THE IMPACT OF NEMATODES ON SUGARCANE CULTIVARS¹

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ABSTRACT

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The susceptibility of sugarcane cultivars to a nematode community comprised of *Mesocriconema xenoplax, Paratrichodorus minor*, and *Tylenchorhynchus annulatus* was evaluated in greenhouse and microplot experiments. In greenhouse tests, across years (1995 and 1996) and cultivars (CP 65-357, CP 70-321, LCP 82-89, HoCP 85-845, and LCP 86-454), plant height, shoot length, shoot and root dry weight, and the number of tillers per plant were suppressed by nematodes. Growth parameters for the LCP cultivars were most affected by the nematodes, and those of cultivars HoCP 85-845 and CP 65-357 were least affected. In microplots, across years (1995, 1996, and 1997) and cultivars (CP 70-321 and LCP 82-89), nematodes reduced shoot and root dry weights and numbers of tillers per plant. LCP 82-89 supported higher nematode community levels and sustained the greatest amount of root damage. The results suggest that the commonly occurring nematode community is adversely affecting sugarcane productivity in Louisiana.

Key words: Mesocriconema xenoplax, nematode, Paratrichodorus minor, ring nematode, Saccharum, stubbyroot nematode, stunt nematode, Tylenchorhynchus annulatus.

RESUMEN

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Se evaluó la susceptibilidad de cultivares de la caña de azúcar a la comunidad de nemátodos incluyendo *Mesocriconema xenoplax, Paratrichodorus minor* y *Tylenchorhynchus annulatus* en experimentos de invernadero y micro parcelas. En los experimentos de invernadero, entre años (1995 y 1996) y cultivares (CP 65-357, CP 70-321, LCP 82-89, HoCP 85-845, y LCP 86-454), la altura de las plantas, la longitud de los brotes, el peso seco de brotes y raíces, y el número de tallos por planta fueron suprimidos por los nemátodos. Los parámetros de crecimiento de los cultivares LCP fueron lo más afectados por los nemátodos, y los de los cultivares HoCP 85-845 y CP 65-357 fueron los que menos se afectaron. En las micro parcelas entre años (1995, 1996, y 1997) y cultivares (CP 70-321 y LCP 82-89), los nemátodos redujeron el peso seco de brotes y raíces y el número de tallos por planta. LCP 82-89 produjo niveles de comunidades de nemátodos más altos y sufrió la cantidad más alta de daño a las raíces. Los resultados sugieren que los nemátodos que ocurren normalmente afectan la producción de la caña de azúcar adversamente en Louisiana.

Palabras clave: Mesocriconema xenoplax, nemátodo, nemátodo del anillo, nemátodo del enanismo, Paratrichodorus minor, Saccharum, Tylenchorhynchus annulatus.

INTRODUCTION

Sugarcane, interspecific hybrids of *Saccharum* L., is a major crop that has been produced for over 200 years in Loui-

siana. Each year, approximately 180,000 hectares of sugarcane are harvested, producing a crop with a total value in excess of 350 million dollars (Anonymous, 2003).

The ring nematode, Mesocriconema xenoplax (Raski) Loof & de Grisse, the stunt nematode, **Tylenchorhynchus** annulatus (Cassidy) Golden, and the stubby-root nematode, Paratrichodorus minor (Colbran) Siddiqui, are commonly found in sugarcane soil in Louisiana (Bond et al., 1997, 2001). Both T. annulatus (Birchfield and Martin, 1956; Roman, 1968; Gargantiel and Davide, 1973) and P. minor (Apt and Koike, 1962a) are pathogenic to sugarcane and cause damage to roots that results in a stubby, stunted appearance with a lack of fine feeder roots. Such damage to the root system can cause significant suppression of growth (Birchfield and Martin 1956; Apt and Koike, 1962a). Nematodes reduce annual sugarcane yield by 4% nationally (Koenning et al. 1999), and 15% worldwide (Sasser and Freckman, 1987).

The effects of individual populations of several nematodes (Birchfield and Martin, 1956; Apt and Koike, 1962a; Roman, 1968; Gargantiel and Davide, 1973; Spaull and Cadet, 1990) on sugarcane have been investigated previously. However, information on the effects of a community of nematode populations on sugarcane is lacking in spite of their usual occurrence together in nature. Therefore, the objectives of this research were to evaluate: (i) the effects of a community of nematode species on sugarcane and (ii) the impact of sugarcane genotype on nematode reproduction.

