appropriate concentrations to achieve final concentrations of 0.01, 0.1, 1, and 10 µg a.i. ml⁻¹ when added to molten PDA. PDA was autoclaved and cooled to 55°C before then being amended with each pesticide. The pesticide-amended medium (15 ml) then was added to standard 9-cm petri dishes. A 5-mm plug taken from the margin of an actively growing 7-day-old culture of *C. minitans* was placed upside down in the center of each test dish. Dishes were incubated at 18°C and radial growth of *C. minitans* was measured in two axes every 48 h for 14 days. There were three replicate dishes for each of the pesticide treatments and experiments were conducted three times. Percentage of mycelial growth inhibition (PMGI) was calculated by the formula $PMGI = 100 \times [(D_{PDA} - D_F)/D_{PDA}]$, where D_{PDA} is the average colony diameter of *C. minitans* after 14 days of growth on PDA and D_F is the colony diameter of *C. minitans* after 14 days of growth on PDA amended with each of the pesticides.

Germination of conidia of *C. minitans* was measured in the presence of the pesticides at 0.01, 0.1, 1, and 10 µg a.i. ml⁻¹ by placing three drops of a conidial suspension (5×10^5 spores/ml) on the surface of each dish of amended agar. The dishes were incubated at 18°C for 32 h, at which time 85% lactic acid was added to each dish to stop conidial germination. Conidial germination was assessed by counting the number of germinated conidia out of 100 per spot on each dish using a compound microscope. A conidium was considered germinated when the germ tube length was equal to or greater than half the length of the conidium. There were three replicate dishes for each pesticide treatment and the experiment was repeated once. Percentage of conidial germination inhibition (PCGI) was calculated by the formula $PCGI = 100 \times [(C_{PDA} - C_F)/C_{PDA}]$, where C_{PDA} is the number of conidia that germinated on PDA after 32 h and C_F is the number of conidia after 32 h that germinated on PDA amended with each pesticide.

Preparation of soil plates. Temperature, moisture, and pesticide effects on mycoparasitism of sclerotia of *S. minor* were studied using a modification of a soil plate technique described by Smith and

Phipps (20). A sample of a Rains fine sandy-loam soil was collected from a peanut field in Gatesville, NC with a history of Sclerotinia blight of peanut. Soil was air dried and sifted through a U.S. standard number 12 sieve (1.7-mm opening) to produce uniform-sized soil particles and to remove organic debris.

In the pesticide experiments, soil was autoclaved for 30 min (121°C at 103 kPa) and allowed to cool; then, 30 g of soil was dispensed into a 9-cm plastic petri dish and 10 ml of sterile distilled water was added to each dish. Next, 20 sclerotia were placed on the soil surface and sprayed with a suspension of spores of *C. minitans* (approximately 1×10^6 spores ml⁻¹) using an airbrush. The dishes were placed on a laboratory bench for 4 h to allow the spores to settle, after which an airbrush was used to apply the maximum labeled field rate of each pesticide across each dish. Maximum rates per hectare were converted to maximum rates per dish using surface area of the dish equal to 63.6 cm². Dishes were incubated at 18°C for 14 days. After 14 days, sclerotia were removed from the soil plate to determine their viability and infection by *C. minitans*. The sclerotia were surface disinfested in 15% NaClO for 30 s, rinsed in sterilized distilled water, plated on PDA amended with tetracycline (25 mg liter⁻¹), and incubated at 18°C for 14 days. Sclerotia were examined with a stereoscope and a compound microscope and those producing mycelium, whether or not they were infected by *C. minitans*, were considered viable. Both viable and nonviable sclerotia showing sporulation of *C. minitans* on the surface of the sclerotia were scored as infected. Each pesticide treatment plus a nontreated control was replicated three times and the experiment was repeated once.

In the temperature experiments, soil was amended with commercial cornmeal (5% wt/wt) and thoroughly mixed, and 50 g of the amended soil was placed in 9-cm glass petri dishes. The amended soil was moistened with 20 ml of distilled water, autoclaved for 30 min at 121°C (103 kPa) (20), and allowed to cool. Then, 20 sclerotia of *S. minor* were placed on the soil surface of each dish and sprayed uniformly with a suspension of spores of *C. minitans* (approximately 1×10^6 spores ml⁻¹) using an airbrush. Dishes with soil and sclerotia then were placed in growth chambers at 14, 18, 22, 28, or 30°C for 14 days, after which time the sclerotia were removed from the soil dishes. The sclerotia were surface disinfested and plated on PDA as described earlier. The number of viable sclerotia and sclerotia infected by *C. minitans* were determined as described previously. For each temperature treatment, there were four replications plus a control with noninoculated sclerotia. The experiment was repeated once.

