Management of Sclerotinia Blight of Peanut with the Biological Control Agent *Coniothyrium minitans*

D. E. Partridge, Former Graduate Research Assistant, T. B. Sutton, Professor, Department of Plant Pathology, D. L. Jordan, Associate Professor, Department of Crop Science, V. L. Curtis, Agricultural Research Specialist, and J. E. Bailey, Professor (deceased), Department of Plant Pathology, North Carolina State University, Raleigh 27695

ABSTRACT

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Sclerotinia blight, caused by *Sclerotinia minor*, is an important disease of peanut in North Carolina. The effectiveness of *Coniothyrium minitans*, a mycoparasite of sclerotia of *Sclerotinia* spp., was studied in a 5-year field experiment and in eight short-term experiments in northeastern North Carolina. The 5-year experiment was initiated in November 1999 to evaluate the effectiveness of repeated soil applications of *C. minitans* (commercial formulation, Contans WG) at 2 and 4 kg ha⁻¹ in reducing Sclerotinia blight. In addition, individual commercial peanut fields were selected in 2001 and 2002 to evaluate a single application of *C. minitans* at 4 kg ha⁻¹. No differences were found between the 2 and 4 kg ha⁻¹ rates of *C. minitans* in reducing Sclerotinia blight. In 2002, there was less disease in plots receiving applications of *C. minitans* for either 1 or 3 years compared with the nontreated control; whereas, in 2003, *C. minitans* applications for 1, 2, or 3 years reduced disease and the number of sclerotia isolated from soil. A single application of *C. minitans* reduced sclerotia in only two of the eight short-term experiments. The integration of consecutive years of soil applications of *C. minitans* at 2 kg ha⁻¹ with moderately resistant cultivars and fungicide applications may aid in the management of Sclerotinia blight in peanut.

Additional keywords: disease control, fluazinam

Sclerotinia blight, caused by the soil-borne fungus *Sclerotinia minor* Jagger, is an important disease of peanut (*Arachis hypogaea* L.). This disease first was reported in Virginia in 1971 and North Carolina in 1972 (32). Since then, it has spread to all the major peanut-producing counties in North Carolina and also is found in Texas, Oklahoma, and New Mexico (5,46). During favorable conditions for Sclerotinia blight, peanut farmers can lose up to 50% yield as a result of the disease (31). After passage of the 2002 Farm Bill, the market value of Virginia-type peanut, the main peanut type grown in North Carolina, fell from \$600 per ton to the current loan rate of \$355 per ton. The requirement of calcium and intensive use of chemicals for disease control cause producers in the Virginia-North Carolina region to have a higher production cost than most growers in the United States. Fungicides for control of Sclerotinia blight alone can cost producers as much as \$104 per hectare for a single application, with up to three appli-

cations made in a season. Consequently, there is a pressing need to reduce the cost of managing Sclerotinia blight.

Overwintering sclerotia of *S. minor* in the soil provide the primary inoculum for Sclerotinia blight on peanut. Sclerotia of *S. minor* germinate myceliogenically, producing a mass of mycelium that directly penetrates peanut stem, leaf, peg, and pod tissues, initiating disease (21,33). As Sclerotinia blight develops in the peanut canopy, numerous sclerotia are produced in and on diseased tissue. Sclerotia then are shed into the soil and increase the inoculum density of the pathogen, which subsequently is available in future growing seasons.

Rotation sequence is very important in managing diseases in peanut (30). Cylindrocladium black rot (CBR; caused by *Cylindrocladium parasiticum*) was found to increase in peanut fields when crop rotation included soybean (*Glycine max* (L.) Merr.) or when peanut was cropped continuously, but not when a nonhost crop was used in the rotation (19,38). However, long-term rotations with nonhost crops such as corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) have shown limited effectiveness in controlling Sclerotinia blight on peanut (33) because the sclerotia are able to remain viable for as many as 4 years in the soil (1,26). Cvs. Perry, VA-C 92R, and VA98R have partial resistance;

however, currently there are no Virginia-type cultivars with complete resistance to Sclerotinia blight (37). Although partial resistance and cultural methods provide limited control, the fungicides fluazinam (Omega 500F; Syngenta Crop Protection, Greensboro, NC) and boscalid (Endura; BASF Corp. Research Triangle Park, NC) are effective if applied properly (30,39,40). These fungicides and host plant resistance are aimed at reducing disease development, but do not directly reduce the population of sclerotia in soil.

