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Interrelationships Among *Macrophomina phaseolina*, *Criconemella xenoplax*, and *Tylenchorhynchus annulatus* on Grain Sorghum[1]

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Abstract: Microplot experiments were established in 1992, 1993, and 1994 to investigate the relationships among *Macrophomina phaseolina*, *Criconemella xenoplax*, and *Tylenchorhynchus annulatus* on grain sorghum in Louisiana. A factorial treatment arrangement of two grain sorghum hybrids (De Kalb DK 50 and Pioneer hybrid 8333), three levels of *M. phaseolina* (0, 10, and 100 colony-forming units (CFU)/g soil), and three nematode inoculum levels (0, 1×, and 2×) were used. Nematode inocula at 1× levels were 929, 1,139, and 1,445 *C. xenoplax* and *T. annulatus*/microplot in 1992, 1993, and 1994, respectively. Plants were harvested after 90–105 days. In all 3 years, grain sorghum root and head dry weights were suppressed as nematode inoculum level increased. These reductions were detected both in the absence and in the presence of *M. phaseolina* at 10 CFU/g. Reproduction of both nematode species was suppressed by *M. phaseolina*. Interactions between *M. phaseolina* and nematodes were antagonistic with regard to plant dry weights, yield, and nematode reproduction, so that combined effects were less than the sum of the effect of each pathogen alone.

Key words: charcoal rot, *Criconemella xenoplax*, grain sorghum, interaction, *Macrophomina phaseolina*, *Sorghum bicolor*, *Tylenchorhynchus annulatus*.

Grain sorghum (*Sorghum bicolor* (L.) Moench.) is a minor crop in Louisiana and is commonly grown in rotation with other crops (Anonymous, 1991). A survey of nematode species and their abundance on grain sorghum in Louisiana indicated that the stunt nematode, *Tylenchorhynchus annulatus* (Cassidy) Golden, and the ring nematode, *Criconemella xenoplax* (Raski) Luc and Raski, were most abundant (Wenefrida, unpubl.)

Symptoms of nematode damage to grain sorghum in the field closely mimic those caused by drought stress, nutrient deficiencies, other diseases, and insects. Severely infected plants are usually smaller and chlorotic, and have a tendency to wilt as the result of extensive root dysfunction. Root cells are destroyed by the nematode during feeding, thereby reducing uptake of water and nutrients. Digestive fluids rich in pectolytic and proteolytic enzymes are secreted during nematode feeding and probably cause much of this root injury (Claflin, 1983). Twenty species of nematodes have been documented as parasites of grain sorghum, and many of these are pathogenic (Claflin, 1983; Starr, 1992). Estimated annual losses of grain and forage sorghum to nematodes averaged 6% in the United States (Thomas and Murray, 1987) and 6.9% worldwide (Starr, 1992).

Charcoal rot, caused by the fungus *Macrophomina phaseolina* (Tassi) Goid., is an important disease of grain sorghum (Frederiksen, 1986; Mughogho and Pande, 1983). Symptoms associated with charcoal rot include decayed stalks; lodged plants; premature dying of stalks; poorly developed panicles with small, inferior-quality grain; and root rot (Hsi, 1956; Mughogho and Pande, 1983). The most diagnostic symptom of charcoal rot disease is lodging, which occurs as plants approach maturity (Hsi, 1956, 1961; Mihail, 1992). Grain yield losses of 23% to 64% have been attributed to *M. phaseolina* (Mughogho and Pande, 1983). There is evidence that disease incidence is directly related to plant stress and that disease is most severe on grain sorghum hybrids with high yield potential (Mughogho and Pande, 1983; Norton, 1958).

The individual effects of the charcoal rot

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fungus (Frederiksen, 1986; Holliday and Punithalingam, 1970; Hsi, 1956, 1961; Mihail, 1992) and common endoparasitic and ectoparasitic nematode species (Claflin, 1983; Cuarezma-Teran et al., 1984; Cuarezma-Teran and Trevathan, 1985; Fortnum and Currin, 1988; Hafez and Claflin, 1982; Mataloate et al., 1987; Orr, 1967; Thomas and Murray, 1987) on grain sorghum are well documented. However, few investigators have evaluated the combined effects of these pathogens on grain sorghum. Our objectives were to evaluate the: (i) individual and combined effects of *M. phaseolina, T. annulatus,* and *C. xenoplax* on growth and yield of grain sorghum, and (ii) interrelationships between these pests.

Materials and Methods

Fungus inoculum production: Isolates of *M. phaseolina* were collected in 1991 from lower stems of grain sorghum plants at Winnsboro, Louisiana, in a field that had been cropped to grain sorghum for 12 years. Cultures of the fungus were maintained at 4 °C as mycelia and microsclerotia on wood toothpicks (Mihail, 1992). Inoculum was produced in quantity by transferring 20 to 25 agar plugs (5-mm diam.) from 5- to 7-day-old cultures to glass trays containing 300 g corn cob grits that were ground to pass through a 250-µm sieve (BIO-SERV, Frenchtown, NJ) and moistened with 1 liter of potato dextrose broth (Difco, Detroit, MI). This mixture of mycelia, microsclerotia, and grits was incubated at 30 °C for 60 days, dried in a forced-air oven at 30 to 35 °C for 3 days, and powdered using a mortar and pestle. Numbers of colony-forming units (CFU)/g of dry inoculum were determined by dilution plating on potato dextrose agar (Difco, Detroit, MI) amended with rifampicin (100 mg dissolved in 1 ml dimethyl sulfoxide), metalaxyl (224 mg a.i. as Ridomil 2E-G), and Tergitol NP-10 (1 ml). Rifampicin, metalaxyl, and Tergitol were added after the medium was autoclaved and cooled to 55 °C (Cloud and Rupe, 1991). Preliminary results indicated that there were no differences among isolates of *M. phaseolina* from eight parishes in Louisiana with regard to root colonization and pathogenicity on several different grain sorghum hybrids. Therefore, a single isolate from Winnsboro (Mp4) was used in all studies.

Nematode inoculum, identification, and production: Populations of ring and stunt nematodes were separated by hand-picking from communities that contained small numbers of lesion and spiral nematodes. Monoxenic cultures of these two parthenogenetic nematode species were derived from single females, maintained in a greenhouse on Pioneer hybrid 8333 grain sorghum, and used in all experiments. The ring nematode was identified as *Criconemella xenoplax* (Raski) Luc & Raski (Raski and Golden, 1965), and the stunt nematode was identified as *Tylenchorhynchus annulatus* (Cassidy) Golden (Anderson and Potter, 1991).

Experiment design and microplot environment: The experiment design was a randomized complete block with factorial treatment arrangement. Treatments involved two grain sorghum hybrids (De Kalb DK 50 [DK 50] and Pioneer hybrid 8333 [P 8333]), three levels of *M. phaseolina* (0, 10, and 100 CFU/g soil), and three nematode infestation levels (0, 1×, and 2×), each replicated four times. Nematode inoculum at the 1× level was 929 (52% stunt, 48% ring), 1,139 (43% stunt, 57% ring), and 1,445 (38% stunt, 62% ring) nematodes/microplot in 1992, 1993, and 1994, respectively. These were similar to preplant populations observed in grain sorghum fields in Louisiana (Wenefrida, unpubl. data).

Experiments were conducted during 1992, 1993, and 1994 in microplots, which consisted of large clay pots (30-cm diam.) containing 15 kg Convent silt loam soil (coarse-silty, mixed, non-acid, thermic Aeric Fluvaquent; 80.3% sand, 5.8% silt, and 13.9% clay). Before use, soil was treated with sodium methyldithiocarbamate (32.7%) at 12.5 ml/kg soil. Soil in each microplot subsequently was infested with *M. phaseolina* at 0, 10, or 100 CFU/g soil. Microplots were buried in soil up to the pot rims. Seventy-two microplots were spaced 1 m apart in a 6-by-12 pattern. The entire area was covered by a

14-m-long by 9-m-wide aluminum quonset hut frame open at both ends that supported polyethylene greenhouse film. This covering was necessary to protect plants in microplots from excessive rainfalls common in southern Louisiana.

Experiment establishment: Planting and harvest dates were 17 July and 28 October, 7 May and 23 August, and 4 May and 19 August for 1992, 1993, and 1994, respectively. Grain sorghum seeds were germinated on moist filter paper in darkness at 30 °C and sown in flats of methyl bromide (98% methyl bromide, 2% chloropicrin) fumigated soil. Two weeks later, seedlings of uniform size were transplanted to 8-cm-diam., plastic pots that contained 150 g fumigated soil infested with *M. phaseolina* at 0, 10, or 100 CFU/g soil. Five days later, the entire contents of the plastic pot (plant and soil) were transferred to appropriate microplots. Soil was amended with 112.5 kg/ha each of N (as $[NH_4]_2NO_3$) and K (as KCl) according to soil tests. One-half of these fertilizers was added to soil at transplanting, and the remainder was added 7 days later.

Nematode inoculum was added 4 days after establishing seedlings in microplots. Water alone or suspensions containing 1× or 2× levels of nematodes were pipetted into four depressions (6 and 10 cm deep by 1.5 cm diam.) surrounding the base of each seedling. Depressions were filled with fumigated soil following infestation.

Data collection and analysis: Plants were harvested 90, 105, and 105 days after transplanting in 1992, 1993, and 1994, respectively. Stems were excised 2.5 cm above the soil line, and weights for stems, roots, and heads were recorded after drying for 72 hours at 60 °C.

Four soil cores (2.5 cm diam. by 30 cm deep) were collected from each microplot at harvest, and samples (150 g) were used to estimate nematode population densities in soil. Soil for nematode analysis was extracted using the centrifugal-flotation technique (Jenkins, 1964). Numbers of mature and immature vermiform life stages of each nematode species were counted, and totals per microplot and reproductive value (R, where R = Pf/Pi and Pf = final population density and Pi = initial inoculum level) were computed. Root systems were separated from soil by gentle washing with water.

Root colonization by *M. phaseolina* was estimated by randomly collecting ten 1-cm-long fragments from each root system. Fragments were surface-sterilized in 0.525% sodium hypochlorite for 3 minutes, rinsed in sterile deionized water for 3 minutes, blotted with sterile filter paper, and arranged uniformly across the surface of a 90-mm-diam. petri dish containing 15 ml of selective medium (Cloud and Rupe, 1991). Cultures were incubated at 30 °C for 5 to 7 days in darkness, and the numbers of root fragments colonized by *M. phaseolina* were counted and multiplied by 10 to estimate colonization percentage for each plant. Density of microsclerotia in the soil was determined at harvest from a 5-g subsample (Cloud and Rupe, 1991).

Data were analyzed with the General Linear Models procedure (SAS Institute, Cary, NC) to test for main treatment effects and interaction among treatments. When the number of treatment levels exceeded two, means were separated using least significant difference (LSD). Interactions that were significant in 2 or more years are presented (Figs. 1–3). Those interactions that occurred in only 1 year are described within the text only.

Results

Plant dry weights: Macrophomina phaseolina reduced grain sorghum root weight in all three tests (Table 1). Root weight was reduced in 1992 at both soil infestation levels, but there was no difference between levels. In 1993 and 1994, stepwise reductions in root weight were detected as levels of the fungus in soil increased. Across tests, mean root weight reductions were 26% and 31% at fungal densities of 10 and 100 CFU/g, respectively. Stem weight was reduced by *M. phaseolina* at both soil infestation levels in 1993 but only at the 100-CFU/g level in 1994. Across tests, mean stem weight reductions were 5% and 14% at *M. phaseolina* levels of 10 and 100 CFU/g, respectively. The

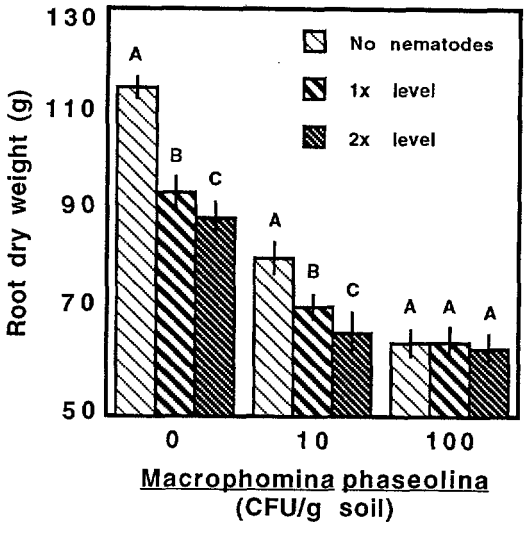

FIG. 1. Grain sorghum root dry weight in 1993 as influenced by nematode inoculum levels of 1× (1,139 nematodes) and 2× (2,278 nematodes) per microplot (43% *Tylenchorhynchus annulatus*, 57% *Criconemella xenoplax*) and by *Macrophomina phaseolina* at infestation levels of 10 and 100 colony forming units (CFU)/g soil. Within each *M. phaseolina* infestation level, letters above bars indicate means that differed significantly ($P \leq 0.05$) according to a Least Significant Difference test. Vertical lines delimit standard errors of means for four replicates.

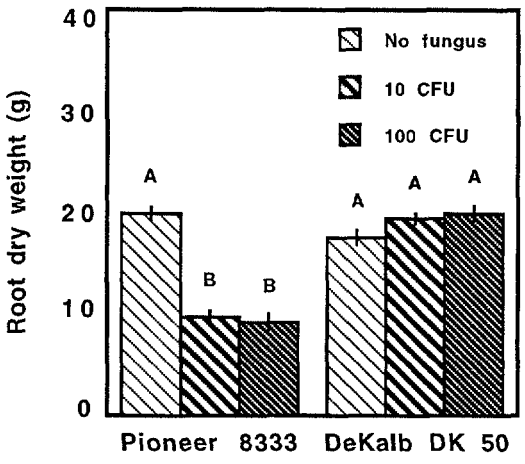

FIG. 2. Grain sorghum root dry weight in 1992 as influenced by host genotype and by *Macrophomina phaseolina* at infestation levels of 10 and 100 colony forming units (CFU)/g soil. Within each hybrid, letters above bars indicate means that differed significantly ($P \leq 0.05$) according to a Least Significant Difference test. Vertical lines delimit standard errors of means for four replicates.

fungus reduced head weight in a stepwise manner in both 1993 and 1994. Reductions across tests were 34.2% and 41.5% at 10 and 100 CFU/g, respectively.

Nematodes reduced root weight in all three tests (Table 1). In 1993 and 1994, root weight reductions were greater as nematode inoculum level increased, but only nematode inoculum at 2× level reduced root weight in 1992. Across tests, mean root weight reductions were 9% and 20%, respectively, at 1× and 2× nematode inoculum levels. In 1993 and 1994, nematodes reduced stem weight at both inoculum levels, but there were no differences between levels. Across tests, stem weights were reduced 7% and 10% at 1× and 2× nematode inoculum levels, respectively. Head weight was reduced by nematodes in both 1993 and 1994. In 1993, there was a stepwise reduction in head weight as nematode inoculum level increased, but there were no differences between inoculum levels in 1994. Reductions across tests averaged 17% and 21% at 1× and 2× nematode inoculum levels, respectively.

DK 50 plants generally were larger than those of P 83333 (Table 1). This was evident for root weight in 1992 and for all plant parameters in 1994.

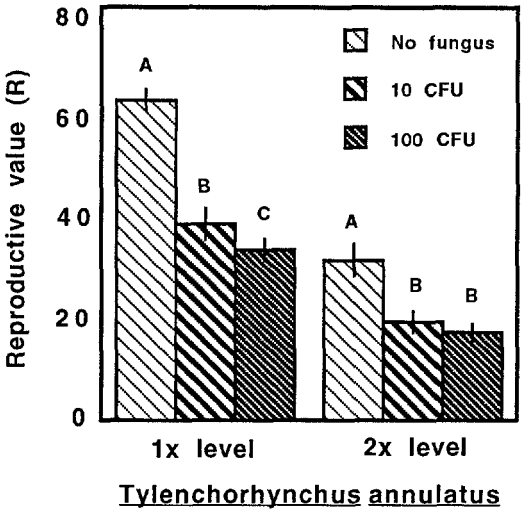

FIG. 3. Reproduction of *Tylenchorhynchus annulatus* in 1993 as influenced by nematode inoculum levels of 1× (1,139 nematodes) and 2× (2,278 nematodes) per microplot (43% *Tylenchorhynchus annulatus*, 57% *Criconemella xenoplax*) and by *Macrophomina phaseolina* at infestation levels of 10 and 100 colony forming units (CFU)/g soil. R = Pf/Pi, where Pf = final population density and Pi = initial inoculum density. Within each nematode inoculum level, letters above bars indicate means that differed significantly ($P \leq 0.05$) according to a Least Significant Difference test. Vertical lines delimit standard errors of means for four replicates.

TABLE 1. Grain sorghum dry weights as influenced by *Macrophomina phaseolina* infestation level, nematode inoculum level, and grain sorghum genotype in microplot studies.

		Dry weight (g)							
		1992		1993			1994		
Factor	Level	Root	Top	Root	Stem	Head	Root	Stem	Head
Fungus	0[a]	18.7 a	64.3 a	98.0 a	49.8 a	44.5 a	89.6 a	59.1 a	105.7 a
	10	14.4 b	64.5 a	72.9 b	45.0 b	25.0 b	62.7 b	58.9 a	81.2 b
	100	15.8 b	63.2 a	63.9 c	45.1 b	21.4 c	51.2 c	48.5 b	74.7 c
	P > *F*	**	ns	**	**	**	**	**	**
Nematode	0[b]	17.0 a	61.7	85.5 a	48.8 a	34.8 a	81.1 a	59.8 a	99.9 a
	1×	17.4 a	69.5	76.5 b	45.8 b	29.3 b	67.1 b	55.2 b	82.5 b
	2×	14.4 b	67.9	72.7 c	45.2 b	26.8 c	57.1 c	52.2 b	80.6 b
	P > *F*	**	ns	**	**	**	**	**	**
Host genotype	DK 50[c]	18.8 a	68.9	78.1	46.9	31.2	73.1 a	59.3 a	92.6 a
	P 8333	13.0 b	64.3	78.4	46.3	29.4	63.5 b	52.0 b	82.5 b
	P > *F*	**	ns	ns	ns	ns	**	**	**
F × N	*P* > *F*	**	ns	**	**	**	**	ns	**
F × H	*P* > *F*	**	ns	ns	ns	ns	**	ns	**
N × H	*P* > *F*	ns	ns	ns	ns	ns	ns	ns	ns
F × N × H	*P* > *F*	ns	ns	ns	ns	ns	ns	ns	ns

Data are means of four replicates. Within each column, values followed by the same letter are not different ($P > 0.05$) according to a Least Significant Difference test.

[a] 0, 10, and 100 colony forming units (CFU)/g soil for *M phaseolina.*

[b] Initial inoculum percentages for *Tylenchorhynchus annulatus : Criconemella xenoplax* were 52:48 (1× level = 929 nematodes per pot, 2× level = 1,859 nematodes per pot), 43:57 (1× level = 1,139 nematodes per pot, 2× level = 2,278 nematodes per dot), and 38:62 (1× level = 1,445 nematodes per pot, 2× level = 2,890 nematodes per pot) for 1992, 1993, and 1994, respectively.

[c] Grain sorghum host genotypes: DK 50 = De Kalb DK 50; P 8333 = Pioneer hybrid 8333.

Consistent fungus by nematode interactions that affected plant weights were detected in all three tests (Table 1). Examination of individual treatment means showed that these interactions were similar. The fungus by nematode interaction for root weight in 1993 is presented as an example (Fig. 1). When *M. phaseolina* was absent, an increase in nematode inoculum level resulted in a stepwise decrease in root weight. This pattern was similar when *M. phaseolina* was present at 10 CFU/g, but the differences were less. At 100 CFU/g, root weight did not change regardless of nematode inoculum level.

Fungus by hybrid interactions were detected, which influenced root weight in 1992 and both root and head weights in 1994 (Table 1). Examination of individual treatment means revealed a similar pattern for all interactions, and the data for root weight in 1992 are presented as an example (Fig. 2). Both levels of *M. phaseolina* in soil reduced root weight of P 8333 by about 50% but had no effect on DK 50.

Root colonization by M. phaseolina: Root colonization increased from 73% to 96% as *M. phaseolina* infestation level increased from 10 to 100 CFU/g soil (Table 2). In each test, low levels (4%) of root colonization by *M. phaseolina* were detected in control plants. Nematodes had minimal effect on root colonization by *M. phaseolina.* Colonization increased slightly at the 2× nematode inoculum level, but only in 1992. Hybrids did not differ in root colonization by *M. phaseolina.* A fungus by nematode interaction that influenced fungus root colonization in 1992 showed that nematodes enhanced colonization by *M. phaseolina,* but only at the 10-CFU/g soil level.

Population density of T. annulatus: In all three tests, reproductive value as well as total *T. annulatus* per microplot were reduced when roots were colonized by *M. phaseolina* (Table 3). For both parameters, stepwise reductions were detected as fungal infestation level increased. Reproduction by *T. annulatus* was greater at 1× than at 2× nematode inoculum level in each test, but total *T. annulatus* per microplot differed only in 1992. DK 50 was a better host for *T. annulatus* than was P 8333, as indicated by greater nematode populations and reproductive values in 1992 and 1994.

Fungus by nematode interactions influ-

TABLE 2. Colonization of grain sorghum roots by *Macrophomina phaseolina* as influenced by fungus infestation level, nematode inoculum level, and grain sorghum genotype in microplot studies.

Factor	Level	Root colonization (%) 1992	1993	1994
Fungus	0[a]	3 c	4 c	4 c
	10	74 b	72 b	73 b
	100	94 a	98 a	98 a
	P > *F*	**	**	**
Nematode	0[b]	56 a	56	58
	1×	55 a	59	57
	2×	60 b	59	59
	P > *F*	*	ns	ns
Host genotype	DK 50[c]	61	58	57
	P 8333	55	59	58
	P > *F*	ns	ns	ns
F × N	*P* > *F*	*	ns	ns
F × H	*P* > *F*	ns	ns	ns
N × H	*P* > *F*	ns	ns	ns
F × N × H	*P* > *F*	ns	ns	ns

Data are means of four replicates. Within each column, values followed by the same letter are not different ($P > 0.05$) according to a Least Significant Difference test.

[a] 0, 10, and 100 colony forming units (CFU)/g soil for *M. phaseolina*.

[b] Initial inoculum percentages for *Tylenchorhynchus annulutus* : *Criconemella xenoplax* were 52:48 (1× level = 929 nematodes per pot, 2× level = 1,859 nematodes per pot), 43:57 (1× level = 1,139 nematodes per pot, 2× level = 2,278 nematodes per pot), and 38:62 (1× level = 1,445 nematodes per pot, 2× level = 2,890 nematodes per pot) for 1992, 1993, and 1994, respectively.

[c] Grain sorghum host genotypes: DK 50 = De Kalb DK 50; P 8333 = Pioneer hybrid 8333.

enced reproductive values of *T. annulatus* in 1992 and 1993. Examination of individual treatment means indicated that the interactions were similar in these tests. Therefore, data for 1993 are presented as an example (Fig. 3). At 1× nematode inoculum level, increasing fungus infestation level from 0 to 100 CFU/g soil resulted in a stepwise reduction in nematode reproduction. At 2× inoculum level, however, *T. annulatus* reproduction was reduced similarly by *M. phaseolina* at either 10 or 100 CFU/g soil. A fungus by nematode interaction that influenced total *T. annulatus* per microplot was detected only in 1992. Population total was greater at 1× than at 2× nematode inoculum level, but only when *M. phaseolina* was absent.

Population density of C. xenoplax: Population density and reproductive value of *C. xenoplax* were reduced by *M. phaseolina* in 1993 and 1994 but not in 1992 (Table 3). Stepwise increases in *M. phaseolina* infestation level resulted in concomitant reductions in total *C. xenoplax* per microplot in 1993 and 1994 and in reproductive value in 1993. However, reproductive value decreased only at *M. phaseolina* level of 100 CFU/g soil in 1994. Total *C. xenoplax* per microplot was greater at 1× than at 2× nematode inoculum level in 1993 and 1994, but reproduction by this species was greater at 1× than at 2× level in all three tests.

Interactions between fungus and nematode were detected for reproductive values of *C. xenoplax* in 1992 and 1993. Examination of individual treatment means indicated that interactions were similar in both years. Therefore, data for 1993 are presented as an example (Fig. 4). At the 1× nematode inoculum level, increasing fungus infestation level in soil from 0 to 100 CFU/g soil resulted in a stepwise reduction in *C. xenoplax* reproductive value. At the 2× level, however, nematode reproduction was reduced similarly at both 10 and 100 CFU/g soil. Interactions between nematode and hybrid for both reproductive value and population total of *C. xenoplax* were detected in 1992 only. On DK 50, reproductive value for *C. xenoplax* was greater at 1× than at 2× nematode inoculum level, which resulted in a higher population total per microplot for this species at 1× inoculum level. Different results were obtained on P 8333. Reproductive value for this species did not differ regardless of nematode inoculum level, so that final population total per microplot at 2× level was about twice that at 1× level. A fungus by hybrid interaction for population total of *C. xenoplax* was detected in 1992 only. *Macrophomina phaseolina* had no effect on nematode population total on DK 50, but population total on P 8333 was higher at an *M. phaseolina* level of 10 CFU/g than at 100 CFU/g.

Combined nematode species: In all three tests, increasing infestation level of *M. phaseolina* from 0 to 100 CFU/g soil resulted in a stepwise reduction of the combined total for both nematode species per microplot (Table 3). Across tests, reductions were 18% and 32% at the fungus levels of 10 and 100

TABLE 3. Population counts of *Tylenchorhynchus annulatus, Criconemella xenoplax,* and combined totals as influenced by *Macrophomina phaseolina* infestation level, nematode inoculum level, and grain sorghum genotype in microplots[a].

		1992					1993					1994				
		T. annulatus per microplot		*C. xenoplax* per microplot		Combined total[b]	*T. annulatus* per microplot		*C. xenoplax* per microplot		Combined total	*T. annulatus* per microplot		*C. xenoplax* per microplot		Combined total
Factor	Level	(× 1,000)	R[c]	(× 1,000)	R	(× 1,000)	(× 1,000)	R	(× 1,000)	R	(× 1,000)	(× 1,000)	R	(× 1,000)	R	(× 1,000)
Fungus	0[d]	477 a	67 a	45	10	522 a	298 a	47 a	239 a	28 a	537 a	518 a	71 a	285 a	24 a	803 a
	10	406 b	52 b	60	11	466 b	180 b	28 b	160 b	19 b	340 b	474 b	67 b	272 b	23 a	746 b
	100	364 c	42 c	51	10	415 c	159 c	25 c	126 c	15 c	285 c	363 c	50 c	221 c	18 b	584 c
	P > *F*	**	**	ns	ns	**	**	**	**	**	**	**	**	**	*	**
Nematode	1×[e]	454 a	79 a	46 b	13	500 a	213	44 a	189 a	29 a	402 a	466	86 a	274 a	31 a	740 a
	2×	386 b	34 b	57 a	8	443 b	212	22 b	161 b	12 b	373 b	442	41 b	246 b	14 b	688 b
	P > *F*	**	**	*	ns	*	ns	**	**	**	**	ns	**	*	**	*
Host genotype	DK 50[f]	431 a	59 a	49	11	480 a	214	33	181	21	395 a	472 a	65 a	270	23	742 a
	P 8333	400 b	50 b	56	10	456 b	211	33	170	20	381 b	436 b	61 b	250	21	686 b
	P > *F*	*	**	ns	ns	*	ns	ns	ns	ns	ns	*	*	ns	ns	*
F × N	*P* > *F*	**	**	ns	*	**	ns	**	ns	**	ns	ns	ns	ns	ns	ns
F × H	*P* > *F*	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × H	*P* > *F*	ns	ns	*	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
F × N × H	*P* > *F*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Data are means of four replicates. Within each column, values followed by the same letter are not different ($P > 0.05$) according to a Least Significant Difference test.

[a] Microplots contained 15 kg soil.

[b] Total *T. annulatus* + *C. xenoplax* per microplot.

[c] R (reproductive value) = Pf/Pi, where Pf = final population density and Pi = initial inocolum density.

[d] 0, 10, and 100 colony forming units (CFU)/g soil for *M. phaseolina.*

[e] Initial inoculum percentages for *T. annulatus* : *C. xenopolax* were 52:48 (1× level = 929 nematodes per microplot, 2× level = 1,859 nematodes per microplot), 43:57 (1× level = 1,139 nematodes per microplot, 2× level = 2,278 nematodes per microplot), and 38:62 (1× level = 1,445 nematodes per microplot, 2× level = 2,890 nematodes per microplot) for 1992, 1993, and 1994, respectively.

[f] Grain sorghum host genotypes: DK 50 = DeKalb DK 50; P 8333 = Pioneer hybrid 8333.

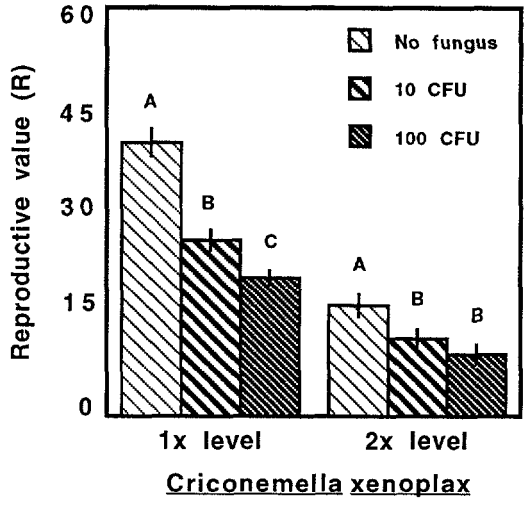

FIG. 4. Reproduction of *Criconemella xenoplax* in 1993 as influenced by nematode inoculum levels of 1× (1,139 nematodes) and 2× (2,278 nematodes) per microplot (43% *Tylenchorhynchus annulatus*, 57% *Criconemella xenoplax*) and by *Macrophomina phaseolina* at infestation levels of 10 and 100 colony forming units (CFU)/g soil. R = Pf/Pi, where Pf = final population density and Pi = initial inoculum density. Within each nematode inoculum level, letters above bars indicate means that differed significantly ($P \leq 0.05$) according to a Least Significant Difference test. Vertical lines delimit standard errors of means for four replicates.

CFU/g soil, respectively. The effect of nematode inoculum level was consistent in all three tests. Combined total for both nematode species per microplot was reduced as nematode inoculum level increased from 1× to 2×. In 1992 and 1994, the combined total for both nematode species was higher on DK 50 than on P 8333.

An interaction between fungus and nematode was detected for the combined total for both nematode species in 1992 only. At 1× nematode inoculum level, the combined total was reduced similarly at both the 10- and 100-CFU/g infestation levels for *M. phaseolina*. At 2× nematode inoculum level, however, the combined total was higher at the 10- than at the 100-CFU/g level. In 1992, an interaction between nematode and hybrid was detected for the combined total for both nematode species. On DK 50, total nematode numbers were higher at the 1× than at the 2× nematode inoculum level. On P 8333, however, nematode numbers were similar regardless of inoculum level.

DISCUSSION

Most reports indicate that grain sorghum yield losses are primarily related to lodging caused by *M. phaseolina* (Frederiksen, 1986; Hsi, 1956). Under severe lodging, yield losses of 23% to 65% on high-yielding grain sorghum hybrids are not unusual (Mughogho and Pande, 1983). Although lodging did not occur in the present study, *M. phaseolina* reduced head dry weight, an expression of yield potential, by as much as 52%. Our study also indicated that *T. annulatus* and *C. xenoplax* in combination reduced grain sorghum head weight as much as 20%.

Endoparasitic nematodes were reported to affect severity of charcoal rot disease (Powell, 1971). Root rot of French bean (*Phaseolus vulgaris* L.) caused by *M. phaseolina* was nearly twice as severe when roots were colonized by the root knot nematode (*Meloidogyne incognita* (Kofoid & White) Chitwood) (Al-Hazmi, 1985). The soybean cyst nematode (*Heterodera glycines* Ichonohe) enhanced severity of charcoal rot on soybean (*Glycine max* (L.) Merr.) cultivars resistant and susceptible to this nematode species (Todd et al., 1987). Species of cyst and root-knot nematodes are known to colonize vascular tissue and directly affect water relations in affected plants (Al-Hazmi, 1985; Melendez and Powell, 1967; Todd et al., 1987). In addition, physiological changes in nematode-infected root tissue, such as alteration of nutrient content or increase in production of growth factors, may provide a more favorable substrate for fungus development (Riedel, 1988). However, the two nematode species in this study primarily are ectoparasites and consequently are less damaging compared to cyst and root knot nematodes. This might explain why root colonization by *M. phaseolina* was enhanced by *T. annulatus* and *C. xenoplax* only in 1992 and only when the fungus was at the highest infestation level.

Several previous reports show combined effects of *M. phaseolina* and nematodes on host plants. Infestation of *Ligustrum japonicum* Thunb. by *M. phaseolina* and *Meloidogyne javanica* (Treub) Chitwood resulted in plant

weight reduction, leaf abscission, and twig dieback that were greater than when either pathogen occurred alone (Alfieri and Stokes, 1971). Similarly, the combined effect of *M. javanica* and *M. phaseolina* on chickpea (*Cicer arietinum* L.) caused greater reductions in fresh and dry weights of shoot and roots than that caused by either organism alone (Goel and Gupta, 1986). Effects of *Pratylenchus hexincisus* Taylor & Jenkins and *M. phaseolina* together on grain sorghum yield were greater than the effect of either pathogen alone (Norton, 1958). Growth of cowpea (*Vigna unguiculata* (L.) Walp.), however, was not affected by *M. phaseolina* and *Heterodera cajani* Koshy in any combination (Walia and Gupta, 1986), and no differences in plant weights among any *M. incognita* and *M. phaseolina* treatment combination were detected on French bean (Al-Hazmi, 1985).

In the present study, combined effects of *M. phaseolina* and nematode species were less than the sum of the effect of each pathogen alone. Such antagonistic interactions can be caused by a spatial occupation or physical alteration or destruction of feeding sites, or by a physical alteration of the host that decreases its suitability (Powell, 1971). *Macrophomina phaseolina* is a root-inhabiting fungus with little or no saprophytic growth in either soil or dead host cells (Collins et al., 1991; Mihail, 1992). *Tylenchorhynchus* commonly feeds on epidermal cells and root hairs (Claflin, 1983; Wyss, 1981). *Criconemella* prefers cortical cells deeper in the root and forms modified cortical food cells (Westcott and Hussey, 1991). The similarity in modes of parasitism of these three pathogens may affect the degree of competition between the species and, consequently, the antagonistic interaction detected in the present study.

Reproduction of plant-parasitic nematodes on grain sorghum and other crops was shown to be inhibited (Al-Hazmi, 1985; Sakhuja and Sethi, 1986), stimulated (Todd et al., 1987), or not affected (Norton, 1958; Carter, 1980) by *M. phaseolina.* Population densities of *Heterodera glycines* related positively to soybean root colonization by *M. phaseolina* (Todd et al., 1987). Simultaneous infestation of soil with *M. javanica* and *M. phaseolina* resulted in lower populations of the nematode in soil as well as reduced galling on roots of peanut (*Arachis hypogaea* L.) (Sakhuja and Sethi, 1986). An antagonistic effect of *M. phaseolina* on *M. incognita* reproduction also was reported (Al-Hazmi, 1985). Infection and reproduction by *M. incognita* on French bean were adversely affected by *M. phaseolina* when the fungus was introduced 2 weeks prior to the nematode. In the present study, reproduction by both *T. annulatus* and *C. xenoplax* generally was suppressed by *M. phaseolina* in all 3 years.

Because co-parasitism of grain sorghum roots by several plant-parasitic nematodes and *M. phaseolina* is common in nature, interactions between and among these pathogens may influence not only their reproduction but also their parasitic ability. Our study found that *T. annulatus* and *C. xenoplax* cause significant damage to grain sorghum. Antagonistic interactions between *M. phaseolina* and these nematodes were observed consistently. Evidence from the present study indicates that an important interaction exists between the two nematode species and *M. phaseolina,* and that the effect of these nematodes in addition to *M. phaseolina* may be of greater importance to sorghum production than the fungus alone. Future work will focus on the interrelationships between *T. annulatus* and *C. xenoplax* on grain sorghum.

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