In the soil moisture experiments, field soil was dried for 24 h at 60°C to completely remove moisture. The soil was allowed to cool and 30 g of soil was dispensed into 9-cm plastic petri dishes. A sample of the field soil was submitted to the Soil Physical Properties Laboratory, North Carolina State University, for a soil water retention analysis under pressures of 0, -0.1, -0.33, -0.5, -1, -8, and -15 kPa $\times 10^2$. Data from the soil water retention analysis were used to develop a soil release curve. Sterile distilled water was added by weight to each soil dish to obtain the moisture treatments of approximately 0, -0.10, -0.33, -1, and -15 kPa $\times 10^2$. Sclerotia were inoculated by dipping them into a *C. minitans* spore suspension (approximately 1×10^6 spores ml⁻¹). The sclerotia were removed from the spore suspension and placed on sterilized filter paper to remove excess water. Then, 20 sclerotia were placed on the soil surface of each petri dish. Static Guard (Alberto-Culver Co., Melrose Park, IL) was applied to the top of each petri dish to prevent condensation on the lid and the dishes were sealed with Parafilm to maintain constant moisture. Preliminary experiments showed that Static Guard did not interfere with the mycoparasitic interaction but did inhibit the condensation of water on the lid. The weight of each dish was recorded to monitor soil moisture levels. Dishes then were placed in an incubator at 18°C for 14 days, after which time a final weight of the dishes was recorded and sclerotia removed. Sclerotia were surface disinfested and plated on PDA, and viable sclerotia and sclerotia infected by *C. minitans* were determined as described previously. Each moisture treatment was replicated four times and included a non-inoculated control. The experiment was repeated once.

Data analyses. The experimental design was a randomized complete block for each experiment. Data from separate experiments were combined when statistical analysis indicated that variances were homogenous. Analysis of variance (ANOVA; version 8; SAS Institute Inc., Cary, NC) was used to separate the treatment effects on sclerotia viability and infection by *C. minitans*. Significant differences among treatments in each experiment were assessed using Fisher's protected least significant difference test at $P \leq 0.05$. Correlation analysis was used to compare the viability and infection of sclerotia. For calculation of EC₅₀s, percentage of mycelial growth inhibition or conidia germination inhibition was plotted against the log₁₀-transformed concentration of each pesticide to determine the linear relationship between the two parameters. EC₅₀ values for each pesticide then were determined using the linear equation given by the general linear model (version 8; SAS Institute Inc.).

RESULTS

Mycelial growth and conidial germination. Of the nine pesticides evaluated, all the fungicides and the herbicide flumioxazin reduced radial mycelial growth of *C. minitans* on PDA as shown by the slope of the regression of PMGI versus pesticide concentration ($P \leq 0.010$; Table 1). Fluazinam was the most active compound tested, with calculated EC_{50} of < 0.01 (Table 1). The calculated EC_{50} values for the remaining fungicides ranged from 0.15 to $0.90 \mu\text{g a.i. ml}^{-1}$ except for azoxystrobin, which had an $EC_{50} > 10 \mu\text{g a.i. ml}^{-1}$ (Table 1). At the higher concentrations, the QoI fungicides azoxystrobin and pyraclostrobin were less active than chlorothalonil, fluazinam, or tebuconazole (Table 1). Flumioxazin was the only herbicide with an EC_{50} for mycelial growth $< 10 \mu\text{g a.i. ml}^{-1}$ (Table 1).

Pyraclostrobin was the most active compound tested on *C. minitans* conidial germination at $0.01 \mu\text{g a.i. ml}^{-1}$, reducing

germination by 85% (Table 2). EC_{50} s for conidial germination for all fungicides except tebuconazole were $\leq 0.01 \mu\text{g a.i. ml}^{-1}$ (Table 2). Flumioxazin was the only herbicide that reduced spore germination (Table 2). EC_{50} s for diclosulam, S-metolachlor, and pendimethalin could not be estimated reliably because they were greater than the highest concentration of a.i. used in this study (Table 2).

