SUGARCANE GROWTH AS INFLUENCED BY NEMATODES AND *PYTHIUM ARRHENOMANES*[1]

J. P. Bond[2], E. C. McGawley[3], and J. W. Hoy[3]

[1]A portion of the first author's Ph.D. dissertation. [2]Assistant Professor, Department of Plant, Soil and Agricultural Systems, Southern Illinois University, Carbondale, IL 62901. [3]Professors, Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

ABSTRACT

Bond, J. P., E. C. McGawley, and J. W. Hoy. 2004. Sugarcane growth as influenced by nematodes and *Pythium arrhenomanes*. Nematropica 34:245-256.

The single and combined effects of nematodes and the sugarcane root-rot pathogen, *Pythium arrhenomanes*, were evaluated in greenhouse trials conducted in 1996, 1997, and 1998. Individually, *P. arrhenomanes* and nematodes both reduced shoot and root growth. A community comprised of *Mesocriconema xenoplax*, *Paratrichodorus minor* and *Tylenchorhynchus annulatus* consistently reduced root growth across two infestation levels. Shoot growth was suppressed only at the highest infestation level. Shoot and root growth were consistently suppressed by both low and high infestation levels of *P. arrhenomanes*. Significant interactions occurred between nematodes and *P. arrhenomanes*. The fungus reduced nematode populations resulting in a less than additive response for plant growth suppression. Root colonization by *P. arrhenomanes* was influenced by nematode infestation in only one year. Temperature had an impact on the pathogens. Reproduction by *T. annulatus* was higher at 30°C than 20°C. The inverse of was observed for reproduction of *M. xenoplax*, *P. minor* and root colonization by *P. arrhenomanes*. The relative importance and interactions among these soil borne pathogens affecting sugarcane will depend on weather and soil conditions.

Key words: interaction, *Mesocriconema xenoplax*, nematode, *Paratrichodorus minor*, *Pythium arrhenomanes*, Pythium root rot, ring nematode, *Saccharum*, stubby-root nematode, stunt nematode, *Tylenchorhynchus annulatus*.

RESUMEN

Bond, J. P., E. C. McGawley, and J. W. Hoy. 2004. Crecimento de caña de azúcar afectado por nemátodos y *Pythium arrhenomanes*. Nematropica 34:245-256.

Se evaluaron los efectos individuales y combinados de nemátodos y el patógeno del pudrimiento de las raíces de la caña de azúcar, *Pythium arrhenomanes*, en experimentos de invernadero en 1996, 1997 y 1998. Individualmente, *P. arrhenomanes* y nemátodos redujeron el crecimiento de brotes y raíces. Una comunidad de nemátodos que incluyó *Mesocriconema xenoplax*, *Paratrichodorus minor* y *Tylenchorhynchus annulatus* redujo el crecimiento de las raíces en dos niveles de infestación. Sin embargo, crecimiento de brotes fue suprimido solamente a un nivel alto de infestación. El crecimiento de brotes y raíces fue consistentemente suprimido por niveles altos y bajos de infestación de *P. arrhenomanes*. Hubo interacción significante entre los nemátodos y *P. arrhenomanes*. El hongo redujo las poblaciones de nemátodos, resultando en una respuesta menos aditiva para la supresión del crecimiento de la planta. Colonización de las raíces por *P. arrhenomanes* fue afectada por la infestación de nemátodos solamente en un año. La temperatura también afectó a los patógenos. La reproducción de *T. annulatus* era más alta a 30°C que a 20°C. Lo opuesto fue observado en la reproducción de *M. xenoplax*, *P. minor* y la colonización de las raíces por *P. arrhenomanes*. La importancia relativa y las interacciones entre los patógenos del suelo que afectan a la caña de azúcar dependen del clima y de las condiciones en el suelo.

Palabras clave: interacción, *Mesocriconema xenoplax*, nemátodo, nemátodo del anillo, nemátodo del enanismo, *Paratrichodorus minor*, pudrimiento de las raíces Pythium, *Pythium arrhenomanes*, *Saccharum*, *Tylenchorhynchus annulatus*.

INTRODUCTION

Sugarcane, interspecific hybrids of *Saccharum* L., is the highest value row crop produced in Louisiana. Annually, approximately 180,000 hectares of sugarcane are harvested with a total value in excess of 350 million dollars (Anonymous, 2003). Louisiana sugarcane production consists of a plant cane crop that is followed with 2-4 subsequent ratoon crops. The number of ratoon crops is limited when compared to production in the tropics due to a multitude of factors including diseases and environmental constraints. The production area in Louisiana is at the northern-most limit for sugarcane cultivation.

