Additive Effects of *Meloidogyne arenaria* and *Sclerotium rolfsii* on Peanut[1]

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Abstract: Field observations have suggested that infection of peanut by *Meloidogyne arenaria* increases the incidence of southern blight caused by *Sclerotium rolfsii*. Three factorial experiments in microplots were conducted to determine if interactions between *M. arenaria* and *S. rolfsii* influenced final nematode population densities, incidence of southern blight, or pod yield. Treatments included four or five initial population densities of *M. arenaria* and three inoculum rates of *S. rolfsii*. Final nematode population densities were affected by initial nematode densities in all experiments ($P = 0.01$) and by *S. rolfsii* in one of three experiments ($P = 0.01$). Incidence of southern blight increased with increasing inoculum rates of *S. rolfsii* in all experiments and by the presence of the nematodes in one experiment ($P = 0.01$). Pod yield decreased with inoculation with *S. rolfsii* in all experiments ($P = 0.05$) and by *M. arenaria* in two of three experiments ($P = 0.05$). In no experiment was the interaction among treatments significant with respect to final nematode population densities, incidence of southern blight, or pod yield ($P = 0.05$). The apparent disease complex between *M. arenaria* and *S. rolfsii* on peanut is due to additive effects of the two pathogens.
Key words: Arachis hypogaea, disease complex, interaction, *Meloidogyne arenaria*, peanut, root-knot nematode, *Sclerotium rolfsii*, southern blight.

Peanut (*Arachis hypogaea* L.) is an important crop in the southern United States, with approximately 600,000 ha planted annually. Two of the most important diseases of peanut are root knot, caused by *Meloidogyne arenaria* (Neal) Chitwood race 1, and southern blight (also called stem rot), caused by *Sclerotium rolfsii* Sacc. *Meloidogyne arenaria* is most prevalent in Alabama (8), Georgia (13), Florida (25), and Texas (26), where as many as 40% of the peanut fields are infested with this pathogen. As much as 50% of the yield potential can be lost to *M. arenaria* in heavily infested fields. In Texas, we have estimated that more than 10% of the fields from five major peanut-producing counties have root-knot nematode population densities that exceed the level needed to cause a 10% yield loss (26). Studies in Florida have estimated the damage threshold in some years to be as low as a single nematode per 100 cm^3 soil (11). Similar data on

the relationships between nematode population densities and peanut yields are reported from Alabama (21), North Carolina (10), and Texas (26).

Sclerotium rolfsii is a soilborne fungal pathogen that attacks numerous crops in addition to peanut. Southern blight incited by this pathogen on peanut usually coincides with peg and pod development when peanut stems spread rapidly across the soil (2,14). Disease development is favored by warm, moist environmental conditions. Sclerotia at or near the soil surface serve as overwintering propagules and primary inoculum. As with many soilborne pathogens, horizontal distribution of sclerotia is aggregated and it has been difficult to develop precise models of the relationship between inoculum density and disease incidence (23). There is a good correlation, however, between number of disease loci, where a locus is defined as "an infected area equal to or less than 30 cm in length," and peanut yields (16). Annual peanut yield reductions due to southern blight in the southern peanut-producing states range from 2% in Florida to a high of 9.5% in Alabama (25). Losses in Texas are usually estimated to be approximately 4% (25).

Meloidogyne arenaria and *S. rolfsii* are frequently found together in peanut fields,

but there are no reports that document a disease complex between these pathogens on peanut, even though root-knot nematodes are well known for their ability to increase the incidence and severity of a number of diseases caused by soilborne fungal pathogens. When root-knot nematode population densities are reduced through application of fumigant nematicides, the incidence of stem rot also is reduced in fields infested with both pathogens (Lee and Starr, unpubl. data). A reduction in the incidence of southern blight on peanuts has been observed by others when there was a significant reduction in nematode populations through applications of nematicides (18,20) or crop rotation systems (16–18). In other instances, nonfumigant nematicides and rotation of peanut with crops that are poor hosts for *M. arenaria* or *S. rolfsii* did not reduce the incidence of southern blight on peanut (16,19,20). Collectively, these observations suggest that an interaction between *M. arenaria* and *S. rolfsii* increases the incidence of southern blight. Interactions between other root-knot nematode species and *S. rolfsii* on tobacco or *Solanum melongena* that result in increased incidence and severity of disease caused by the fungal pathogen are known (7,15). The incidence of *S. rolfsii* on soybean was reported to be increased by damaging population densities of *M. arenaria* (12). Additionally, *M. arenaria* interacts in a synergistic manner with *Pythium myriotylum* on peanut in the pod rot syndrome (6).