Mycoparasitic activity. All fungicides and the herbicides diclosulam, flumioxazin, and pendimethalin reduced infection of *S. minor* sclerotia by *C. minitans* compared with the nontreated control (Table 3). Viability of sclerotia was inversely related to the proportion infected by *C. minitans* ($r = -0.823$, $P < 0.0001$); as the percentage of infected sclerotia decreased, the percent viable sclerotia increased (Table 3). None of the nine pesticides tested completely prevented infection by *C. minitans* (Table 3). Azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, and flumi-

oxazin treatments had the greatest effect on infection of the sclerotia by *C. minitans* (Table 3).

Infection of sclerotia of *S. minor* by *C. minitans* increased as soil moisture level decreased from 0 to $-1 \text{ kPa} \times 10^2$ (Fig. 1). The largest percentage of sclerotia were infected between -1 and $-0.33 \text{ kPa} \times 10^2$. Infection declined as soil moisture was reduced to $-15 \text{ kPa} \times 10^2$ (Fig. 1). Viability of inoculated *S. minor* sclerotia was inversely related to the proportion of sclerotia infected by *C. minitans* ($r = -0.572$, $P < 0.0001$). As the percentage of sclerotia infected by *C. minitans* increased from 0 to $-1 \text{ kPa} \times 10^2$ after 14 days, viability of sclerotia decreased from 98 to 90% (Fig. 1). Viable sclerotia then increased to 98% at $-15 \text{ kPa} \times 10^2$ while the number of infected sclerotia was reduced (Fig. 1).

Mycoparasitic activity of *C. minitans* remained high (98% sclerotia infected) at temperatures ranging from 14 to 22°C , but decreased dramatically at temperatures

Table 1. Colony diameter, regression parameters, and 50% effective concentration (EC_{50}) values ($\mu\text{g a.i. ml}^{-1}$) of *Coniothyrium minitans* isolate CON/M/91-08 on potato dextrose agar (PDA) amended with fungicides or herbicides commonly used in peanut production^x

	Colony diameter (mm) for pesticide concentration (µg a.i. ml ⁻¹)								
Pesticide	0	0.01	0.1	1	10	Slope ^y	R ²	P slope	MG EC ₅₀ ^z
Fungicide									
Azoxystrobin	76.8	55.6	50.8	47.1	44.4	5.03	0.179	0.0101	>10 (–)
Chlorothalonil	76.8	60.1	33.8	19.3	16.4	19.54	0.775	<0.0001	0.15
Fluazinam	76.8	38.5	21.1	16.4	14.4	10.35	0.679	<0.0001	<0.01
Pyraclostrobin	76.8	51.0	47.6	34.5	29.9	10.24	0.592	<0.0001	0.90
Tebuconazole	76.8	69.8	48.7	25.6	10.8	26.83	0.934	<0.0001	0.38
Herbicide									
Diclosulam	76.8	77.4	71.4	78.9	78.1	–0.13	0.019	0.4329	>10 (–)
Flumioxazin	76.8	76.9	66.8	35.5	33.1	21.88	0.719	<0.0001	2.93
S-Metolachlor	76.8	79.8	78.2	74.1	77.0	1.65	0.043	0.2263	>10 (–)
Pendimethalin	76.8	78.9	74.5	77.5	76.7	0.50	0.005	0.6879	>10 (–)

^x Growth (mm) after 14 days of incubation. Data represent the mean of three repetitions with three replicates each.

^y Slope of inhibition determined from the regression of percent mycelial growth inhibition (PMGI) versus $\log_{10}$ -transformed concentration of each pesticide, where $PMGI = 100 \times [(D_{PDA} - D_F)/D_{PDA}]$, where D_{PDA} is the average colony diameter of *C. minitans* after 14 days of growth on PDA and D_F is the colony diameter of *C. minitans* after 14 days of growth on PDA amended with each of the pesticides.

^z MG = mycelial growth. Calculation of EC_{50} determined from the linear relationship between PMGI versus $\log_{10}$ -transformed concentration of each pesticide. The EC_{50} s for each pesticide then were determined using the linear equation given by the general linear model; (–) = no effect within confines of experiment.