Many sugarcane fields in Louisiana are infested with the ring nematode, *Mesocriconema xenoplax* (Raski) Loof & de Grisse, the stubby-root nematode, *Paratrichodorus minor* (Colbran) Siddiqui, and the stunt nematode, *Tylenchorhynchus annulatus* (Cassidy) Golden. These nematodes are often found as co-inhabitants in the sugarcane rhizosphere (Bond *et al.*, 1997, 2001), and they have been shown to be pathogenic to sugarcane (Apt and Koike, 1962a; Birchfield and Martin, 1956; Roman, 1968; Gargantiel and Davide, 1973). Nematodes have been estimated to reduce annual yield of sugarcane by 4% in Hawaii, Florida, Louisiana and Texas (Koenning *et al.* 1999).

In Louisiana, the causal agent of sugarcane root-rot is *Pythium arrhenomanes* Drechs. (Rands, 1929; Hoy and Schneider, 1988a). Following infection, mycelium of the pathogen grows inter- and intra-cellularly in young root tissue. Distinct aboveground symptoms of sugarcane root rot usually are not observed. Damage to the root system can result in reduced tillering and stalk weight. Symptoms of root damage include reddish-brown tissue at the infection zone, root tips with flaccid and water-soaked appearances, and lesions on the primary roots. Infected primary roots often exhibit reddish-black discolorations and may have rotted root tips. Lateral roots, which are critical for water and nutrient uptake, are destroyed by *P. arrhenomanes*. Disease severity is intensified by moderate temperature and wet soil, both of which favor growth and sporulation of the pathogen (Flor, 1930; Rands and Dopp, 1938; Hoy and Schneider, 1988a). Yield reductions caused by *P. arrhenomanes* can be a high as 20% (Hoy and Schneider, 1988b).

The individual effects of *P. arrhenomanes* (Rands, 1961; Hoy and Schneider, 1988a, 1988b; Croft and Magarey, 1984) and 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 combined effects of nematodes and *Pythium* on sugarcane is inadequate, in spite of their occurrence together in nature. Therefore, the objectives of this research were to evaluate: (i) the effects of a community of nematode species and *P. arrhenomanes* on sugarcane and (ii) the interrelationship between nematodes and *P. arrhenomanes*.

MATERIALS AND METHODS

The interrelationship between the nematodes and *Pythium arrhenomanes* was evaluated on the cultivar LCP 82-89 in three greenhouse trials. Plants of LCP 82-

89 were derived from cuttings of sugarcane stalks that consisted of 1-2-cm of internode tissue on either side of the node. Single node cuttings were selected for uniformity and subjected to hot water (50°C) for 50 minutes to eliminate fungal pathogens. Cuttings were germinated in fumigated (67% methyl bromide: 33% chloropicrin at the rate of 0.91 kg per 1.42 m^3 soil) Convent silt loam soil (Aeric Fluvaquent, coarse-silty, mixed, nonacid, thermic) in trays (Speedling, Inc., Sun City, FL) containing 7.5-cm by 7.5-cm cells. After 3 weeks, plants were selected for uniform height and vigor and transplanted.

Pythium arrhenomanes isolate ATCC 96526 was obtained from the collection of J.W. Hoy and used for all experiments. Agar discs (3 mm) containing *P. arrhenomanes* were revived from sterile water storage by plating on V8 medium (200 ml of V8 vegetable juice, 2 g of $CaCO_3$, 17 g of Bacto agar, 800 ml of distilled water (dH_2O)). Inoculum was produced following a method described by Mircetich and Matheron (1976). Canning jars (473 ml) that contained 450 ml of medium grade vermiculite, 300 ml of V8 vegetable juice broth (200 ml of V8 juice, 800 ml dH_2O, and 2 g $CaCO_3$), and 20 ml of oat seeds were autoclaved on 2 consecutive days. Four agar discs containing *P. arrhenomanes* were added to each jar and incubated 4 weeks at room temperature (24-26°C). Prior to incorporation into soil, the mixture was placed in cheese-cloth and thoroughly washed to remove excess nutrients. Inoculum from all jars was bulked, mixed, and added to the soil at the appropriate levels.

Populations of *M. xenoplax*, *T. annulatus*, and *P. minor* were obtained from soil of nematode-infested production fields on the Cinclare Sugarcane Plantation in West Baton Rouge Parish. These populations were maintained in monoxenic culture using the sugarcane cultivars CP 70-321 and LCP 82-89. Inoculum was prepared by extracting juveniles and adults 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 the cuttings were transplanted, soil was infested by pipetting suspensions of the nematodes into four depressions (1.5-cm-diam. by 3- and 6-cm deep) approximately 4 cm from the base of the cutting. Controls received suspending fluid minus nematodes. Depressions were filled with fumigated soil following infestation.