The objective of this research was to test the hypothesis that infection of peanut by *M. arenaria* and *S. rolfsii* increases the incidence of southern blight.

MATERIALS AND METHODS

The isolate (no. 82-4) of *M. arenaria* race 1 used for these experiments was originally isolated from peanut and maintained on *Lycoperscion esculentum* Mill 'Rutgers'. The isolate of *S. rolfsii* used was also isolated from peanut as a single hyphal-tip culture on potato dextrose agar (PDA).

Mature sclerotia of *S. rolfsii* were preserved in vials at room temperature. The peanut cv. Florunner was the host plant for all experiments.

Three microplot experiments were conducted to test for possible interactions between *M. arenaria* and *S. rolfsii*, one in 1992 and two in 1993. The 1992 and one of the 1993 (1993-A) experiments were conducted in 54-cm-d × 45-cm-deep microplots containing a loamy sand soil (pH 7.8). Each was a three-by-five factorial experiment in a randomized complete block design with six replications of each treatment. Treatments were five initial densities of *M. arenaria* (0, 1, 10, 50, and 100 eggs and second-stage juveniles [J2]/500 cm^3 soil) and three initial densities of *S. rolfsii* (0, 15, and 150 sclerotia/microplot in 1992 and 0, 25, and 100 sclerotia/microplot in 1993). The second experiment in 1993 (1993-B) was conducted in 60-cm × 84-cm × 45-cm-deep microplots containing a sandy loam soil (pH 7.9). This experiment was a three-by-four factorial with five replications of each treatment. Treatments were four initial densities of *M. arenaria* (0, 1, 10, and 100 eggs and J2/500 cm^3 soil) and three initial densities of *S. rolfsii* (0, 37, and 150 sclerotia/microplot). The sclerotia population densities for the second microplot test in 1993 were equivalent to the population densities in the other 1993 experiment in terms of numbers of sclerotia per m^2 in the two differently shaped microplots. Microplots were treated with 1 kg of methyl bromide (98%) and chloropicrin (2%)/m^2 of soil in 1992 and with 12 ml of 1,3-dicloropropene (83%) and chloropicrin (17%)/m^2 of soil in 1993 to eliminate existing populations of plant-parasitic nematodes and soilborne plant-pathogenic fungi. Microplots were covered with 50-mil plastic for 7 days following application of methyl bromide and chloropicrin.

Inoculum of *M. arenaria* was prepared as infested soil from cultures maintained on tomato. Ten weeks after tomato plants were each inoculated with 10,000 eggs, plants were harvested and the roots

shaken free of soil. The infected roots were cut into segments 2 to 3 cm long and mixed with the nematode-infested soil from those cultures. Samples (300 cm^3) of this infested soil and infected root fragments were processed by elutriation (5) and centrifugation (9) to estimate the numbers of J2 present. Root fragments recovered during elutriation were treated with 1.0% NaOCl to extract eggs of *M. arenaria*. Combined counts of J2 and eggs were used to determine amounts of infested soil to be added to each microplot to achieve desired population densities. The nematode-infested soil was added to the microplot soil immediately before planting. Six peanut seeds were added to the circular plots (1992 and 1993-A), and 10 seeds were added to the rectangular plots (1993-B). Plant stands were thinned to three and six plants/microplot, respectively, 1 week after emergence.

Nonsterile sclerotia of *S. rolfsii* were prepared according to the method of Beute and Rodríguez-Kábana (4). Sclerotia from permanent storage vials were placed on 15-cm-d petri dishes of PDA and incubated at 27 C. Agar plugs (5-mm d) were collected from the advancing margin of the resulting colony and used to infest 500-ml flasks containing 50 g of autoclaved oat grains. After allowing 7 days for *S. rolfsii* to colonize the oat grains at room temperature, the infested oat grains were spread on paper towels and air-dried. The air-dried, infested oat grains were stored at −4 C. To produce sclerotia for inoculum, infested oat grains were spread on a 23 × 32 × 2.5-cm layer of moist, sandy loam field soil, covered with plastic wrap, and incubated at room temperature. Mature sclerotia were collected from the trays of soil and infested oat grains by wet sieving and then air-dried on paper towels at room temperature. These non-sterile sclerotia were added to the surface of the microplot soil, near the crown of the peanut plants, at the time of peanut peg initiation.