Table 2. Percent conidia germination, regression parameters, and 50% effective concentration (EC_{50}) values ($\mu\text{g a.i. ml}^{-1}$) of *Coniothyrium minitans* isolate CON/M/91-08 after 32 h at 18°C on potato dextrose agar (PDA) amended with pesticides commonly used in peanut production^x

Pesticide	Germinated spores (%) for pesticide concentration (µg a.i. ml ⁻¹)					Slope ^y	R ²	P slope	CG EC ₅₀ ^z
	0	0.01	0.1	1	10				
Fungicide									
Azoxystrobin	69.7	40.3	16.5	6.3	3.8	12.05	0.781	<0.0001	<0.01
Chlorothalonil	69.7	33.8	5.7	1.8	0.7	10.12	0.644	<0.0001	<0.01
Fluazinam	69.7	49.0	8.5	2.0	0.3	15.11	0.640	<0.0001	0.01
Pyraclostrobin	69.7	8.3	5.3	2.0	0.7	2.68	0.667	<0.0001	<0.01
Tebuconazole	69.7	50.3	40.0	13.7	6.8	16.40	0.900	<0.0001	0.25
Herbicide									
Diclosulam	69.7	48.0	47.3	47.8	48.8	−0.04	0.011	0.6336	(−)
Flumioxazin	69.7	46.3	23.0	12.8	10.3	11.58	0.713	<0.0001	0.03
S-Metolachlor	69.7	45.2	46.2	46.3	40.3	1.34	0.105	0.1233	>10 (−)
Pendimethalin	69.7	48.2	52.5	51.2	50.2	−0.04	0.013	0.5999	(−)

^x Percentage of germinated spores out of 100. Data represents the mean of two repetitions with three replicates each.

^y Slope of inhibition determined from the regression of percent inhibition of conidia germination versus $\log_{10}$ transformed concentration of each pesticide. Where percentage of conidia germination inhibition (PCGI) was calculated by the formula $PCGI = 100 \times [(C_{PDA} - C_F)/C_{PDA}]$, where C_{PDA} is the number of conidia that germinated on PDA after 32 h and C_F is the number of conidia after 32 h that germinated on PDA amended with each pesticide.

^z CG = conidia germination. Calculation of EC_{50} determined from the linear relationship between PCGI versus $\log_{10}$ -transformed concentration of each pesticide. The EC_{50} s for each pesticide were then determined using the linear equation given by the general linear model; (–) = no effect within confines of experiment.

$\geq 28^{\circ}\text{C}$, where only 9% sclerotia were infected. (Fig. 2). Viability of *S. minor* sclerotia was inversely related to proportion of those sclerotia infected by *C. minitans* ($r = -0.963$, $P < 0.0001$). At all temperatures, the noninoculated sclerotia were 100% viable (Fig. 2).

DISCUSSION

In this study, mycelial growth, spore germination, and mycoparasitic activity of *C. minitans* were inhibited by most fungicides and one herbicide commonly used in peanut production. *C. minitans* was most sensitive to the fungicides azoxystrobin, fluazinam, chlorothalonil, pyraclostrobin, and tebuconazole and the herbicide flumi-

oxazin. Each of these fungicides have broad-spectrum activity against basidiomycota, ascomycota, and deuteromycota (16). Azoxystrobin, chlorothalonil, and pyraclostrobin have good activity on web blotch of peanut caused by *Phoma arachidicola* (9). The genera *Phoma* and *Coniothyrium* are anamorphs of fungi in the family *Leptosphaeriaceae* (2); therefore, it is not unexpected that *C. minitans* is sensitive to the same fungicides used to control *P. arachidicola* and leaf spot (*Cercospora arachidicola*).

The herbicide flumioxazin, used to control a number of problematic weeds in peanut, including Florida beggarweed (*Desmodium tortuosum* (Sw.) DC.), com-

mon ragweed (*Ambrosia artemisiifolia* L.), and eclipta (*Eclipta prostrata* L.) (3), was found to have activity similar to that of many of the fungicides tested in reducing fungal growth and mycoparasitic activity of *Coniothyrium minitans*. The herbicide lactofen, an inhibitor of protophyrinogen oxidase, has been found to reduce early leaf spot on peanut (caused by *Cercospora arachidicola*) by reducing spore germination and decreasing sporulation in lesions (4). Because lactofen has fungicidal activity, it was not unexpected that flumioxazin, a herbicide that also inhibits protophyrinogen oxidase, showed fungicidal activity in reducing *Coniothyrium minitans* growth, spore germination, and mycoparasitic activity.