Planting and harvest dates for 1996, 1997, and 1998 were 8 January and 16 April, 1 March and 10 June, and 9 May and 7 July, respectively. Each trial was arranged in a randomized complete block design with a factorial treatment structure. Treatments included three levels of nematodes (0, 1×, or 10×) and three levels of *Pythium* (0, 22, or 220 g of inoculum mixture) in all possible combinations for a total of nine treatments with six replications. Prior research indicates that 220 g of inoculum mixture will result in sufficient infection and disease for variety evaluations and for chemical trials (Hoy, unpublished). Therefore, this level and one decreased by 10 fold were selected to insure disease but not overwhelm the system. To maintain the same soil consistency in all pots, all treatments received similar levels of growth medium without *P. arrhenomanes*. The inoculum was mixed thoroughly with three parts steamed (100°C for 12 hours) Commerce silt loam soil (Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) and one part steamed sand and placed in 20-cm-diam. clay pots. Nematode inoculum at the 1× level contained 491 (35% *T. annulatus*, 58% *M. xenoplax*, 6% *P. minor*), 495 (28% *T. annulatus*, 69% *M. xenoplax*, and 3% *P. minor*), and 495 (42% *T. annulatus*, 46% *M. xenoplax*, and 12% *P. minor*) nema-

todes per pot in 1996, 1997, and 1998, respectively. In 1998, temperature was included as a main effect in addition to the levels of *P. arrhenomanes* and nematodes previously described. Two temperatures (20 and 30°C) were selected to simulate spring and summer conditions, respectively. This $3 \times 3 \times 2$ factorial treatment arrangement was replicated six times. Nematodes were extracted and enumerated and plant dry weights were determined as described previously (Bond *et al.*, 2004).

Root colonization by *P. arrhenomanes* was determined by evaluating a total of 180 cm of root length for each treatment. Six, 5-cm root segments were selected randomly from each root system. Root pieces for each treatment were combined and placed into a 250-ml Erlenmeyer flask and agitated in dH_2O for 12 hours. Roots then were rinsed, blotted, and placed in 100 × 15-mm plastic petri plates. Three root segments per plate were covered with molten pimaricin-vancomycin-pentachloronitrobenzene-spectinomycin (PVPS) medium (Dissanayake *et al.*, 1998), a modified *Pythium*-selective medium (Mircetich and Matheron, 1976). To prepare PVPS, 10 g each of Difco cornmeal agar and Bacto agar were autoclaved in 1 liter of dH_2O. The medium was allowed to cool (40-45°C) and was amended with 300 mg of vancomycin dissolved in 10 ml of sterile dH_2O, 300 mg of spectinomycin dissolved in 10 ml of sterile dH_2O, 0.4 ml of pimaricin (10 mg a.i./liter), and 15 mg of pentachloronitrobenzene dissolved in 2 ml of 95% ethyl alcohol. Roots were covered with the medium and incubated at room temperature (24-26°C). After 24 and 48 hours, quantitative estimates of root segments from which *Pythium* growth emanated were recorded. A subset of four *Pythium* colonies were removed from the leading edge of randomly selected colonies from each plate (eight isolates for each plant) and transferred to V8 medium and incubated for 24 hours. After incubation, five, 3-mm-diam agar disks were transferred to 60 × 15-mm petri plates, covered with 5-ml filter-sterilized soil extract (10 g of soil/liter of dH_2O, agitated for 24 hours), and incubated at 20-24°C. Sexual and asexual structures were produced within 48 hours. Isolates were verified as *P. arrhenomanes* by comparing reproductive structures to species descriptions in a monograph on the genus *Pythium* (Van der Plaats-Niterink, 1981).

Statistical Analysis

Nematode population data (log (x+1)) and Pythium colonization data (square root) were transformed and 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, differences noted are significant at $P \leq 0.05$. 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

Trial by treatment interactions occurred; therefore, data for each trial were analyzed separately. In each of the three trials, nematodes limited sugarcane plant growth (Table 1). In 1996, nematodes suppressed shoot growth at the higher infestation level and root growth at both infestation levels. In 1997, nematodes suppressed shoot and root growth only at the high infestation level. In 1998, growth of both shoots and roots were limited by nematodes at both infestation levels. Shoot growth was suppressed by an average of

Table 1. Sugarcane dry weights as influenced by nematodes and *Pythium arrhenomanes* infestation levels and temperature in greenhouse experiments during 1996-98.

		Dry weight (g)					
		1996		1997		1998	
Factor	Level	Shoot	Root	Shoot	Root	Shoot	Root
Nematode[y]	0	19 a	18 a	37 a	30 a	25 a	30 a
	1×	17 ab	14 b	32 ab	27 ab	22 b	23 b
	10×	13 b	13 b	27 b	23 b	21 b	18 b
	P > F	**	**	*	*	**	***
Pythium[z]	0	31 a	34 a	38	33 a	32 a	42 a
	22	8 b	6 b	29	25 b	16 c	15 b
	220	9 b	6 b	30	24 b	19 b	15 b
	P > F	***	***	ns	***	***	***
Temp.	20°C	—	—	—	—	11	23
	30°C	—	—	—	—	33	25
	P > F	—	—	—	—	***	ns
N × P	P > F	**	***	ns	***	ns	**
N × T	P > F	—	—	—	—	ns	ns
P × T	P > F	—	—	—	—	*	*
N × P × T	P > F	—	—	—	—	ns	ns

Data are means of six replications. For each factor and column, *, **, and *** indicate differences at $P \leq 0.05$, 0.01, 0.001 respectively; ns indicates that means are not significantly different. For treatments with three levels, means followed by the same letter are not different ($P \leq 0.05$), according to Tukey's HSD test.