Data collected from each experiment were nematode population densities at crop maturity, proportion of plants killed by *S. rolfsii* per microplot, and peanut pod yield per microplot. Time of first appearance of stem blight symptoms was recorded also. To estimate nematode population densities, eight soil cores (2.5 cm d × 25 cm deep) were collected and composited from each microplot at crop maturity. Subsamples of 500 cm^3 were processed by elutriation and centrifugation to extract J2 nematodes. Root fragments recovered during elutriation were treated with 1.0% NaOCl to extract nematode eggs (3). To confirm that symptomatic plants were infected by *S. rolfsii*, arbitrarily selected symptomatic peanut stem segments were placed on petri dishes containing PDA for re-isolation of the pathogen. Pods were hand-picked from vines and air-dried before weighing.

All data were subjected to analysis of variance using the SAS general linear models procedure (22). Mean separations were by least significance difference. Regression analysis was used to determine the relationships between initial nematode population densities and pod yield, and between initial nematode population densities and incidence of southern blight.

RESULTS

Population densities of *M. arenaria* increased up to 8,000-fold from planting until harvest in these experiments. Final mean population densities of *M. arenaria* were similar for the 1992 and 1993-B experiments, with maximum densities of 9,400 and 11,300 eggs and J2/500 cm^3 soil, respectively, in plots initially infested with 100 eggs and J2/500 cm^3 soil (Fig. 1). Final nematode mean population densities were lower in the 1993-A experiment, with maximum population densities of 1,400 eggs and J2/500 cm^3 soil in plots initially infested with 100 eggs and J2/500 cm^3 soil (Fig. 1A). Only in the 1992 experiment did the presence of *S. rolfsii* affect the final nematode population densities ($P = 0.05$; Table 1); final nematode population densities, in general, were lower in the presence of the fungal pathogen than in its ab-
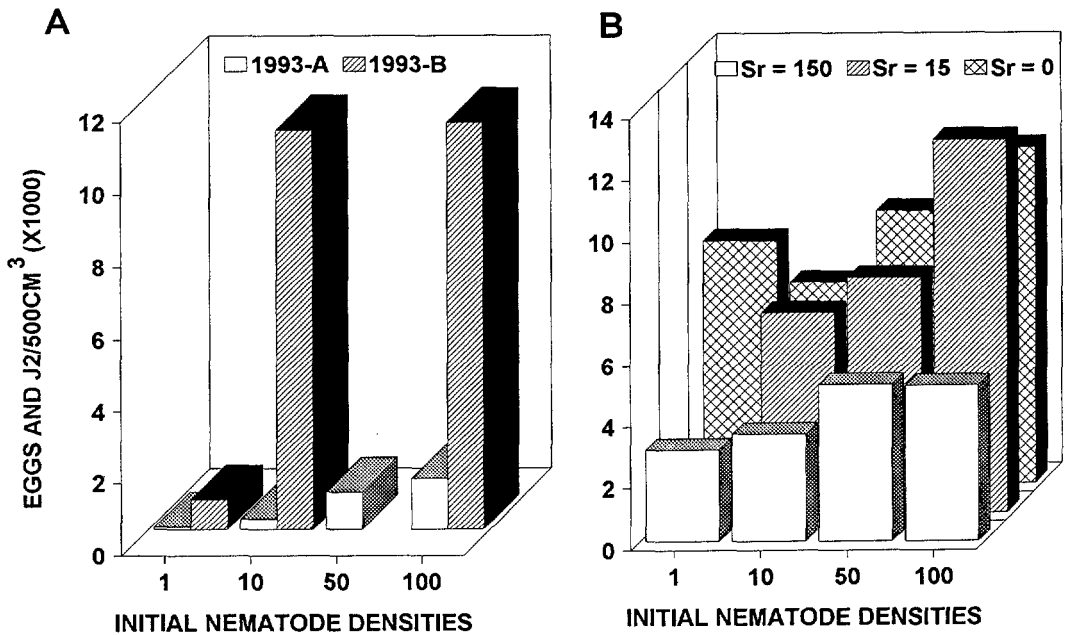
FIG. 1. Final population densities of *Meloidogyne arenaria* on peanut in microplots also infested with *Sclerotium rolfsii.* A) From the 1993-A and 1993-B experiments, in which there was no effect of *S. rolfsii* on nematode population densities and data from different *S. rolfsii* treatments were pooled. B) From the 1992 experiment, in which both initial population densities (eggs and J2/500 cm^3 soil) of *M. arenaria* and inoculation rates with *S. rolfsii* affected final nematode population densities. Sr = numbers of sclerotia of *S. rolfsii* per plot.