A number of microorganisms have been reported to parasitize sclerotia of *Sclerotinia* spp., including *Coniothyrium minitans* Campbell. *C. minitans* first was identified as a mycoparasite of sclerotia of *Sclerotinia* spp. by Campbell in 1947, when isolated from *S. sclerotiorum* (9). Subsequently, a number of studies have shown that sclerotia of *Sclerotinia* spp. in soil are readily infected and colonized by *C. minitans* (14-16,25,42,47) and have demonstrated the effectiveness of *C. minitans* in controlling *S. sclerotiorum* in several crops in the greenhouse and field (6,7,14,23,24,48,49) and, in one case, the effects have been shown to be long term (12). A spore suspension of *C. minitans* applied across field crops infected by *S. sclerotiorum* at the first appearance of apothecia resulted in a reduced population of sclerotia in soil at the end of a 7-year period even when susceptible crops were planted (12). Less information is known about the effectiveness of *C. minitans* on *S. minor*. Adams (1) reported that the viability of sclerotia of *S. minor* declined when soaked in a spore suspension of *C. minitans* but was not affected when spores of *C. minitans* were applied to soil containing sclerotia of *S. minor*. However, studies in Europe and New Zealand have reported the effectiveness of *C. minitans* in controlling disease caused by *S. minor* in lettuce production (35,41). If *C. minitans* is active on *S. minor*, as has been found in some studies, then it may provide a way to reduce sclerotia numbers in peanut fields heavily infested by *S. minor*, thereby reducing losses.

The objective of this study was to determine the effectiveness of soil application of *C. minitans*, applied as Contans WG, in reducing the sclerotial population of *S. minor* and subsequent disease in peanut while integrating it with current cultivars and chemical treatments.

Corresponding author: D. Partridge
E-mail: departri@vt.edu

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MATERIALS AND METHODS

Long-term field site. A commercial peanut field near Gatesville, NC with a long-term history of peanut production and presence of Sclerotinia blight was selected for this study in 1999. The same field and the plots defined within it were used through the 2003 season. The rotation sequence in this field from 1998 to 2003 was peanut, cotton, cotton, peanut, peanut, and peanut.

C. minitans, commercial formulation Contans WG strain CON/M/91-08, with a minimum of 1×10^9 CFU g⁻¹ (Prophyta Biologischer Pflanzenschutz GmbH, Germany), was applied at 2 and 4 kg ha⁻¹ each for 1, 2, or 3 consecutive years (Table 1). Soil applications were made across crop residue on the soil surface with an N-pressurized tractor-mounted sprayer at 276 kPa and aqueous suspension at 142 liters/ha using hollow cone nozzles spaced 31 cm apart. The first application of *C. minitans* was made after cotton harvest on 3 December 1999 and repeated on 9 February 2001, establishing the one- and two-consecutive-year soil treatments. *C. minitans* was applied again on 12 November 2001 following the first year of peanut, establishing the final one-, two-, and three-consecutive-year treatments (Table 1).

Virginia-type peanut cvs. NC-V 11 (susceptible to Sclerotinia blight; 37) and Perry (moderately resistant to Sclerotinia blight;

37) were planted on 2 May 2001, 16 May 2002, and 18 June 2003. Fluazinam at 0.625 kg a.i. ha⁻¹ was applied according to the weather-based Sclerotinia advisory warning system (28–30) using the N-pressurized tractor-mounted sprayer described previously. The interval between fluazinam sprays was no shorter than 3 weeks, no more than three applications were made per year, and a 30-day preharvest interval was used (Table 2). Standard cultural and pest management practices such as tillage, fertilization, herbicide and insecticide applications, and fungicide applications to control early leaf spot (*Cercospora arachidicola*) and southern stem rot (*Sclerotium rolfsii*) were used as recommended by the North Carolina Cooperative Extension Service (19).

The experimental design was a split plot. Main-plot factors consisted of *Coniothyrium minitans* application at 2 and 4 kg ha⁻¹ each applied in one, two, or three consecutive years and a nontreated control (Table 1). Main plots were 29 m wide (28 rows) by 41 m long with 4.6-m alleys. Subplot factors consisted of combinations of the two cultivars (NC-V 11 and Perry) and two fluazinam rates (0 and 0.625 kg a.i. ha⁻¹). Subplots were 9 m wide (14 rows) by 18 m long, with the two center rows used for disease and pod yield evaluations.

Disease and yield assessment. Disease ratings were initiated in July each year and

taken biweekly, with a final disease assessment taken just prior to harvest. The center two rows in each subplot each were divided into 60 30-cm long increments per row. Sclerotinia blight incidence was estimated by counting the number of 30-cm increments showing signs of *S. minor* or symptoms of Sclerotinia blight (8). Area under disease progress curve (AUDPC) for each treatment was calculated as a measure of the cumulative incidence of Sclerotinia blight during the season (36).

Plants were dug based on mesocarp color determination (50) and vines inverted. Peanut plants were combined with conventional harvesting equipment from the two center rows of each plot on 22 October 2001, 8 November 2002, and 10 November 2003. A 500-g sample of pods from each plot was collected in 2002 to determine the percentages of extra large kernels (%ELK), total sound mature kernels (%TSMK), and fancy pods (%FP) using United States Department of Agriculture Grading Service Guidelines (45).