[y]Infestation levels for 1996, 1997, and 1998 were: (1×) 491, 495, and 495 nematodes per pot, respectively. Ratios of *T. annulatus*:*M. xenoplax*:*P. minor* in inocula were 35:58:6 in 1996, 28:69:3 in 1997, and 42:46:12 in 1998.

[z]0, 22, or 220 g of inoculum mixture containing *P. arrhenomanes*.

25% at the highest infestation level. Across all three trials of this experiment, root growth was limited by an average of 22% and 30%, respectively, at low and high nematode levels.

Both levels of *P. arrhenomanes* suppressed shoot and root growth in 1996 and 1998 (Table 1). Only root growth was affected in 1997. In 1996 and 1998, suppression of shoot growth averaged 47% and 56% at low and high *Pythium* levels, respectively. Across the three trials, suppression in root growth was 48% and 58% at low and high *Pythium* levels, respectively.

Interactions between nematodes and *Pythium* occurred in each year and consistently impacted root growth. Examination of individual treatment means for root weights in each year showed that the interactions were similar. The nematode by *Pythium* interaction for root weight in 1997 is presented as an example (Fig. 1). In the absence of *Pythium*, nematodes limited root growth at both infestation levels.

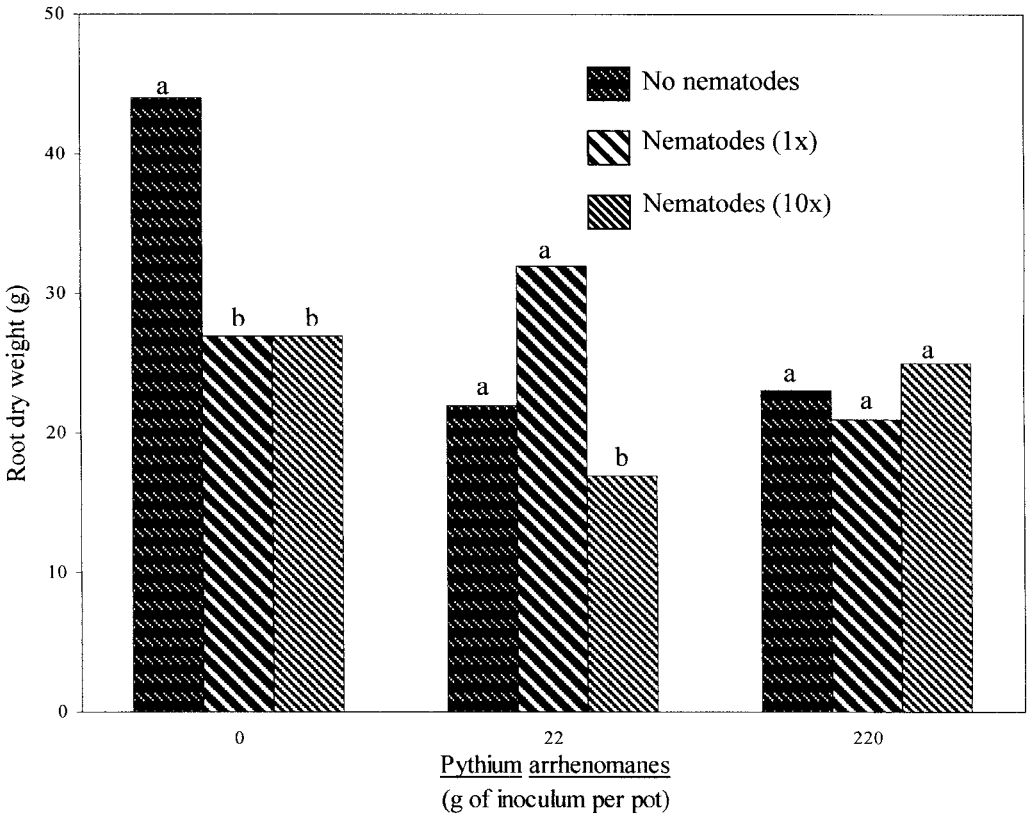

Fig. 1. Root dry weight as influenced by nematode and *P. arrhenomanes* infestation levels in 1997. Nematode infestation levels were 495 (1×) and 4,950 (10×) nematodes per pot (inoculum was 27% *T. annulatus*, 69% *M. xenoplax* and 3% *P. minor*), and *P. arrhenomanes* infestation levels were 0, 22 or 220 g of inoculum mixture per pot. Within each *Pythium* level, bars with the same letter indicate means that do not differ significantly ($P < 0.05$) according to Tukey's HSD test.

When *Pythium* was present at low levels, root growth was restricted at the highest nematode infestation level when compared to the low nematode infestation level. At the highest *Pythium* level, differences in root weight were not observed.