In our study, mycelial growth and spore germination of *C. minitans* was highly sensitive to chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and flumioxazin. Spore germination also was highly sensitive to azoxystrobin. Mycelial growth and spore germination were less sensitive to the herbicides diclosulam, S-metolachlor, and pendimethalin. Budge and Whipps (7) found that *C. minitans* growth and spore germination were sensitive in vitro to pesticides, including the fungicides iprodione, thiram, mancozeb, metalaxyl, tolclofos-methyl, and zineb, along with a number of insecticides and the herbicide combination chloropropham + cetrymide used in lettuce production. Li et al. (15) also found that *C. minitans* was highly sensitive in vitro to benomyl and vinclozolin ($\text{EC}_{50}\text{s} < 1 \mu\text{g a.i. ml}^{-1}$).

Although most pesticides tested in soil plates reduced the number of sclerotia infected by *C. minitans*, sufficient inocula survived to infect at least 10% of the sclerotia of *S. minor*. This activity may be a result of escapes because of incomplete coverage of the sclerotia by the pesticides. In the field, these pesticides are applied to the peanut plant or the soil surface, and residuals of the active ingredient that eventually enter the soil likely would be much lower than those used in our test. Nevertheless, the EC_{50}s for inhibition of spore germination and mycelial growth were less than $1 \mu\text{g a.i./ml}$ for all the fungicides except azoxystrobin, indicating that *C. minitans* is very sensitive to these pesticides. *C. minitans* was found to successfully parasitize sclerotia of *S. minor* in field trials where traditional peanut production practices were implemented, including the application of fluazinam and other fungicides for disease control (19). However, *C. minitans* is very sensitive to these pesticides and care should be taken in the timing of the application of the biological control agent relative to pesticide application, because growth, spore germination, and mycoparasitic activity of *C. minitans* potentially could be reduced in their presence. *C. minitans* should not be applied with pesticides or during times when there

Table 3. Mycoparasitic activity of *Coniothyrium minitans* on sclerotia of *Sclerotinia minor* in soil plates in the presence of the maximum recommended field concentration of pesticides commonly used in peanut production

Pesticide ^w	Rate (g a.i. ha ⁻¹) ^x	<i>S. minor</i> sclerotia ^y	
		Infected ^y	Viable ^z
Nontreated control	...	50.8 a	60.8 c
Fungicides			
Azoxystrobin	450	12.5 d	83.3 ab
Chlorothalonil	1,260	14.2 d	90.0 a
Fluazinam	875	21.7 cd	80.8 ab
Pyraclostrobin	275	10.8 d	90.0 a
Tebuconazole	229	35.8 b	73.3 cb
Herbicides			
Diclosulam	27	31.7 bc	70.8 cb
Flumioxazin	107	18.3 d	80.0 ab
S-Metolachlor	2,140	38.3 ab	64.2 c
Pendimethalin	1,070	37.5 b	71.7 cb
<i>F</i> (<i>Pr</i> > <i>F</i>)	...	9.3 (<0.0001)	4.0 (0.0008)
LSD	...	12.6	14.3

^v Values followed by the same letter in each column are not significantly different at a $P \leq 0.05$ according to the Fisher's protected least significant difference (LSD).

^w Pesticides used were from commercial formulations.

^x Maximum application rate.

^y Infected by *C. minitans*. Percentage of both viable and nonviable sclerotia infected by *C. minitans* after 14 days. Data represents average results from two trials with 20 sclerotia each.

^z Percentage of sclerotia germinating after 14 days whether or not they were infected by *C. minitans*. Data represent average results from two trials with 20 sclerotia each.

Fig. 1. Effects of soil moisture on infection of *Sclerotinia minor* sclerotia by *Coniothyrium minitans*. Percentage of sclerotia that were viable or infected by *C. minitans* after 14 days of incubation at 18°C with soil moistures ranging from 0 to $-15 \text{ kPa} \times 10^2$.

is a high risk of direct contact with azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and flumioxazin.