Data were subjected to analysis of variance using the PROC GLM procedure of SAS (version 8; SAS Institute Inc. Cary, NC) for AUDPC, pod yield, %TSMK, %ELK, and %FP. Means for appropriate treatment main effects and interactions were separated using Fisher's protected least significant difference test at $P \leq 0.05$. Contrast statements were used to separate rate and yearly effects of *C. minitans* treatments at $P \leq 0.05$.

Sclerotia isolation and mycoparasitism. Soil samples were taken across main plots to determine populations of *S. minor* on 11 September and 10 October in 2002 and 16 September and 16 October in 2003. Soil samples were taken under the peanut canopy in each plot. The top 12 cm of soil was sampled with a shovel that removed soil cores (10 cm in diameter) from four locations in each plot, which then were combined into one sample. Sub-

Table 1. Main plot treatments applied in long-term study located in Gatesville, NC

Treatment	Rate (kg ha ⁻¹)	<i>Coniothyrium minitans</i> ^z		
		Fall 1999	Fall 2000	Fall 2001
<i>C. minitans</i>	2	Yes	Yes	Yes
<i>C. minitans</i>	4	Yes	Yes	Yes
<i>C. minitans</i>	2	No	Yes	Yes
<i>C. minitans</i>	4	No	Yes	Yes
<i>C. minitans</i>	2	No	No	Yes
<i>C. minitans</i>	4	No	No	Yes
Control	NA	No	No	No

^z *C. minitans* applied as Contans WG (Prophyta Biologischer Pflanzenschutz GmbH, Germany).

Table 2. Field experiments (Exp), locations (loc), cultivars, and dates of planting, *Coniothyrium minitans* soil incorporation, fluazinam application, and harvest

Exp, loc, year	Cultivar	Planting	<i>C. minitans</i> ^y	Fluazinam application ^z			Harvest
				1st	2nd	3rd	
Long-term							
Gatesville	...	...	3 December 1999	...	...	...	...
2001	NC-V 11, Perry	2 May	9 February 2001	26 July	29 August	22 October	22 October
2002	NC-V 11, Perry	16 May	12 November 2001	30 July	12 September	...	8 November
2003	Perry	18 June	...	21 August	10 September	...	10 November
Short-term							
2001							
Windsor	NC-V 11, Perry	10 May	15 January 2001	25 July	23 August	...	5 October
Tyner	VA 98R	12 May	15 January 2001	25 July	23 August	...	2 October
Scotland Neck	NC-V 11, Perry	8 May	15 January 2001	25 July	21 August	...	16 October
Jackson	NC-V 11, Perry	7 May	15 January 2001	24 July	30 August	...	3 October
Hertford	VA 98R	10 May	15 January 2001	25 July	23 August	...	2 October
2002							
Tyner	NC-V 11, Perry	8 May	21 November 2001	31 July	04 September	...	...
Corapeake	NC-V 11, Perry	15 May	12 November 2001	30 July	11 September	...	8 November
Gatesville	NC-V 11, Perry	28 May	22 November 2001	31 July	06 September	...	9 November

^y *C. minitans* applied as Contans WG (Prophyta Biologischer Pflanzenschutz GmbH, Germany).

^z Fluazinam formulated as Omega 500F (Syngenta Crop Protection, Greensboro, NC) and applied at 0.625 kg a.i. ha⁻¹.

samples of 500 g were subjected to a modified soil elutriation technique to trap sclerotia on a 425- μ m sieve placed in a four-unit semiautomatic elutriator and allowed to sieve for 8 min (34). Sieves used included a U.S. standard number 10 sieve (2-mm opening) nested on top of U.S. number 35 sieve (425- μ m opening). The organic matter collected on the sieve was dried at room temperature (22°C) for 24 h and then placed in 5°C until enumeration of sclerotia. Sclerotia in each sample were isolated from organic matter and enumerated under a stereomicroscope. Sclerotia viability and infection by *C. minitans* were determined after surface disinfecting in 15% NaOCl for 30 s; then, sclerotia were rinsed in sterilized distilled water, plated on potato dextrose agar (PDA) amended with tetracycline (25 mg liter⁻¹), and incubated at 18°C for 14 days. The number of sclerotia showing mycelial growth, whether or not they were infected by *C. minitans*, were considered viable. The percent sclerotia infected by *C. minitans* included both viable and nonviable sclerotia.