Greater reproductive values were observed for the low infestation level for each nematode in each of three years (Table 2). *Pythium arrhenomanes* limited reproduction of both *T. annulatus* and *M. xenoplax* in all three trials of this experiment. In 1996 and 1998, similar effects on reproduction of *T. annulatus* occurred with both *Pythium* levels. In 1997, reproduction was suppressed only at the high *Pythium* level. With the exception of reproduction in 1996, reproduction of *M. xenoplax* was suppressed similarly at both *Pythium* levels. With the exception of 1997, reproduction of *P. minor* was not affected by *Pythium*.

Temperature influenced all three nematodes. In 1998, reproduction of *T. annulatus* was greater at 30°C than at 20°C. Reproduction for *M. xenoplax* and *P. minor* were greater at 20°C than at 30°C.

Interaction between the two types of pathogens affected reproduction of *T. annu-*

Table 2. Reproduction of *Tylenchorhynchus annulatus*, *Mesocriconema xenoplax*, and *Paratrichodorus minor*, as influenced by nematode and *Pythium arrhenomanes* infestation levels and temperature in greenhouse experiments, 1996-98.

		Reproductive values[x]								
		1996			1997			1998		
Factor[y]	Level	*T. annulatus*	*M. xenoplax*	*P. minor*	*T. annulatus*	*M. xenoplax*	*P. minor*	*T. annulatus*	*M. xenoplax*	*P. minor*
Nematode[z]	1×	41	7	39	58	5	75	206	5	133
	10×	6	1	5	6	1	15	16	2	14
	P > F	***	**	**	***	***	***	***	***	***
Pythium	0	47 a	4	13	40 a	4 a	74 a	194 a	6 a	62
	22	15 b	6	37	39 a	2 b	30 b	61 b	3 b	72
	220	7 b	1	13	16 b	2 b	29 b	77 b	2 b	84
	P > F	***	ns	ns	**	*	**	***	**	ns
Temp.	20°C	—	—	—	—	—	—	49	5	93
	30°C	—	—	—	—	—	—	173	2	53
	P > F	—	—	—	—	—	—	***	*	**
N × P	P > F	*	ns	ns	**	ns	ns	***	ns	*
N × T	P > F	—	—	—	—	—	—	***	*	*
P × T	P > F	—	—	—	—	—	—	***	*	ns
N × P × T	P > F	—	—	—	—	—	—	***	ns	ns

Data are means of six replicates. For each factor and column, *, **, and *** indicate differences at $P \leq 0.05$, 0.01, 0.001, respectively; ns indicates that means are not significantly different. For treatments with three levels, means followed by the same letter are not different ($P \leq 0.05$), according to Tukey's HSD test.

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

[y]Infestation levels for 1996, 1997, and 1998 were: (1×) 491, 495, and 495 nematodes per pot, respectively. Ratios of *Tylenchorhynchus*:*Mesocriconema*:*Paratrichodorus* in inocula were 35:58:6 in 1996, 28:69:3 in 1997, and 42:46:12 in 1998. 0, 22, or 220 g of inoculum mixture containing *P arrhenomanes*.

[z]Nematodes were not recovered from controls.

latus consistently across all three trials. Data from 1997 are presented as an example (Fig. 2). At the low nematode infestation level, reproduction was suppressed only at the highest *Pythium* infestation level. However, at the high nematode level, reproduction was not influenced by either level of *Pythium*.

Root colonization by *P. arrhenomanes* was influenced by nematode infestation only in 1996 (Table 3). When compared to the low nematode infestation level, a 22% reduction in colonization by *P. arrhenomanes* was observed at the high nematode level. As was the case for nematodes, colonization of sugarcane roots by *P. arrhenomanes* was greater at the low *Pythium* infestation level in 1996 and 1998. Additionally, colonization was greater at 20°C than at 30°C.

DISCUSSION

An experiment with three trials was conducted to evaluate the interactions and damage potential to sugarcane of an indigenous nematode community and the fungus *P. arrhenomanes*. In these studies, the nematode community and *P. arrhenomanes* suppressed the growth of sugarcane both alone and in combination. When nematodes and *P. arrhenomanes* were together, antagonistic interactions occurred.