Soil temperature and moisture influenced the ability of *C. minitans* to infect sclerotia of *S. minor*. Optimum infection of sclerotia by *C. minitans* occurred with soil moisture ranging from -0.33 to -1 kPa $\times 10^{-2}$ and temperatures ranging from 14 to 22°C. These temperatures and moisture conditions are typical throughout most of the year in peanut fields in North Carolina, where the 30-year average daily maximum temperatures from 1971 to 2000 was 22.0°C and the average minimum was 9.7°C (30-year weather data obtain from the State Climate Office of North Carolina from weather stations located in Lewiston and Edenton, NC).

Both extremely wet and dry soil moisture reduced the ability of *C. minitans* to infect sclerotia of *S. minor*. *S. minor* sclerotia viability decreased from 98 to 90% and *C. minitans* infection increased from 10 to 45% with increasing soil moisture. Other studies also have shown the importance of moisture on the activity of *C. minitans* (7,23,24). Sclerotia of *S. sclerotiorum* in celery and lettuce crops grown in a glasshouse were infected by *C. minitans* at all times of the year; however, the sclerotia failed to degrade during the summer months when the soil was dry (7). In culture, mycelial extension of *C. minitans* decreased as PDA was osmotically adjusted from -0.7 to -7.0 MPa (27), and relative humidity $\geq 90\%$ is required for *C. minitans* spore germination, with $\geq 95\%$ required for maximum germination and infection of *S. sclerotiorum* (23).

C. minitans was active at temperatures ranging from 14 to 22°C, infecting more than 98% of the sclerotia and significantly

reducing their viability. These results are consistent with the findings of Budge et al. (8) and Trutmann et al. (23), who found that infection of sclerotia of *S. sclerotiorum* by *C. minitans* occurred between 4 and 25°C, with the optimum occurring at 20°C. We mainly were interested in the effect of high temperatures on the mycoparasitic activity of *C. minitans* on sclerotia of *S. minor*. Previous studies have shown that spores of *C. minitans* are able to survive in cold storage at 5°C for up to 64 weeks (18), and spore germination occurs at temperatures as low as 4°C (23). Also, extremely low temperatures in North Carolina usually are short lived and probably would not be detrimental to *C. minitans* spore survival and germination (minimum average soil temperatures ranged from 5.9 to 26.5°C; 21).

Spores of *C. minitans* can germinate at 30°C (23), but temperatures exceeding 26°C have been shown to inhibit infection of *S. sclerotiorum* by *C. minitans* (12). In our study, infection of sclerotia of *S. minor* by our isolate of *C. minitans* was significantly reduced at temperatures $\geq 28^\circ\text{C}$, but some activity (9% of sclerotia infected) was still detected at 30°C after 14 days. During the summer months in northeastern North Carolina, soil temperature can reach 32°C (21). Both air and soil temperatures consistently exceed 28°C during the months of June, July, and August (21). Therefore, *C. minitans* should persist and infect sclerotia of *S. minor* in the soil throughout most of the year in North Carolina.

After peanut harvest in the fall or early spring may be the best time to apply *C. minitans* to the soil for the biological control of *S. minor*. After harvest, in early to mid-October, temperatures remain favor-

able for infection for at least 6 weeks. However, October tends to be a dry month in North Carolina; therefore early spring (March) applications may be more effective. Because *C. minitans* survives cold temperatures, application during the winter also is likely to be effective as long as there is adequate soil moisture. In a long-term study, we found that multiple applications of *C. minitans* made in November, December, and February were effective in reducing numbers of sclerotia of *S. minor* and Sclerotinia blight of peanut (19). Therefore, if *C. minitans* is applied in the fall and has a chance to become established in the soil, by spring and summer months, when herbicides and fungicides are used in peanut production, the effects on *C. minitans* mycoparasitic activity will be minimized.

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Fig. 2. Effect of temperature on mycoparasitism of *Sclerotinia minor* sclerotia by *Coniothyrium minitans*. Percentage of sclerotia that were viable or infected by *C. minitans* after 14 days of incubation at temperatures ranging from 14 to 30°C.

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