sence (Fig. 1B). No interaction between initial population densities of *M. arenaria* and presence of the fungal pathogen was observed ($P = 0.05$).

Symptoms of southern blight were observed in each experiment beginning 2 to

TABLE 1. F-values for main treatment effects and treatment interactions in three microplot experiments to determine effects of *Meloidogyne arenaria* and *Sclerotium rolfsii* on nematode population densities, incidence of southern blight, and pod yield of peanut.

Treatments	1992	1993-A	1993-B
Final population densities of *M. arenaria*			
M. arenaria Pi	7.29**	5.23**	7.23**
Sclerotia per plot	3.45*	0.25	1.50
Interaction	0.92	0.27	0.86
Proportion of plants infected by *S. rolfsii*			
M. arenaria Pi	1.16	1.76	4.94**
Sclerotia per plot	56.34**	28.47**	25.59**
Interaction	0.68	0.46	0.91
Pod yield			
M. arenaria Pi	6.83**	1.63	9.41**
Sclerotia per plot	34.66**	5.41**	2.59*
Interaction	0.77	0.86	1.67

*, ** indicate significance at $P = 0.05$ and 0.01 levels, respectively.

3 weeks after microplots were infested with *S. rolfsii.* The presence of the fungal pathogen was confirmed by re-isolation from symptomatic tissues. No effect of numbers of sclerotia per microplot or presence of root-knot nematodes was observed with respect to time of initial symptom development. Increased numbers of sclerotia per microplot increased total disease incidence at crop maturity ($P = 0.01$) (Table 1; Fig. 2). In the 1992 and 1993-A experiments, the presence of root-knot nematodes did not affect incidence of southern blight ($P = 0.05$). Nematodes did increase disease incidence in the 1993-B experiment ($P = 0.01$). The relationship between incidence of southern blight and initial nematode population densities in the 1993-B experiment was linear and described by the models $Y = 0.14 + 0.11$ $\log_{10} (x + 1)$ ($R^2 = 0.88$) at 37 sclerotia/ plot and $Y = 0.32 + 0.13 \log_{10} (x + 1)$ ($R^2 = 0.99$) at 150 sclerotia/plot. No southern blight was observed in microplots not inoculated with *S. rolfsii.* In no case was the interaction between *M. arenaria* and *S. rolf-*
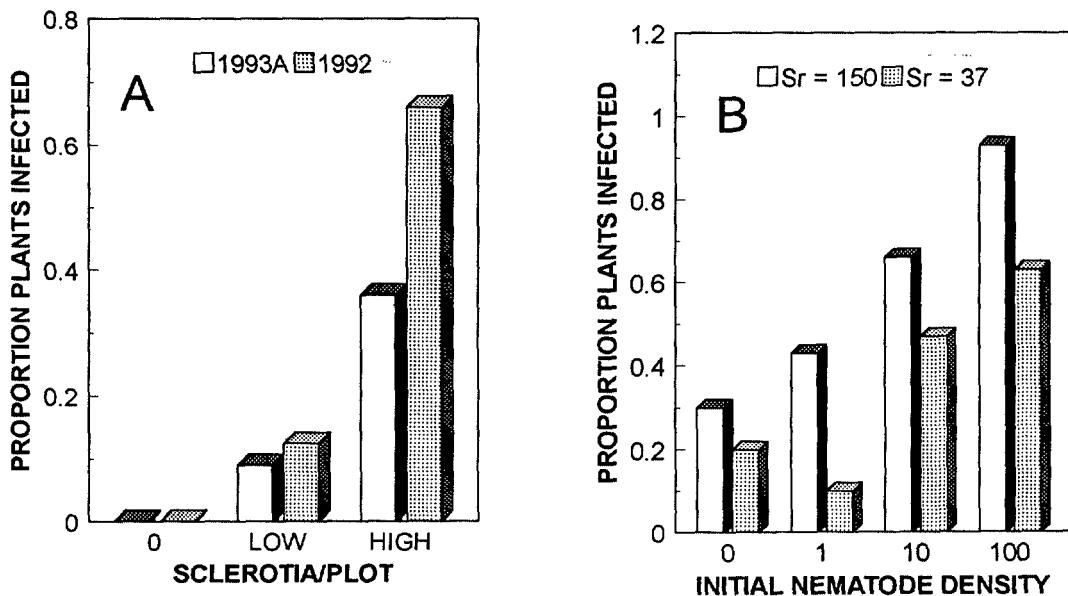
Fig. 2. Incidence of southern blight in microplots infested with *Meloidogyne arenaria* and *Sclerotium rolfsii*. A) From the 1992 and 1993-A experiments in which there was no effect of *M. arenaria* on incidence of southern blight. Inoculum densities of *S. rolfsii* were 0, 15, and 150 sclerotia/plot in 1992 and 0, 25, and 100 sclerotia/plot in 1993-A. B) Effects of numbers of sclerotia per plot (Sr) and initial nematode population densities (eggs and J2/500 cm³ soil) on the incidence of southern blight.