The three nematode species used in this study are commonly found in sugarcane field soil in Louisiana (Bond *et al.*, 1997, 2000). This community of nematodes was shown to be highly pathogenic to sugarcane in a previous study, and results from the research detailed herein

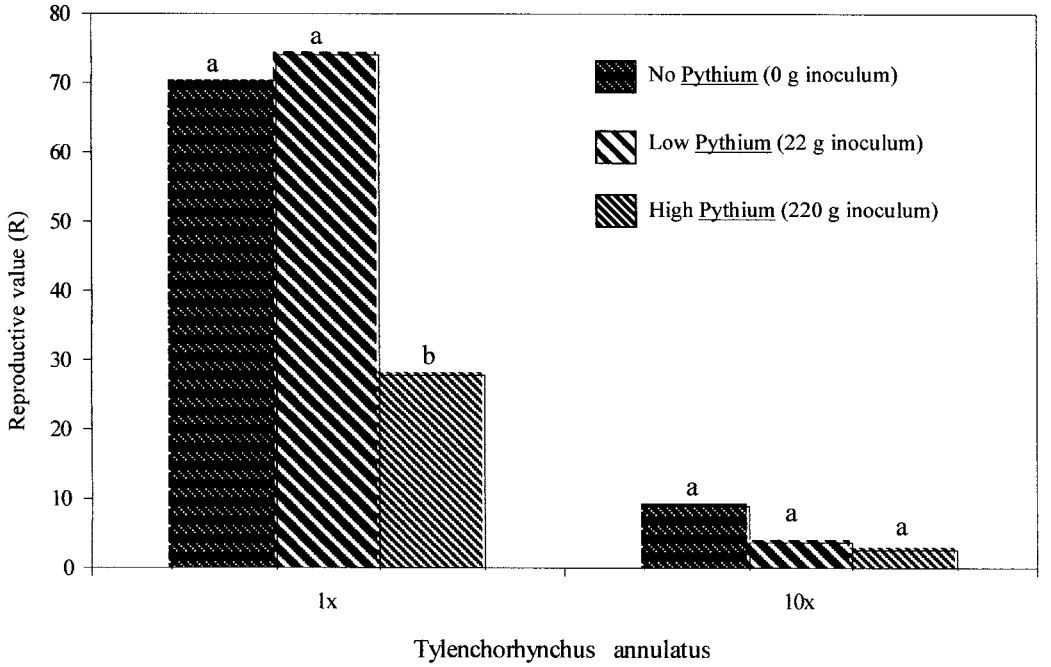

Fig. 2. Reproduction of *T. annulatus* as influenced by nematode and *P. arrhenomanes* infestation levels in 1997. Nematode infestation levels were 495 (1×) and 4,950 (10×) nematodes per pot (inoculum was 27% *T. annulatus*, 69% *M. xenoplax* and 3% *P. minor*), and *P. arrhenomanes* infestation levels were 0, 22 or 220 g of inoculum mixture per pot. Within each nematode infestation level, bars with the same letter indicate means do not differ significantly ($P < 0.05$) according to Tukey's HSD test.

Table 3. Colonization of sugarcane roots by *Pythium arrhenomanes* as influenced by nematode (N) and *P. arrhenomanes* (P) infestation levels and temperature (T) in greenhouse experiments during 1996-98.

		Root colonization (%)		
Factor	Level	1996	1997	1998
Nematode[y]	0	53	7	27
	1×	62	13	23
	10×	41	16	14
	P > F	*	ns	ns
Pythium[z]	0	0	0	0
	22	63	10	30 a
	220	40	13	20 b
	P > F	**	ns	*
Temp.	20°C	—	—	33 a
	30°C	—	—	17 b
	P > F	—	—	*
N × P	P > F	*	ns	ns
N × T	P > F	—	—	ns
P × T	P > F	—	—	ns
N × P × T	P > F	—	—	ns

Data are means of six replications. For each factor and column, * and ** indicate differences at $P \leq 0.05$, and 0.01,; ns indicates that means are not significantly different. For treatments with three levels, means followed by the same letter are not different ($P \leq 0.05$), according to Tukey's HSD test.

[y]Infestation levels for 1996, 1997, and 1998 were: (1×) 491, 495, and 495 nematodes per pot, respectively. Ratios of *T. annulatus*:*M. xenoplax*:*P. minor* in inocula were 35:58:6 in 1996, 28:69:3 in 1997, and 42:46:12 in 1998.

[z]0, 22, or 220 g of inoculum mixture containing *P. arrhenomanes*.

are in agreement (Bond *et al.*, 2004) with results of those studies. In greenhouse trials, the nematode community caused significant damage to the cultivar LCP 82-89.

Pythium arrhenomanes is a production constraint in subtropical sugarcane areas, such as the Mississippi delta, which experience marked seasonal changes and occasional cool growing conditions (Matherne *et al.*, 1977). In Louisiana, root rot caused by *P. arrhenomanes* has been shown to be both temperature and moisture dependent. Research indicates that when temperatures are between 15-20°C, root rot is more severe. At higher temperatures, symptoms are less severe (Flor, 1930; Rands and Dopp, 1938; Hoy and Schneider, 1988a). Results from experiments detailed herein are in agreement with these findings.