Sclerotia baiting. An isolate of *S. minor* (#20, obtained from Dr. Barbara Shew, North Carolina State University; [13]) originally obtained from a diseased peanut plant in Gates County, NC, was used to produce sclerotia required for baiting. The sclerotia were produced on carrot disks that were incubated for 30 days, separated, and dried at room temperature (22°C) prior to placement in terylene net bags. Twenty sclerotia (0.03 g) were placed in bags, which then were buried 10 cm below the surface of the soil underneath the peanut canopy. Four bags were buried in each main plot in the long-term field study on

23 July 2002 and 25 July 2003. Following burial for 2, 4, 8, or 12 weeks, the sclerotia baits were recovered on 7 and 21 August, 9 September, and 10 October in 2002 and 5 and 21 August, 16 September, and 6 October in 2003. The sclerotia remaining in each bag were surface disinfested and plated on PDA amended with tetracycline as described previously for determination of viability and/or infection by *C. minitans* and other fungi.

Short-term field studies. Experiments examining single-year applications of *C. minitans* were conducted at five locations in 2001 and at three locations in 2002 in North Carolina (Table 2). Eight treatments were established at each location to examine the interactions between soil incorporation of *C. minitans*, cultivar (Perry or NC-V 11), and fungicide on the development of Sclerotinia blight in peanut, except at two locations, where only cv. VA 98R was planted. Assessments of disease incidence; sclerotial isolation, viability, and infection by *C. minitans*; and peanut yield and quality were the same as described for the long-term field site. Six of the short-term field sites had a split-plot experimental design with six replicates. *C. minitans* treatments were main plots and fungicide-cultivar treatments were subplots. Only one peanut cultivar (VA 98R) was planted in plots in Tyner and Hertford in 2001, resulting in a randomized complete block design of *C. minitans* treatment by fungicide. Data for AUDPCs, sclerotial number, viability and infection, and peanut yield and market grade characteristics were subjected to analysis of variance appropriate for each factorial treatment arrangement. Means for appropriate treatment main effects and interactions were sepa-

rated using Fisher's protected least significant difference test at $P \leq 0.05$.

RESULTS

Long-term field site. Interactions among whole-plot treatments of *C. minitans* and subplot treatments of fungicide and cultivars on the AUDPC for Sclerotinia blight incidence were not significant (Table 3). However, there were several significant interactions between the main effects on peanut yield in 2001 and 2002 (Table 3). Further examination of the simple effects on yield found that they were not significant; therefore, the mean yield data are presented for each main effect only. Contrasts were used to separate the effects of *C. minitans* rate and years of application (Table 3). Less disease, as reflected by the AUDPC, developed in the moderately resistant cv. Perry than in very susceptible cv. NC-V 11 in both 2001 and 2002 (Table 4). In 2003, only Perry was planted due to heavy rains, which flooded the plots and necessitated replanting. Seed of NC-V 11 was not commercially available for replanting. Fluazinam applied according to the Sclerotinia blight advisory reduced disease in all years (Table 4). In 2001, there was no significant difference between no *C. minitans* and *C. minitans* rates of 2 or 4 kg ha⁻¹ (Table 4). The application of *C. minitans* did not affect yield in 2001 (Table 4). *C. minitans* at 2 or 4 kg ha⁻¹ reduced disease across both cultivars in 2002 and on Perry in 2003 (Table 4). Peanut pod yield was highest in plots treated with *C. minitans* at 4 kg ha⁻¹ in 2002; however, in 2003, all plots treated with *C. minitans* had lower yield compared with the nontreated control (Table 4).

Table 3. Summary of analyses of variance for main effects of *Coniothyrium minitans* treatment, fungicide, cultivar, their interactions, and contrasts of *C. minitans* treatment on Sclerotinia disease incidence as measured by the mean area under disease progress curve (AUDPC) and peanut yield in long-term field study for 2001, 2002, and 2003^a

Source	df	2001				2002				2003			
		AUDPC		Yield		AUDPC		Yield		AUDPC		Yield	
		F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Rep	3	0.97	ns	6.06	**	0.48	ns	0.36	ns	7.48	***	7.48	***
Contans treatment	6	1.46	ns	1.34	ns	7.74	**	1.20	ns	2.62	ns	2.62	ns
Fungicide (fung)	1	8.79	**	7.77	**	26.12	****	1.45	ns	295.73	****	295.73	****
Cultivar (cult)	1	25.08	****	2.05	ns	91.42	****	1.40	ns	—	—	—	—
Fung × cult	1	0.80	ns	0.13	ns	0.32	ns	4.88	*	—	—	—	—
Contans × fung	6	0.77	ns	3.01	*	0.48	ns	2.14	ns	0.86	ns	0.86	ns
Contans × cult	6	0.91	ns	0.75	ns	1.99	ns	2.29	*	—	—	—	—
Contans × fung × cult	7	0.37	ns	2.86	*	1.70	ns	1.27	ns	—	—	—	—
Contrasts													
Contans year	1	0.41	ns	1.09	ns	19.04	***	0.39	ns	38.84	****	38.84	****
Control vs. 3 years	1	—	—	—	—	26.35	***	0.15	ns	23.61	****	23.61	****
Control vs. 2 years	1	0.04	ns	1.43	ns	3.66	ns	2.32	ns	27.62	****	27.62	****
Control vs. 1 year	1	4.05	*	0.06	ns	6.71	*	1.52	ns	36.89	****	36.89	****
1 year vs. 2 years	1	3.67	ns	0.69	ns	0.46	ns	0.09	ns	0.67	ns	0.67	ns
1 year vs. 3 years	1	—	—	—	—	6.47	*	2.63	ns	1.48	ns	1.48	ns
2 years vs. 3 years	1	—	—	—	—	10.36	**	3.67	ns	0.16	ns	0.16	ns
Contans rate	1	1.46	ns	0.47	ns	14.35	**	1.88	ns	42.20	****	42.20	****
Control vs. 2 kg ha ⁻¹	1	0.34	ns	1.28	ns	14.21	**	0.08	ns	35.65	****	35.65	****
Control vs. 4 kg ha ⁻¹	1	1.50	ns	0.09	ns	10.68	**	4.02	ns	34.23	****	34.23	****
2 vs. 4 kg ha ⁻¹	1	0.30	ns	0.52	ns	0.32	ns	6.51	*	0.02	ns	0.02	ns