sii significant with respect to incidence of southern blight ($P = 0.05$; Table 1).

Pod yield of peanut was suppressed by *S. rolfsii* in all three experiments and suppressed by *M. arenaria* in the 1992 and 1993-B experiments (Table 1). In no case was the interaction between *S. rolfsii* and *M. arenaria* significant. The relationship between the transformed $\log_{10}(x + 1)$ initial population densities of *M. arenaria* and pod yield was linear at all inoculum rates of *S. rolfsii* for the 1992 and 1993-B experiments (Fig. 3; Table 2). The higher inoculum rates of *S. rolfsii* resulted in lower pod yields in all three experiments (Fig. 3).

DISCUSSION

In none of the three experiments was an interaction between *M. arenaria* and *S. rolfsii* observed with respect to incidence of southern blight, final nematode population densities, or pod yield. In only one instance each did initial nematode population densities affect the incidence of southern blight (1993-B) or did inoculum levels of *S. rolfsii* affect the final population den-

sities of the nematode (1992). In both of these cases, the interaction statements were not significant. Additionally, in two of three experiments when both pathogens suppressed pod yield, the interaction statements were, likewise, not significant. Collectively, these observations from microplot experiments contradict our previous field observations and those reported by others (16–20) that suggested an interaction exists between *M. arenaria* and *S. rolfsii* with respect to incidence of southern blight. It is possible that the additive effects of the two pathogens observed with respect to disease incidence and pod yield in some previous experiments were interpreted as an interaction in less controlled field situations. Alternatively, it is possible that an interaction could have occurred at different nematode or fungal population densities than were tested in these experiments. In interactions between *M. incognita* and forma specialis of *Fusarium oxysporum* in development of vascular wilts, the population densities of both pathogens affect the development of the interaction; no interaction is observed if population densi-
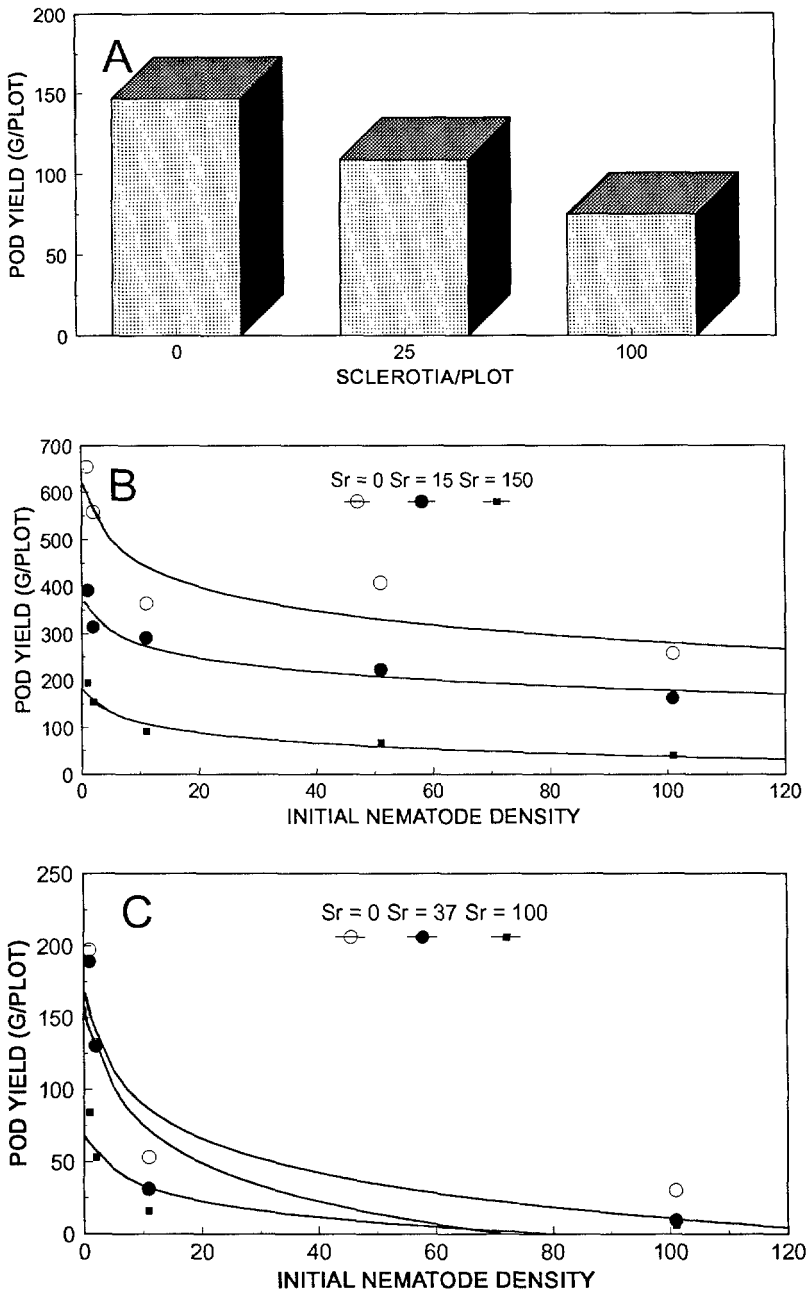
FIG. 3. Effects of *Meloidogyne arenaria* and *Sclerotium rolfsii* on pod yield of peanut in microplots. A) From the 1993-A experiment, in which nematodes had no effect on pod yield (LSD 0.05 = 43). B, C) From the 1992 and 1993-B experiments, respectively, in which effects of *M. arenaria* and *S. rolfsii* were significant (P = 0.05), but interactions were not. See Table 2 for regression models.

ties of either pathogen are high or low (1,24).

Development of southern blight resulting from infestations of the plots with *S. rolfsii* suppressed nematode population densities in only one of three experiments. In the interaction of *F. o. vasinfectum* and *M. incognita* on cotton, the presence of the two organisms commonly suppressed final nematode population densities (24); it was

TABLE 2. Regression analysis of the relationship between initial population densities of *Meloidogyne arenaria* (X) and pod yield (Y) of peanut at different infestation rates with *Sclerotium rolfsii.*

S. rolfsii inoculum	Model	R-squared
	1992	
0 Sclerotia/plot	$Y = 618.8 - 73.2 \log_{10}(x + 1)$	0.85*
15 Sclerotia/plot	$Y = 375.4 - 42.6 \log_{10}(x + 1)$	0.93*
150 Sclerotia/plot	$Y = 182.8 - 31.3 \log_{10}(x + 1)$	0.97**
	1993-B	
0 Sclerotia/plot	$Y = 168.9 - 34.3 \log_{10}(x + 1)$	0.86*
37 Sclerotia/plot	$Y = 163.5 - 38.2 \log_{10}(x + 1)$	0.87*
150 Sclerotia/plot	$Y = 70.6 - 16 \log_{10}(x + 1)$	0.85*

*, ** indicate significance at $P = 0.05$ and 0.01, respectively.

suggested that this was due to increased levels of plant mortality, which occurred earlier in the season, thereby suppressing reproduction by the biotrophic nematode late in the season. In the current study, the onset of southern blight, which is typically a late-season disease, was not affected by population densities of either the nematode or the fungus. Thus, in two of three experiments, there was insufficient plant mortality due to southern blight early enough in the season to affect nematode population densities.

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