Apt and Koike (1962b) demonstrated that sugarcane top growth was reduced to a greater extent when both *Pythium graminicola* and *Meloidogyne incognita* were present than when either pathogen was alone. Valle-Lamboy and Ayala (1980) indicated that *M. incognita*, *Pratylenchus zeae*, and *P. graminicola* separately reduced the growth of sugarcane. However, when soil was infested with

either nematode species plus *Pythium*, the effect on plant growth was less than additive. The species *P. arrhenomanes* and *P. graminicola* are so similar in morphology and ecology (Van der Plaats-Niterink, 1981) that it is possible some confusion in identification has occurred in the literature. Birchfield and Martin (1956) indicated that the pathogenicities of *T. annulatus* and *P. arrhenomanes* were in-dependent of one another on sugarcane, although no data were presented to support this contention. Results of this study are in contrast to this report. In the present study, interactions between the nematode community and *P. arrhenomanes* were antagonistic. The sum of damage caused separately by the nematodes and *P. arrhenomanes* was greater than damage caused when both organisms were together.

Antagonistic interactions, such as those observed between *P. arrhenomanes* and nematodes in this work, can be explained by destruction or competition for available root space, or by fungal production of nematicidal or nematistatic metabolites (Evans and Haydock, 1993). *Tylenchorhynchus annulatus* (Johnson, 1970), *P. minor* (Apt and Koike, 1962a), and *P. arrhenomanes* (Hendrix and Campbell, 1973) all parasitize young root tissue and therefore may compete for the same feeding sites. *Pythium arrhenomanes* was added to the soil just prior to the addition of the sugarcane cuttings, and nematodes were added 72 hours after transplanting. It is possible that this sequence of infestation provided *P. arrhenomanes* with a competitive advantage over the nematodes. Research indicating the rate at which *P. arrhenomanes* infects and successfully colonizes host tissue is lacking. However, in the present study, *P. arrhenomanes* inoculum contained actively growing mycelium capable of immediate direct infection or developing sporangia within 24 hours. Therefore, the antagonistic interaction that was detected could have resulted from *Pythium* destroying or altering root tissue rendering it less suitable for the nematodes. A final possible factor affecting the interactions between *P. arrhenomanes*, nematodes, and sugarcane would be limitations imposed by the experimental system. Substantial damage to the plant root system occurs in greenhouse experiments. In order for the effects of *P. arrhenomanes* and nematodes to be additive, nearly the entire root system would have to be destroyed, and given the characteristics of infection of both pathogens, this would be unlikely.

This work constitutes the first report that *P. arrhenomanes* influences nematodes on sugarcane or any other crop. Nematode reproduction has been shown to be either suppressed (Santo and Holtzman, 1970) or unaffected (Valle Lamboy and Ayala, 1980) by several other *Pythium* species. In the present study, reproduction of *T. annulatus* and *M. xenoplax* were suppressed by *P. arrhenomanes*. However, *Pythium* had variable effects on reproduction of *P. minor*. In these tests, suppression of nematode reproduction may have resulted not only from competition with *P. arrhenomanes* for feeding sites but also from alteration of the nutritional substrate. Toxic metabolites are known to be produced by this pathogen (Mojdehi *et al.*, 1990).

The effects of nematodes on root colonization by *P. arrhenomanes* have not been investigated. Valle-Lamboy and Ayala (1980) indicated that as a result of root damage caused by *M. incognita*, colonization by *P. graminicola* was inhibited. The data from the 1996 trial is in agreement with the work of Valle-Lamboy and Ayala. In this study, reduced colonization by *P. arrhenomanes* was observed only at the high nematode infestation level suggesting a high level of nematode infection is necessary to inhibit *Pythium*.

The cultural practices of sugarcane production throughout the world rely on establishing a monoculture for 3 to 16 or

more years. Obligate parasites, such as nematodes, are aided by this production technique. In Louisiana, acreage used in sugarcane production is rarely rotated with other crops, although many producers do include a fallow season after removing the crop. This experiment demonstrates the potential adverse impact of nematodes in combination with *P. arrhenomanes* to sugarcane production in Louisiana. Based on evidence from this and previous research, there is a strong indication that the total nematode community and Pythium root rot are factors in progressively decreasing sugarcane ratoon crop yields commonly observed in Louisiana. The relative importance of each pathogen type and the degree of interaction may vary depending on seasonal weather and soil conditions.

ACKNOWLEDGMENTS

The authors thank J. S. Russin, and W. P. Bond for critical review of this manuscript.

LITERATURE CITED

ANONYMOUS. 2003. 2003 Louisiana Agricultural Statistics. Louisiana Agricultural Statistics Service (2001-2003). Online. http://www.nass.usda.gov/la/annbullmain.htm.

APT, W. J., and H. KOIKE. 1962a. Influence of the stubby-root nematode on growth of sugarcane in Hawaii. Phytopathology 52:963-964.

APT, W. J., and H. KOIKE. 1962b. Pathogenicity of *Meloidogyne incognita acrita* and its relation with *Pythium graminicola* on sugarcane in Hawaii. Phytopathology 52:1180-1184.

BIRCHFIELD, W., and W. J. MARTIN. 1956. Pathogenicity on sugarcane and host plant studies of a species of *Tylenchorhynchus*. Phytopathology 46:277-280.