MATERIALS AND METHODS

General Procedures

Plants were obtained by excising singlebud cuttings from the middle portion of sugarcane stalks. Cuttings were trimmed to 1-2-cm of internode tissue on either side of the node. Prior to planting, single node cuttings were selected for uniform size and immersed in hot (50°C) water for 50 minutes to eliminate fungal pathogens. Heattreated cuttings were germinated in fumigated (67% methyl bromide: 33% chloropicrin at the rate of 0.91 kg per 1.42 m³ soil) Convent silt loam soil (Coarse-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) in trays (Speedling, Inc., Sun City, FL) containing 7.5-cm by 7.5-cm cells. After 3 weeks, plants with uniform height and vigor were selected and transplanted.

Populations of Mesocriconema spp., Tylenchorhynchus spp., and Paratrichodorus spp. were derived from communities present in sugarcane field soil obtained from the Cinclare Sugarcane Plantation in West Baton Rouge Parish, Louisiana. Populations of M. xenoplax, T. annulatus, and P. minor were propagated in monoxenic culture in a greenhouse on the sugarcane cultivars CP 70-321 and LCP 82-89. Inoculum for all tests consisted of juveniles and adults extracted from cultures by wet-sieving through nested 250-µm-pore and 38-µm-pore sieves followed by sugar flotation and centrifugation (Jenkins, 1964). Five days after transplanting, soil was infested by pipetting suspensions containing known numbers of nematodes into depressions (1.5-cm-diam. by 3- and 6-cm deep) around the base of the sugarcane cutting. Controls received suspending fluid minus nematodes. Following infestation, depressions were filled with fumigated soil. At harvest, 3-g subsamples from each root system were placed on Baerman funnels for collection and enumeration of endoparasitic nematodes.

At 2-week intervals throughout the experiment, plant height, shoot length and tiller numbers were recorded. Plant height was obtained by measuring the distance from the soil line to the tip of the longest leaf. Shoot length was obtained by measuring the distance from the soil line to the highest ligule. Growth of shoot and root systems was determined by measuring dry weight of plant material at harvest.

Greenhouse

Experiments were conducted under conditions where air temperature ranged from 25-33°C. Three-week-old cuttings were transplanted to the center of 20-cmdiam. clay pots, each containing 4 kg of a soil mixture composed of three parts MeBr-fumigated Convent silt loam (Coarse-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) and one part steamed sand. Pots received 120 ml of 23-19-17 N-P-K fertilizer solution (RapidGro; Chevron, San Ramon, CA) every 14 days until harvest.

Five sugarcane cultivars commonly planted in Louisiana (CP 65-357, CP 70-321, LCP 82-89, HoCP 85-845, and CP 86-454) were evaluated in two experiments. Planting and harvest dates were 21 February and 1 June 1995 and 24 November and 11 March 1996. Treatments were arranged in a randomized complete block design with a factorial treatment structure. Treatments included three levels of nematodes $(0, 1\times, \text{ or } 4\times)$ and five cultivars in all possible combinations for a total of 15 treatments with six replications. Nematode inoculum at the 1× level contained 925 (32% T. annulatus, 61% M. xenoplax, and 7% P. minor) and 911 (43% T. annulatus, 46% M. xenoplax, and 11% P. minor) nematodes per pot in 1995 and 1996, respectively. Infestation levels and community composition were selected to reflect nematode community structure and density commonly encountered in sugarcane fields in Louisiana at planting (Bond et al., 1997).

At harvest, each pot and its entire contents were placed into a 19-liter capacity plastic bucket containing 6 liters of water. After soaking for 5 minutes, the empty pot was removed and rinsed with an additional 2 liters of water. The root system then was agitated to dislodge soil and an additional 2 liters of water were employed to rinse the root system, bringing the final soil:water slurry to a volume of 10 liters. The slurry was stirred vigorously for 10 seconds and a 500-ml subsample was removed and extracted as described previously. Immature and mature life-stages of each nematode species were enumerated at $50\times$ using an Olympus CK-2 inverted microscope. Final population density per pot (Pf) and reproductive values (R, where R = Pf/Pi and Pf = the final population level and Pi = infestation level) were determined for each nematode species and for the total nematode community.

Microplots

Each microplot consisted of a 40.6-cmdiam. clay pot that contained 35 kg MeBrtreated Commerce silt loam soil (Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) obtained from the Sugar Research Station of the Louisiana Agricultural Experiment Station at St. Gabriel, LA. Microplots received 3.5 g of 33-0-0 (N-P-K) every 4 weeks. Each microplot was placed into a preformed depression in the soil with only the rim of the pot exposed. The 42 microplots were spaced 1 m apart in a five-by-nine pattern. The entire area was covered with a 14 m-long by 6.5 m-wide aluminum quonset hut frame that was open at both ends and covered with 4-ml polyethylene plastic. Reflective shade cloth was placed over the plastic cover so that soil and air temperatures in microplots were within 2-3°C of those in the field. Light intensity under the reflective cloth was measured as 512 $\mu E \cdot s^{-1} \cdot m^{-2}$, which is approximately 68% of full sunlight.

Planting and harvest dates were 4 April and 10 October, 12 April and 27 October, 7 May and 17 November for 1995, 1996, and 1997, respectively. Treatments were arranged in a randomized complete block design with a factorial treatment structure. Treatments included three levels of nematodes (0, 1×, or 10×) and two cultivars (CP 70-321 and LCP 82-89) in all possible combinations for a total of six treatments with seven replications. Nematode inoculum at the 1× level contained 1,256 (30% *T. annulatus*, 30% *M. xenoplax*, and 40% *P. minor*), 1,247 (32% *T. annulatus*, 29% *M. xenoplax*, and 39% *P. minor*) and 1,200 (44% *T. annulatus*, 50% *M. xenoplax*, and 6% *P. minor*) nematodes per pot for 1995, 1996, and 1997, respectively.

At harvest, six soil cores (2.5-cm diam. by 30-cm deep) were collected from each microplot, bulked, and nematodes were extracted from a 150 g composite subsample. Nematodes were counted and reproductive values determined as in greenhouse experiments.

Statistical Analysis

Pathogen population data were transformed log (x+1) and were subjected to analysis of variance using the General Linear Models procedure of the Statistical Analysis System version 6.12 for Macintosh (SAS Institute, Inc., Cary, NC). Non-transformed means are presented for clarity. Unless otherwise stated, all experiments were repeated at least once and differences noted are significant at $P \le 0.05$. For treatments with three or more levels, means were separated using Tukey's HSD test. Interactions that were significant in two or more years are presented, whereas those that occurred in only one year are described in the text.

RESULTS

Greenhouse

The absence of year by treatment interactions allowed data for the two greenhouse tests to be combined. Under fullseason field conditions, there are differences in the growth rates of most sugarcane cultivars. However, statistical analysis of growth data for non-infested controls of each cultivar showed there were no significant differences in growth habit among the cultivars in our greenhouse-based, short duration trials (data not presented). All five cultivars were susceptible to the nematodes examined (Table 1). At both low and high infestation levels, plant height, stalk length, shoot and root weights, and the number of tillers per plant were reduced below those of controls. Stalk length and shoot growth were suppressed more as nematode infestation level increased. At the highest infestation level, shoot and root growth were reduced by 28% and 56%, respectively. The nematodes were most damaging to the sugarcane cultivars LCP 82-89 and LCP 86-454. Shoot growth of LCP 86-454, LCP 82-89, and CP 70-321 was suppressed more than that of CP 65-357 and HoCP 85-845. Shoot lengths for LCP 82-89, CP 70-321, and LCP 86-454 were shorter as a result of nematode damage. Differences in shoot and root growth were observed among the five cultivars at harvest. Shoot growth and numbers of tillers per plant for HoCP 85-845 were affected least by nematodes.

There was abundant nematode reproduction on all cultivars. Reproductive values ranged from 10 for M. xenoplax at the high infestation level to 305 for P. minor at the low infestation level (Table 2). Final density for each of the three individual nematode populations and the community reflected greater reproduction at the low infestation level. Population totals per pot ranged from a low of 13,200 M. xenoplax to a high of 19,200 P. minor. Similarly at the high infestation level, population totals ranged from 17,400 T. annulatus per pot to 18,800 M. xenoplax. Totals for the nematode community at the conclusion of the trial (approximately four months after inocula-

		Heigh	t (cm) ^x	Dry wei			
Factor	Level	Plant	Shoot	Shoot	Root	 Tillers per plant 	
Nematode ^z	0	147 a	36 a	32 a	34 a	3 a	
	$1 \times$	$138 \mathrm{b}$	32 b	26 b	17 b	2 b	
	$4\times$	$135 \mathrm{b}$	30 с	23 с	15 b	2 b	
	P > F	***	***	***	***	**	
Cultivar	CP 65-357	155 a	35 b	27 b	25 a	3 a	
	CP 70-321	$140 \mathrm{b}$	29 cd	25 bc	21 ab	2 b	
	LCP 82-89	131 с	27 d	23 с	20 b	2 b	
	HoCP 85-845	153 a	39 a	34 a	24 a	3 a	
	LCP 86-454	123 d	32 c	24 bc	19 b	2 b	
	P > F	***	***	***	ns	**	
N×C	P > F	ns	ns	ns	ns	ns	

Table 1. Sugarcane growth parameters as influenced by nematode infestation levels and sugarcane genotype in greenhouse experiments during 1995 and 1996.

Data are means of six replicates in each of 2 years. For each factor and column, ** and *** indicate differences at $P \le 0.01$, and 0.001, respectively; ns indicates that means are not significantly different. For treatments with three or more levels, means followed by the same letter are not different ($P \le 0.05$), according to Tukey's HSD test. *Plant height is the distance from the soil line to the tip of the longest leaf; shoot height is the distance from the soil line to the highest leaf ligule.

Weight after drying for 2 weeks at 70°C.

¹Infestation levels for 1995 and 1996 were: (1×) 925 and 911 nematodes per pot, respectively. Ratios of *T. annulatus:M. xenoplax:P. minor* in inocula were 32:61:7 in 1995 and 43:46:11 in 1996.

tion) averaged 55,100 individuals from the low and 54,800 from the high infestation levels. Generally, CP 65-357 was the most suitable host. Final community density for CP 65-357 averaged 75,900 nematodes per pot while the next most suitable host, HoCP 85-845, averaged 30% less. Only for *M. xenoplax* were nematode by cultivar interactions detected which influenced population density and reproduction.

Microplots

The absence of year by treatment interactions allowed data for all microplot experiments to be combined. There were no significant differences in any of the plant growth parameters between the noninfested controls for each variety (data not presented). Therefore, differences observed are attributed to nematode pathogenicity. As in the greenhouse experiments, lower shoot and root growth of inoculated plants of CP 70-321 and LCP 82-89 indicated susceptibility to the nematodes (Table 3). Reductions in plant and stalk height were non-significant for both. Shoot and root growth were suppressed by nematodes similarly at both infestation levels. At the 10× infestation level, shoot and root growth were suppressed by 11% and 21%, respectively. The number of tillers per plant was limited only at the 10× nematode infestation level. Susceptibility to nematodes difTable 2. Population densities and reproductive values for *Tylenchorhynchus annulatus, Mesocriconema xenoplax,* and *Paratrichodorus minor,* and community totals as influenced by infestation level and sugarcane genotype in greenhouse experiments during 1995 and 1996.

Factor	Level	T. annulatus		M. xenoplax		P. minor		Community	
		Pf ^w	R×	Pf	R	Pf	R	Pf	R
Nematode ^{yz}	1×	21	61	13	26	21	305	55	60
	$4 \times$	17	12	19	10	19	64	55	15
	P > F	ns	***	***	***	ns	***	ns	***
Cultivar	CP 65-357	21 a	37 ab	29 a	32 a	26 a	225	76 a	48 a
	CP 70-321	13 b	26 b	16 b	20 b	16 b	152	$45 \mathrm{b}$	32 b
	LCP 82-89	20 ab	37 ab	11 b	13 с	22 ab	212	53 b	37 ab
	HoCP 85-845	22 a	42 a	12 b	13 с	19 ab	186	53 b	37 ab
	LCP 86-454	20 ab	40 ab	13 b	12 c	15 b	150	48 b	33 b
	P > F	*	*	***	***	***	ns	***	**
$N \times C$	P > F	ns	ns	*	***	ns	ns	ns	ns

Data are means of six replicates in each of 2 years. For each factor and column, *, ** and *** indicate differences at $P \le 0.05$, 0.01, and 0.001, respectively; ns indicates that means are not significantly different. For treatments with three or more levels, means followed by the same letter are not different ($P \le 0.05$), according to Tukey's HSD test. *Pf = final population density in 1000s per 20-cm-diam. clay pot containing 4 kg of MeBr treated soil mixture.

 ^{x}R (reproductive value) = Pf/Pi, where Pf = final population density and Pi = infestation level.

¹Infestation levels for 1995 and 1996 were: (1×) 925 and 911 nematodes per pot, respectively. Ratios of *T. annulatus:M. xenoplax:P. minor* in inocula were 32:61:7 in 1995 and 43:46:11 in 1996.

^zNematodes were not recovered from controls.

fered between the two cultivars with greater suppression of root growth and more tillers per plant for LCP 82-89 than for CP 70-321.

As in greenhouse experiments, both cultivars were good hosts for each of the three nematode species under microplot conditions (Table 4). In greenhouse experiments, the final community was comprised of approximately equivalent numbers of each of the three nematode species. However, in the microplot environment there was an overwhelming dominance by *T. annulatus* which comprised 93 to 96% of the final communities. Of the two sugarcane cultivars, LCP 82-89 was a better host supporting an average of 500,000 *T. annulatus* per microplot com-

pared to 340,000 on CP 70-321. Nematode by cultivar interactions were detected which influenced reproductive values for both *T. annulatus* and the nematode community. These interactions are accounted for in that means for each of these two parameters were greater at the low infestation level on LCP 82-89 than on CP 70-321, but not at the high infestation level.

DISCUSSION

During the course of this study, five individual experiments were conducted evaluating the damage potential of a combination of nematodes typically found in Louisiana sugarcane fields. None of the

		Heigh	t (cm) ^x	Dry wei			
Factor	Level	Plant	Stalk	Shoot	Root	– Tillers per plant	
Nematode ^z	0	222	93.0	688 a	435 a	12 a	
	$1 \times$	216	87.0	$617 \mathrm{b}$	355 b	11 ab	
	$10 \times$	220	89.0	609 b	344 b	10 b	
	P > F	ns	ns	*	**	*	
Cultivar	CP 70-321	220	88.8	652	398	10	
	LCP 82-89	218	90.4	623	352	12	
	P > F	ns	ns	ns	*	*	
$N \times C$	$\mathbf{P} > \mathbf{F}$	ns	ns	ns	ns	ns	

Table 3. Sugarcane growth parameters as influenced by nematode infestation levels and sugarcane genotype in microplot experiments during 1995-97.

Data are means of seven replications in each of 3 years. For each factor and column, * and ** indicate differences at $P \le 0.05$ and 0.01, respectively; ns indicates that means are not significantly different. For treatments with three levels, means followed by the same letter are not different ($P \le 0.05$), according to Tukey's HSD test. *Plant height is the distance from the soil line to the tip of the longest leaf; stalk length is the distance from the soil line to the highest leaf ligule.

Weight after drying for 2 weeks at 70°C.

¹Infestation levels for 1995, 1996, and 1997 were: (1×) 1,256, 1,247, and 1,200 nematodes per pot, respectively. Ratios of *T. annulatus:M. xenoplax:P. minor* in inocula were 30:30:40 in 1995, 32:29:39 in 1996, and 44:50:6 in 1997.

three species are known to be highly pathogenic alone, but in all cases, the nematode community was shown to cause significant injury to sugarcane. In greenhouse tests, all five cultivars were damaged significantly by nematodes. The degree of susceptibility differed among the five cultivars with LCP 82-89 and LCP 86-454 being most sensitive.

Damage caused by nematodes was more severe in the greenhouse environment compared to that observed in microplots. In the greenhouse, shoot and root growth were suppressed by almost twice that recorded in microplots. Suppression in shoot growth in this study is similar to that observed in field studies when a similar nematode community was not controlled by nematicides (Bond *et al.*, 2000). Differences in the magnitude of damage and the nematode community structure between the two environments were probably most closely related to differences in soil type and growing conditions. The soil used in the microplot tests was a Commerce silt loam, which is typical for sugarcane production. Soil used in the greenhouse environment was three parts Convent silt loam and one part sand, a mixture which optimizes nematode reproduction. The latter soil type favors reproduction of all three nematodes. However, trichodorids (Winfield and Cooke, 1975; Hall and Irey, 1990) are more prevalent in sandier soils than finer texture soils, and Tylenchorhynchus spp. generally are numerous in loam and clay soils (Hu et al., 1968; Hall and Irey, 1990). Mesocriconema spp. generally are widespread regardless of soil type (Hall and Irey, 1990).

Table 4. Population densities and reproductive values for *Tylenchorhynchus annulatus*, *Mesocriconema xenoplax*, *Paratrichodorus minor*, and community totals as influenced by infestation level and sugarcane genotype in microplot experiments during 1995-97.

Factor		T. annulatus		M. xenoplax		P. minor		Community	
	Level	$\mathbf{P}\mathbf{f}^w$	R×	Pf	R	Pf	R	Pf	R
Nematode ^{yz}	1×	398	919	8	15	10	71	412	343
	$10 \times$	443	103	15	3	13	9	470	38
	P > F	ns	***	**	***	ns	***	ns	***
Cultivar	CP70-321	340	389	13	10	9	29	360	146
	LCP82-89	500	634	8	8	13	51	522	235
	P > F	**	***	ns	ns	ns	ns	**	***
$N \times C$	P > F	ns	***	ns	ns	ns	ns	ns	***

Data are means of seven replications in each of 3 years. For each column and factor, ** and *** indicate differences at $P \le 0.01$ and 0.001, respectively; ns indicates that means are not significantly different.

"Pf = final population density in 1000s per 40.6-cm-diam. clay pot containing 35 kg MeBr treated soil.

R (reproductive value) = Pf/Pi, where Pf = final population density and Pi = infestation level.

*Infestation levels for 1995, 1996, and 1997 were: $(1\times)$ 1,256, 1,247; and 1,200 nematodes per pot, respectively. Ratios of *T. annulatus:M. xenoplax:P. minor* in inocula were 30:30:40 in 1995, 32:29:39 in 1996, and 44:50:6 in 1997. *Nematodes were not recovered from controls.

Results from microplot experiments suggest that the pathogenic effect on sugarcane growth due to *T. annulatus* alone is not as great as that caused by the complete nematode community. When the three nematode species that comprise most of the naturally occurring community in sugarcane soils were present at approximately equal levels, their combined effect on plant growth was more severe. In a separate greenhouse experiment, shoot growth of plants grown in soil infested with *T. annulatus* did not differ from those of non-inoculated controls (Bond, unpubl.).

In nematological research, there is not always a direct correlation between host suitability and host sensitivity (Rhode, 1965). In our greenhouse tests, greater sensitivity to nematode damage did not reflect greater host suitability. The cultivar CP 65-357 supported higher community levels than all other cultivars. However, it was this cultivar that was one of the least affected by the nematodes. In the microplot environment, the cultivar which supported the highest nematode community level sustained the greatest amount of root damage.

As evidenced by all experiments represented herein, nematodes are significant constraints to the production of sugarcane in Louisiana. Previously, it has been assumed that the 14-year duration breeding program in Louisiana would eliminate cultivars highly susceptible to unknown soil factors, biotic and abiotic. This projection may not be the case, especially with nematodes. Greenhouse and microplot tests highlight the need to incorporate nematode screening activities into sugarcane cultivar selection in Louisiana. Most of the current cultivars used in sugarcane production in Louisiana are susceptible to nema-

todes. Currently, the best nematode management strategy available is to incorporate a fallow season in the crop cycle. Even though this tactic is currently employed by many producers, it is not uncommon to find fields in the fallow cycle that are infested with numerous weed species. Many of these weed species are excellent hosts for the nematodes and provide a means of bridging the fallow period during spring and summer. Research conducted by Birchfield and Martin (1956) showed that many weeds in sugarcane fields, especially johnsongrass (Sorghum halepense), are excellent hosts for nematodes. The results of this study indicate that this nematode community is an important sugarcane production constraint and management strategies would benefit producers in Louisiana.

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