^a *, **, ***, and **** = Significant at the 0.05, 0.01, 0.001, and <0.0001 probability levels, respectively; ns = not significant at $P \leq 0.05$.

In 2001, the plots receiving a single application of *C. minitans* had less disease than the nontreated control, but disease incidence in plots receiving two applications was not significantly different from the nontreated control (Table 4). In 2002, three consecutive years of *C. minitans* applications had the least amount of disease (Table 4). The single application of *C. minitans* also reduced disease over the nontreated control, whereas there was no difference between the two consecutive years of application and the nontreated control (Table 4). By 2003, applications of *C. minitans* for 1, 2, and 3 years significantly reduced disease when compared with the nontreated plots, but there was no difference between the number of years *C. minitans* was applied and the amount of Sclerotinia blight that developed (Table 4). There was no effect of *C. minitans* or the application of fluazinam on yields or peanut quality as measured by %ELK and %FP (*data not shown*), consistent with previous research. Cv. Perry has a significantly larger number of extra larger kernels and fancy pods than NC-V 11 (*data not shown*).

Isolation of sclerotia and mycoparasitism. There were no significant interactions between *C. minitans* treatment and the time of soil sampling; therefore, the number of sclerotia isolated from soil was averaged across sampling dates (Table 5). One, two, and three consecutive applications of *C. minitans* reduced recovery of sclerotia from soil in the long-term field study compared with the nontreated control when averaged across all sampling dates (Table 5). Viability of recovered sclerotia ranged from 23 to 67%, but there were no statistically significant differences between the various treatments. *C. minitans* was isolated from recovered sclerotia in all plots and infection ranged from 2 to 31% of the sclerotia infected, but no statis-

tically significant differences in infection were found between the various treatments. Soil sampled on 11 September 2002 had fewer sclerotia when *C. minitans* was applied for two and three consecutive years and, by 2 October 2002, soil in all three treatments of *C. minitans* had significantly fewer sclerotia than the nontreated control (Table 5). When soil was sampled on 16 September 2003, there was no difference between the *C. minitans* treatments and the control, but the number of sclerotia was lower than in the previous sample taken in 2002 (Table 5). By 16 October 2003, the plots treated with *C. minitans* had slightly fewer sclerotia, whereas the number of sclerotia isolated in the control increased (Table 5).

Sclerotial baits. There were no significant interactions between the length of burial and treatment on viability or infection by *C. minitans* of the sclerotia in baits placed in the long-term field plot. Therefore, the data were combined across recovery dates for 2002 and 2003. In both years, there were no significant treatment effects on the percentage of sclerotia infected by *C. minitans*, even though *C. minitans* was isolated from 6 to 27% of the sclerotia. Viability of the sclerotia placed in the baits ranged from 5 to 28% after recovery from the soil. There were no statistically significant treatment effects on viability except

for the single treatment of *C. minitans* at 2 kg ha⁻¹ in 2003, which had only 5% viability compared with the remaining treatments with 19% or greater viability (*data not shown*). Several other fungi also were isolated from the sclerotia in baits, including species in the genera *Fusarium*, *Trichoderma*, *Penicillium*, *Aspergillus*, *Sporidesmium*, and *Mucor*.

Short-term field sites. Sufficient Sclerotinia blight occurred in six of the eight locations selected in 2001 and 2002 to evaluate the *C. minitans* treatment. There were no significant interactions among *C. minitans*, fluazinam, and cultivar treatment effects for all locations and dates. Moderate resistance and fungicide provided adequate control of Sclerotinia blight at the six locations (Tables 6 and 7). Fungicide treatments did not have any effect on peanut quality (*data not shown*), but the %ELK was higher where Perry was planted in Windsor, Jackson, Scotland Neck, and Gatesville compared with NC-V 11 (*data not shown*). *C. minitans* slightly reduced disease in four locations, Scotland Neck, Hertford, and Tyner, in 2001 and at Corapeake in 2002, though these reductions were not significant (Tables 6 and 7). There was no effect of *C. minitans* application on peanut yield in all locations and years (Tables 6 and 7). A higher %ELK was found in plots treated with *C. minitans*

Table 5. Average number, viability, and infection by *Coniothyrium minitans* of sclerotia recovered from soil in the long-term field study

<i>C. minitans</i> treatment	Number of sclerotia ^z				
	11 Sep 2002	10 Oct 2002	16 Sep 2003	16 Oct 2003	Average
Control	9 a	21 a	9 a	13 a	13 a
1 year	7 ab	13 b	8 a	8 a	9 b
2 years	4 b	13 b	9 a	8 a	8 b
3 years	5 b	11 b	9 a	8 a	8 b

^z Sclerotia recovered from 500 g dried soil. Values followed by the same letter in each column are not significantly different at a $P \leq 0.05$ according to Fisher's protected least significant difference.

Table 4. Mean area under disease progress curve (AUDPC) and yield (kg ha⁻¹) of main effects in the long-term field study for 2001, 2002, and 2003 peanut growing seasons^y

Main effect	Treatment	2001		2002		2003	
		AUDPC	Yield	AUDPC	Yield	AUDPC	Yield
<i>Coniothyrium minitans</i> rate							
	0	188 a	5,810 a	842 a	3,050 b	874 a	1,950 a
	2 kg ha ⁻¹	167 a	5,640 a	591 b	3,000 b	642 b	1,430 b
	4 kg ha ⁻¹	152 a	5,770 a	625 b	3,370 a	646 b	1,440 b
Years of <i>C. minitans</i> ^z							
	0	181 a	5,810 a	842 a	3,050 a	874 a	1,950 a
	1	133 b	5,780 a	653 b	3,270 a	615 b	1,370 b
	2	188 ab	5,630 a	702 ab	3,310 a	650 b	1,450 b
	3	...	...	469 c	2,980 a	667 b	1,490 b
Fluazinam							
	0	207 a	5,660 b	749 a	3,050 a	1089 a	2,430 a
	0.625 kg a.i. ha ⁻¹	132 b	5,850 a	584 b	3,260 a	313 b	700 b
Cultivar							
	NC-V 11	234 a	5,810 a	826 a	3,080 a	...	...
	Perry	106 b	5,700 a	507 b	3,220 a	701	1,560

^y Data pooled over other treatment factors and contrasts were used to separate the main effects of *C. minitans* rate and yearly treatment, values followed by the same letter in each column are not significantly different at a $P \leq 0.05$ for each main effect. There were no significant interactions between main effects in all years for disease. AUDPC was calculated using disease incidence taken from two center rows of each plot and yield estimated from two center rows.

^z Only 1 and 2 years of application of *C. minitans* were made in 2001, and 2003 is the year following the yearly treatments.

in Windsor, Scotland Neck, and Gatesville locations; there was no effect of *C. minitans* on the %FP (data not shown). In 2002, *C. minitans* reduced the number of sclerotia recovered 22% at both Gatesville and Corapeake where disease pressure was high (Table 7). Subsequently, only one of these plots had slightly less disease where *C. minitans* was applied, which was not significantly different from the nontreated control. *C. minitans* was isolated from a small number of the sclerotia recovered from soil in both Gatesville and Corapeake short-term field plots, but not in Tyner.

DISCUSSION

The number of sclerotia of *S. minor* in the soil and the incidence of Sclerotinia blight of peanut was reduced in our 5-year field study by applications of *C. minitans*. However, this activity was not observed until at least 1 year after *C. minitans* was introduced. *C. minitans* was active against the sclerotia of *S. minor* in the soil even when favorable environmental conditions and continuous planting of peanut promoted disease. Both the 2 and 4 kg ha⁻¹ rate of *C. minitans* applied to the soil decreased the number of sclerotia recovered and disease incidence. The lack of increased control with the higher application

of *C. minitans* supports the hypothesis that there is a carrying capacity for this mycoparasite in the soil we examined (18). Once a certain population size is achieved in the soil, the addition of additional biological control agent will not reduce disease further, as is seen often with chemical application. Most previous studies have focused on the activity of *C. minitans* on sclerotia of *S. sclerotiorum* (6,7,14,23,24, 48,49). Our results support other studies (33,40) that found that *C. minitans* has useful activity on *S. minor* in the field. Additionally, our laboratory studies clearly demonstrated that *C. minitans* has good activity on sclerotia of *S. minor* under a wide range of environmental conditions (27).

No significant reduction in disease was observed in our long-term study until approximately 18 months after the first application of *C. minitans* in December 1999. Many sclerotia may have escaped infection by *C. minitans* and were available to germinate and infect the first peanut crop in 2001, resulting in the disease spreading quickly through the canopy in both treated and nontreated plots. However, in 2002, there was less disease in plots receiving one or three consecutive years of application. By 2003, one, two, and three con-

secutive years of *C. minitans* soil application reduced disease. These results suggest that more than 1 year is needed for the mycoparasite to lower the inoculum density enough to reduce disease. This also was observed in the short-term plots, where the numbers of sclerotia recovered from the soil were reduced by *C. minitans* treatment but subsequent reduction in disease was not observed. Spores of *C. minitans* must come in contact with the sclerotia in order for infection to occur (47). Movement of *C. minitans* spores can be augmented by the movement of soil in field preparation and by water or by soil fauna (43,44,52–54), increasing the chance of exposure of the sclerotia to spores of *C. minitans*, because *C. minitans* does not grow saprophytically through soil (51). *C. minitans* will produce conidia after infection of sclerotia; therefore, secondary spread of the mycoparasite also can contribute to increased distribution in the soil. The longer *C. minitans* is present in the soil, the more likely it will come in contact with, infect, and reduce the inoculum density of sclerotia, ultimately decreasing disease incidence (7,12).

Although the effects of *C. minitans* on reducing numbers of sclerotia recovered in soil were promoted with longer exposure,

Table 6. Mean area under disease progress curve (AUDPC) and yield (kg ha⁻¹) of main treatments applied to short-term field studies located in peanut production areas of North Carolina in 2001^x

		Windsor		Jackson		Scotland Neck		Hertford		Tyner	
Main effect	Treatment	AUDPC ^y	Yield ^z	AUDPC	Yield	AUDPC	Yield	AUDPC	Yield	AUDPC	Yield
<i>Coniothyrium minitans</i>											
Fluazinam	0	237 a	4,332 a	12 a	2,503 a	6 a	4,351 a	436 a	4,595 a	3 a	3,645 a
	4 kg ha ⁻¹	224 a	4,215 a	19 a	2,782 a	1 a	4,437 a	357 a	4,730 a	0 a	3,950 a
	0	326 a	4,211 a	23 a	2,557 a	2 a	4,247 b	557 a	4,456 b	0 a	3,719 a
	0.625 kg a.i. ha ⁻¹	136 b	4,363 a	9 a	2,728 a	0 a	4,541 a	235 b	4,869 a	0.3 a	3,875 a
Cultivar											
	Perry	233 a	4,269 a	7 b	2,573 a	0 a	3,949 b	...	...	...	...
	NC-V 11	228 a	4,283 a	24 a	2,712 a	2 a	4,839 a	...	...	...	...

^x For each main effect, values followed by the same letter are not significantly different at a $P \leq 0.05$ according to Fisher's protected least significant difference.

^y AUDPC was calculated using disease incidence taken from two center rows of each plot.

^z Yield estimated from two center rows.

Table 7. Mean area under disease progress curve (AUDPC) yield (kg ha⁻¹), and number of sclerotia isolated from soil of main treatments applied to short-term field studies located in peanut production areas of North Carolina in 2002^w

Main effect	Treatment	Tyner			Gatesville			Corapeake		
		AUDPC ^x	Yield ^y	Sclerotia ^z	AUDPC	Yield	Sclerotia	AUDPC	Yield	Sclerotia
<i>Coniothyrium minitans</i>										
Fluazinam	0	66 a	...	2 a	774 a	3,040 a	9 a	467 a	1,217 a	9 a
	4 kg ha ⁻¹	65 a	...	2 a	799 a	2,900 a	7 b	434 a	1,222 a	7 b
	0	119 b	...	2 a	898 b	2,952 a	16 a	556 b	1,068 b	8 a
Cultivar	0.625 kg a.i. ha ⁻¹	11 a	...	1 a	676 a	2,989 a	9 b	323 a	1,372 a	8 a
	Perry	67 a	...	...	505 a	3,077 a	...	434 a	1,224 a	...
	NC-V 11	64 a	...	...	1,069 b	2,864 a	...	464 a	1,216 a	...

^w For each main effect, values followed by the same letter are not significantly different at a $P \leq 0.05$ according to Fisher's protected least significant difference.

^x AUDPC was calculated using disease incidence taken from two center rows of each plot.

^y Yield estimated from two center rows.

^z Number of sclerotia isolated from 500 g of dry soil taken from under the NC-V11 peanut canopy.

disease incidence of *Sclerotinia* blight increased each year. This was expected because planting of peanut three consecutive years greatly increases inoculum density as a result of sclerotia which are shed into the soil from diseased tissue. Continuous cropping was used in our study because we wanted to examine the long-term effect of *C. minitans* on *Sclerotinia* blight. Current production practices of rotating peanut with corn or cotton, nonhosts of *S. minor*, take this into account, limiting the number of consecutive years peanut is planted in a field (26,33). Long-term rotation has been shown to help reduce disease and allows the sclerotia to be broken down by various soil microorganisms during periods when there is no host to infect (30,43,44).

A greater reduction in disease will be needed in order to significantly increase peanut yield as found in the fungicide treatments in 2001 and 2003. In 2003, disease was reduced 71% by fluazinam, leading to a 1,730 kg/ha increase in yield, whereas *C. minitans* yearly application did not show an affect in peanut on yield in both 2001 and 2002. In 2002, the 4 kg ha⁻¹ rate of *C. minitans* was found to increase peanut yield; however, in 2003, lower peanut yield occurred in all *C. minitans*-treated plots. Lower yields following multiple applications of *C. minitans* in 2003 were not expected. However, yields were low regardless of the rate (2 or 4 kg ha⁻¹) of *C. minitans*, which reflects both the late planting date and poor harvest conditions. Overall yields averaged 4,100 kg/ha less compared with the average yield in 2001 (5,740 kg/ha). Nevertheless, additional research is needed to determine the cause of the lower yield observed in *C. minitans*-treated plots in 2003 before long-term intensive programs are recommended.

The current control measures used for *Sclerotinia* blight, including moderate resistance and fungicide applications, did not influence the activity of *C. minitans*. Application of fluazinam to the peanut canopy reduced disease compared with nontreated plots and significantly increased yield in both 2001 and 2003. Fluazinam is a very effective fungicide against *S. minor* when applied at proper times by preventing infection, (10,11, 39,40), but it does not directly reduce the numbers of sclerotia. The integration of *C. minitans* with fluazinam aims to further disrupt the disease cycle of *S. minor* by reducing the inoculum density. Laboratory experiments have shown that fluazinam limits spore germination, mycelial growth, and mycoparasitic activity of *C. minitans* (27). Even though we detected no treatment interactions of fluazinam and *C. minitans* in the field, applications of *C. minitans* should be made cautiously when fluazinam, azoxystrobin, chlorothalonil, pyraclostrobin, and tebuconazole are being used in the field. These fungicides may

compromise the performance of *C. minitans* and, therefore, application of *C. minitans* over the peanut canopy is not recommended for peanut production as it is in horticultural crops (12). Less disease also was seen in plots planted with cv. Perry. Perry has partial resistance that limits the amount of infection by *S. minor* in the peanut canopy (17). Integration of the mycoparasite with fluazinam and moderate resistance may help further manage this disease by reducing initial inoculum and rate of infection, thereby slowing the disease epidemic in peanut.

Sclerotia were isolated from soil each month during the peanut growing season. The ability to predict disease incidence by enumerating sclerotia has been difficult for *Sclerotinia* blight. Numbers of sclerotia tend to fluctuate throughout the growing season as the sclerotia germinate to infect peanut plants and as they shed back into the soil from diseased tissue. Averaging samples taken in September and October did show a reduction of sclerotia with *C. minitans* treatment. However, 2 to 31% of the sclerotia recovered in samples from each treatment were infected by *C. minitans*. These percentages only represent those sclerotia that were recovered from the soil, whereas the sclerotia that were degraded by *C. minitans* would not be represented in the sample. *C. minitans* also was isolated from sclerotia placed in the soil during 2002 and 2003. Reduced numbers of sclerotia and their colonization demonstrate the ability of *C. minitans* to infect sclerotia and persist in peanut fields. The baits also showed that there are a number of other fungi present in the soil that can aid in the destruction of sclerotia, including species of *Fusarium*, *Trichoderma*, *Penicillium*, *Aspergillus*, *Sporidesmium*, and *Mucor*. These genera all have been shown to be mycoparasites of *Sclerotinia* spp.; therefore, potential exists to reduce the number of sclerotia in soil if natural mycoparasitic populations are promoted (2–4,20,22,55).

Results from these field studies indicate that soil applications of *C. minitans* may aid in reducing losses to *Sclerotinia* blight in peanut. This mycoparasite easily can be integrated with moderately resistant cultivars and fungicide applications that are used in managing *Sclerotinia* blight on peanut. We have found that *C. minitans* is able to persist and infect sclerotia of *S. minor* in peanut fields of North Carolina. This biological control agent can be applied in the fall across crop residue and incorporated into the top layers of the soil where it will most effectively parasitize the sclerotia of *S. minor*. *C. minitans* will not provide a rapid cure to fields heavily infected by *S. minor*; however, these studies provide evidence that, over time, *C. minitans* can help reduce inoculum levels of *S. minor* and may aid in the management of *Sclerotinia* blight of peanut. Studies need

to be conducted in fields with low inoculum levels to see whether applications of *C. minitans* will reduce the disease low enough so that fungicides are no longer needed, which could reduce input costs for controlling *Sclerotinia* blight.

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