BOND, J. P., E. C. McGAWLEY, and J. W. HOY. 1997. Phytoparasitic nematode occurrence and effect on sugarcane growth in Louisiana. Journal of Nematology 29:571 (Abstr.).

BOND, J. P., E. C. McGAWLEY, and J. W. HOY. 2001. Distribution of plant parasitic nematodes on sugarcane in Louisiana and efficacy of nematicides. Journal of Nematology 33:493-501.

BOND, J. P., E. C. McGAWLEY, and J. W. HOY. 2004. The impact of nematodes on sugarcane cultivars. Nematropica 34:235-243.

CROFT, B. J., and R. C. MAGAREY. 1984. Pathogenic fungi associated with northern poor root syndrome of sugarcane. Proceedings of the Australian Society of Sugar Cane Technologists 6:55-61.

DISSANAYAKE, N., J. W. HOY, and J. L. GRIFFIN. 1998. Herbicide effects on sugarcane growth, Pythium root rot, and *Pythium arrhenomanes*. Phytopathology 88:530-535.

EVANS, K., and P. P. J. HAYDOCK. 1993. Interactions of nematodes with root-rot fungi. Pp. 104-133 *in* M. W. Khan, ed. Nematode interactions. Chapman and Hall, New York.

FLOR, H. H. 1930. Relation of environment factors to growth and pathogenicity of *Pythium* isolated from roots of sugarcane. Phytopathology 20:319-328.

GARGANTIEL, F. T., and R. G. DAVIDE. 1973. Survey and pathogenicity tests of plant-parasitic nematodes associated with sugarcane in Negros Occidental. Philippine Phytopathology 9:59-66.

HENDRIX, F. F., JR., and W. A. CAMPBELL. 1973. *Pythium* as plant pathogens. Annual Review of Phytopathology 11:77-98.

HOY, J. W., and R. W. SCHNEIDER. 1988a. Role of *Pythium* in sugarcane stubble decline: Pathogenicity and virulence of *Pythium* species. Phytopathology 78:1688-1692.

HOY, J. W., and R. W. SCHNEIDER. 1988b. Role of *Pythium* in sugarcane stubble decline: Effects on plant growth in field soil. Phytopathology 78:1692-1696.

JENKINS, W. R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

JOHNSON, A. W. 1970. Pathogenicity and interaction of three nematode species on six bermuda grasses. Journal of Nematology 2:36-41.

KOENING, S. R., C. OVERSTREET, J. W. NOLING, P. A. DONALD, J. O. BECKER, and B. A. FORTNUM. 1999. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. Supplement to the Journal of Nematology 31:587-618.

MATHERNE, R. J., R. D. BREAUX, and R. W. MILLHOLLON. 1977. Culture of sugarcane for sugar production in the Mississippi delta. USDA Agricultural Handbook, No. 417.

MIRCETICH, S. M., and M. E. MATHERON. 1976. Phytophthora rot and crown rot of cherry trees. Phytopathology 66:549-558.

MOJDEHI, H., L. L. SINGLETON, H. A. MELOUK, and G. R. WALLER. 1990. Reproduction of disease symptoms from toxic metabolites produced

by two *Pythium* species and their partial characterization. Journal of Phytopathology 128:246-256.

RANDS, R. D. 1929. Fungi associated with root rots of sugar cane in the southern United States. Proceedings of the International Society of Sugarcane Technologists 3:119-131.

RANDS, R. D. 1961. Root rot. Pp. 289-309 *in* J. P. Martin, E. V. Abbot, and C. G. Hughes, eds. Sugar cane diseases of the world, Vol. 1. Elsevier Publishing, New York.

RANDS, R. D., and E. DOPP. 1938. Pythium root rot of sugarcane. United States Department of Agricultural Technical Bulletin. 666.

Roman, J. 1968. Nematode problems of sugarcane in Puerto Rico. Pp. 61-67 *in* G. C. Smart, Jr., and V. G. Perry, eds. Tropical Nematology, University of Florida Press, Gainesville.

SANTO, G. S., and O. V. HOLTZMANN. 1970. Interrelationship of *Pratylenchus zeae* and *Pythium graminicola* on sugarcane. Phytopathology 60:1536 (Abstr.).

SPAULL, V. W., and P. CADET. 1990. Nematode parasites of sugarcane. Pp. 461-491 *in* M. Luc., R. A. Sikora and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. Oxon, CAB International, United Kingdom.

VALLE-LAMBOY, S., and A. AYALA. 1980. Pathogenicity of *Meloidogyne incognita* and *Pratylenchus zeae*, and their association with *Pythium graminicola* on roots of sugarcane in Puerto Rico. Journal of Agriculture, Puerto Rico 64:338-347.

VAN DER PLAATS-NITERINK, A. J. 1981. Monograph of the genus *Pythium*. Studies in Mycology, Vol. 21. Baarn, Centraalbureau voor Schimmelcultures, Netherlands.

Received: *14.VII.2004*

Recibido:

Accepted for Publication: *17.I.2005*

Aceptado para publicacion: