Interactions Among *Pythium* Species Affecting Root Rot of Sugarcane

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ABSTRACT

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Several *Pythium* species are isolated commonly from roots of sugarcane plants (interspecific hybrids of *Saccharum*) grown in field soils. To compare the effects of infection by one or several *Pythium* species, pathogenicity tests were conducted with *P. arrhenomanes, P. catenulatum, P. irregulare, P. spinosum*, and two unidentified *Pythium* species. Isolates of *P. arrhenomanes* caused significant growth reductions and severe root rot. Isolates of *P. irregulare* and *P. spinosum* caused significant reductions in root weight but varying effects on shoot number and weight. Root rot symptoms induced by these species were mild or not evident. *P. catenulatum* and the unidentified *Pythium* species were nonpathogenic. Severity of root rot caused by *P. arrhenomanes* was unaffected by combinations with other species. Combinations of *P. irregulare* and *P. spinosum* generally did not increase disease severity over that caused by each species alone. Root rot severity, total *Pythium* isolation frequency, *P. arrhenomanes* isolation frequency, and percentage of all *Pythium* isolates consisting of *P. arrhenomanes* varied among plants grown experimentally in different field soils. The percentage of all *Pythium* isolates from roots consisting of *P. arrhenomanes* correlated positively with disease severity.

From research conducted in Louisiana during the 1920s and 1930s, *Pythium arrhenomanes* Drechs. was determined to be the causal agent of a severe root rot affecting the sugarcane (*Saccharum officinarum* L.) cultivars grown at that time (2,11). Pythium root rot, together with seed piece rots and a new strain of sugarcane mosaic virus, nearly caused the demise of the Louisiana sugarcane industry during the 1920s. However, economic yields were once again obtained after the introduction of interspecific hybrid cultivars with increased resistance to these diseases.

A yield decline now known as stubble decline is an important limiting factor for sugarcane production in Louisiana. Stubble decline results from an interaction of cultivars, diseases, low winter temperatures and freezes, poor soil aeration and drainage, the physiological maturity of cane plants at the time of harvest, and weed competition (1,3,8,15). Yields of the ratoon or stubble crops progressively decline, and the crop cycle is generally limited to the plant cane crop and two ratoon crops. Recently, Pythium root rot was shown to be capable of causing significant reductions in growth (6,7) and ratoon crop yields (7) of currently used hybrid sugarcane cultivars.

Different *Pythium* species were tested recently for pathogenicity on sugarcane (6), but, as in the 1930s (11), *P. arrhenomanes* was found to be the only highly pathogenic species. However, several other *Pythium* species typically are isolated from roots of sugarcane plants grown in field soils. The effects of infections by mildly pathogenic or nonpathogenic *Pythium* species on root rot are unknown. The severity of root rot caused by *P. arrhenomanes* in greenhouse pathogenicity tests would probably result in wilting or death of plants in the field. These symptoms are not observed in commercial fields, however.

Pathogenicity tests were conducted to determine the effects of infection by one or several *Pythium* species on sugarcane root rot. In addition, the isolation frequencies for total *Pythium* species and *P. arrhenomanes* were correlated with root rot severity in plants grown in different field soils. A preliminary report of these results has been published (9).

MATERIALS AND METHODS

Pathogenicity tests. Three isolates of *P. arrhenomanes*, six isolates of *P. spinosum* Sawada, four isolates of *P. irregulare* Buisman, two isolates of *P. catenulatum* Matthews, and single isolates of two unidentified *Pythium* species were tested for pathogenicity on sugarcane cultivar CP 70-321 in one of two separate experiments, each of which was repeated once. *Pythium* isolates were obtained from roots of sugarcane plants grown in field soil or from sugarcane field soil, with the exception of *P. spinosum* isolate 897, which was isolated from a rice (*Oryza sativa* L.) root. In addition, several combinations of these *Pythium* isolates were tested for effects on root rot severity.

Inoculum of each *Pythium* isolate was prepared by a modification of a method described previously (18) in which 250 cm^3 of vermiculite and 20 cm^3 of whole oat kernels were moistened with 175 ml of V8 juice in a 473 ml-canning jar and autoclaved twice. Agar disks from a colony of the appropriate isolate were then transferred to individual jars. Fungi were allowed to colonize the substrate for 2–3 wk at room temperature (approximately 24 C). The inoculum was then placed in cheesecloth, thoroughly rinsed with water, and mixed with a steam-pasteurized sand/silt loam soil mixture (1:1, v/v) at a rate of approximately 20 cm^3 per 1,000 cm^3 of soil/sand mix. With the *Pythium* species combination treatments, these amounts were divided equally among the different species. Controls consisted of plants grown in sterile, nonamended soil/sand mix.

Plants of sugarcane cultivar CP 70-321 were obtained from single-bud cuttings taken from the middle portion of cane stalks. Cuttings were trimmed so that about 2 cm of internode tissue remained on each side of the node, dipped in hot water (45 C) for 30 min, and planted in a pasteurized sand/silt loam soil mixture (1:1, v/v) in Styrofoam trays with 7.5 × 7.5 cm cells. Plants were selected for uniformity after growing for 3 wk in the greenhouse. Individual plants were placed into each of seven pots containing soil infested with a single isolate or combinations of isolates of *Pythium* species, and pots were placed on a greenhouse bench. Inorganic nutrients were provided by the addition of a 4-mo slow-release fertilizer (24-24-24, Osmocote). Plants were watered as needed to maintain a soil moisture content near field capacity. After 8–9 wk, the plant root systems were gently washed free of soil, and plant growth and disease severity were compared for the different treatments.

Pathogenicity of single isolates or combinations of isolates of *Pythium* was determined by comparing control plants with plants grown in infested soil. Data collected from individual plants for comparison included shoot number, total shoot dry weight, and root system dry weight. Two subjective ratings for root rot severity were assigned to each

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plant based on the extent of lateral root rotting and root system discoloration. Ratings were made on a scale of 1–4, where 1.0 = normal, healthy appearance, 1.5 = occasional lesions or slight discoloration, 2.0 = ≤25% of the primary and lateral roots with lesions or discolored, 2.5 = 26–50% of the primary or lateral roots with lesions or discolored plus some lateral roots pruned, 3.0 = 51–75% of the roots with lesions or discolored plus many lateral roots pruned, 3.5 = 76–90% of lateral roots missing or completely rotted and primary roots extensively discolored, and 4.0 = >90% of lateral roots missing and primary roots completely discolored.

Recovery and identification of *Pythium* species. Fifty sections of roots with lesions were collected from experimental plants, washed in deionized water for 1 hr, blotted dry with a paper towel, and placed onto plates of pimaricin-vancomycin-PCNB (PVP) medium (10) to reisolate *Pythium* species. The plates were incubated at 34 C, and the number of colonies was determined by totaling counts made at 24 and 48 hr.

The key and species descriptions contained in a recent monograph of the genus *Pythium* (17) were used to identify the isolates. Sporangia and zoospore release were observed on hyphae extending into filter-sterilized soil extract (10 g of soil stirred overnight in 1.5 L of deionized water) from disks of V8 agar (200 ml of V8 juice, 2 g of $CaCO_3$, 17 g of agar, and 800 ml of deionized water) removed from the edge of a rapidly growing colony. Flooded agar disks were incubated at 20–24 C, and sporangia and zoospores were produced within 48 hr. Sexual structures were observed on a hyphal mat growing into sterile water from a 2-ml agar slant poured against the edge of a 9-cm-diameter petri dish (4). The agar slant was composed of V8 agar amended with 20 mg/L of β-sitosterol, and the bottom of the petri dish was flooded with 7 ml of sterile, deionized water (enough to cover the edge of the agar slant).

Interactions among *Pythium* species affecting plant growth in field soil. Soil samples consisting of eight to 10 subsamples collected at random in a single field were obtained from 10 scattered sugarcane fields. Each soil sample was sieved through a 1-cm-mesh screen, and a portion of each soil was steam-pasteurized. Five 16-cm-diameter pots of sterile and nonsterile soil were prepared by mixing one pot of each field soil with four pots of sterile soil/sand mix (1:1, v/v). Inorganic nutrients were provided by the addition of the 4-mo slow-release fertilizer. Controls for each field soil consisted of the five plants grown in soil amended with corresponding pasteurized soil. Plants of sugarcane cv. CP 70-321 were obtained from single-bud cuttings as described previously.

All plants were allowed to grow 7 wk in the greenhouse, at which time the plant root systems were gently washed free of soil. Data collected for individual plants included shoot number, total shoot weight, root weight, and lateral root rot and root system discoloration ratings. Symptomatic root segments were collected from plants and plated on PVP medium to isolate *Pythium* species. To identify isolates, colonies on PVP medium were transferred to V8 medium. After 24 hr, five agar disks were taken from the edge of an actively growing colony and transferred to a 5-cm-diameter petri dish, and 7 ml of filter-sterilized soil extract was added. Flooded agar disks were incubated at 20–24 C. Sporangia and sexual structures were observed after 48–72 hr, and *Pythium* species were identified by morphological characteristics (17).

Growth data and ratings of each type for each individual nonsterile and pasteurized field soil combination were compared with a *t* test to determine significant differences. Percent reductions for shoot number, shoot weight, and root weight for plants grown in all field soils were then correlated with the corresponding *Pythium* isolation frequency data, including total *Pythium* isolation frequency, *P. arrhenomanes* isolation frequency, and percentage of total *Pythium* isolates consisting of *P. arrhenomanes*.

RESULTS

Pathogenicity tests. Results of the two separate experiments and of the repeated experiments of each type were similar. Three isolates of *P. arrhenomanes* were highly pathogenic to CP 70-321 and caused significant reductions in all plant growth parameters (Tables 1 and 2). Three isolates of *P. spinosum* (11-2, 1034, and 1045) and one isolate of *P. irregulare* (26-6) were pathogenic and caused significant reductions in all growth parameters (Table 1). All six isolates of *P. spinosum* caused significant reductions in root weight, and all four isolates

Table 1. Effects of *Pythium arrhenomanes*, *P. spinosum*, and *P. irregulare*, alone and in combination, on growth and root rot severity in sugarcane cv. CP 70-321

		Plant growth means			Root rot rating means[z]	
***Pythium* species**	**Isolates**	**Shoot no.**	**Shoot wt (g)**	**Root wt (g)**	**Lateral roots**	**Discoloration**
P. arrhenomanes	147	2.2	6.9	2.7	2.6	2.8
	156	1.2	6.7	2.2	3.0	3.0
	1031	1.2	4.8	1.7	2.6	2.7
P. spinosum	11-2	2.9	10.3	3.5	1.5	2.0
	26-1	3.9	12.7	3.9	1.5	1.6
	897	6.1	17.0	6.4	1.5	1.5
	1027	4.6	16.0	5.5	1.5	2.0
	1034	3.4	11.3	3.5	1.5	2.0
	1045	3.6	14.1	4.1	1.4	1.6
P. irregulare	7-4	4.6	12.7	5.1	1.5	1.5
	26-6	3.1	10.6	3.3	2.1	2.4
	1028	2.9	12.8	5.1	1.4	1.6
	1037	3.9	9.7	3.5	1.5	1.5
P. arrhenomanes + *P. spinosum*	147 + 11-2	1.6	5.1	2.0	2.7	2.7
	1031 + 1027	1.1	6.6	2.4	2.0	2.1
P. arrhenomanes + *P. irregulare*	147 + 7-4	1.4	5.4	2.1	2.8	3.8
	1031 + 1028	2.0	5.9	2.3	2.6	3.2
P. spinosum + *P. irregulare*	11-2 + 7-4	3.9	15.2	6.0	1.5	1.5
	1027 + 1028	1.9	9.7	4.3	1.5	1.5
P. arrhenomanes + *P. spinosum* + *P. irregulare*	147 + 11-2 + 7-4	1.3	5.2	1.7	3.6	3.5
	156 + 11-2 + 7-4	1.7	6.2	2.2	3.3	3.5
	1031 + 1027 + 1028	1.1	7.3	2.8	2.4	2.4
Control		4.7	19.7	10.4	1.0	1.5
LSD ($P = 0.05$)		1.1	2.8	1.4	0.2	0.2

[z] Extent of lateral root rot symptoms and root system discoloration was rated on a scale of 1–4, where 1 = normal, healthy appearance and 4 = severe symptoms.

of *P. irregulare* caused significant reductions in shoot weight and root weight (Table 1). Plant growth was unaffected by the two isolates of *P. catenulatum* or the two unidentified *Pythium* isolates (Table 2). Isolates of *P. arrhenomanes* caused obvious lateral root rot and root system discoloration. The isolates of *P. spinosum* and *P. irregulare* caused only slight discoloration, and the extent of lateral root development was generally similar to that of control plants.

Plants grown in soil infested with combinations of *P. arrhenomanes* and isolates of other *Pythium* species showed significant reductions in all growth parameters when compared with control plants (Tables 1 and 2). Combinations of *P. spinosum* isolates with *P. irregulare* isolates resulted in significant reductions in shoot and root weight (Table 1). One combination of *P. spinosum* and *P. irregulare* resulted in significant reductions in shoot number. Growth reductions caused by combinations of two different *P. spinosum* and *P. irregulare* isolates were generally greater than those caused by the single isolates in one combination and less in another. Growth reductions caused by combinations of *P. arrhenomanes* and any other *Pythium* species were not significantly different from those caused by *P. arrhenomanes* alone, although, in some cases, root rot symptoms were rated as more severe for plants grown in soil infested with combinations of *P. arrhenomanes* and other *Pythium* species (Tables 1 and 2).

The isolation frequencies of *P. arrhenomanes, P. spinosum*, and *P. irregulare* from roots of plants inoculated with single isolates ranged from 80 to 100% (mean = 87%), 50 to 90% (mean = 69%), and 65 to 85% (mean = 75%), respectively. *Pythium* species were not isolated from control plants. In plants inoculated with combinations of isolates of *Pythium* species, the frequency of isolation of all *Pythium* species ranged from 80 to 97%. The percentage of all *Pythium* isolates representing each species ranged from 48 to 92%, 4 to 62%, 4 to 50%, and 22 to 52% for *P. arrhenomanes, P. irregulare, P. spinosum*, and the unidentified *Pythium* species, respectively (Table 3).

Interactions among *Pythium* species affecting plant growth in field soils. Plants grown in soils amended with nonsterile soil from sugarcane fields showed varying degrees of root rot symptoms (Table 4). Growth of plants in soil amended with nonsterile soil from eight of 10 sugarcane fields was significantly less for one to three of the growth parameters than the growth of plants in soil amended with the corresponding pasteurized soil (Table 4). Percent growth reductions ranged from 0 to 42, 10 to 64, and 3 to 43% for shoot number, shoot weight, and root weight, respectively (Table 5).

The frequency of isolation of *Pythium* from symptomatic roots of plants grown in soils amended with the 10 nonsterile field soils ranged from 20 to 78% (Table 5). The percentage of all *Pythium* isolates consisting of *P. arrhenomanes* ranged from 0 to 95%, and the isolation frequency for *P. arrhenomanes* ranged from 0 to 72% (Table 5).

The isolation frequency of all *Pythium* species correlated poorly with plant growth reductions in the field soil experiments (Table 6). Although there was a positive relationship between the isolation frequency of *P. arrhenomanes* and reductions in plant growth, the percentage of all *Pythium* isolates consisting of *P. arrhenomanes* was the infection variable that correlated significantly with disease severity (Table 6). Comparisons of infection variables indicated that the isolation frequency of *P. arrhenomanes* was highly correlated with the total *Pythium* isolation frequency and the percentage of all isolates that were *P. arrhenomanes*. However, the percentage of all *Pythium* isolates that were *P.*

Table 2. Effects of individual *Pythium* species and combined *P. arrhenomanes* and unidentified *Pythium* species on growth and root rot severity in sugarcane cv. CP 70-321

Pythium species	Isolates	Plant growth means			Root rot rating means[z]	
		Shoot no.	Shoot wt (g)	Root wt (g)	Lateral roots	Discoloration
P. arrhenomanes	156	3.0	8.1	2.0	2.8	2.8
	1031	1.5	4.4	1.2	3.5	3.4
P. catenulatum	13-5	4.7	14.4	3.7	2.1	2.2
	16-8	6.0	16.9	5.7	1.1	1.1
Unidentified *Pythium* spp.	29-57	4.5	13.1	3.8	1.6	1.6
	29-58	6.3	14.1	3.4	1.0	1.0
P. arrhenomanes	156 + 29-57	2.2	6.6	1.6	3.4	3.4
+ unidentified	156 + 29-58	2.3	6.2	2.1	3.2	3.2
Pythium spp.	1031 + 29-57	2.5	5.2	1.4	3.4	3.5
	1031 + 29-58	1.6	3.7	1.1	3.6	3.4
Control		6.0	14.1	4.5	1.0	1.0
LSD ($P = 0.05$)		1.3	3.2	1.2	0.6	0.5

[z]Extent of lateral root rot symptoms and root system discoloration was rated on a scale of 1–4, where 1 = normal, healthy appearance and 4 = severe symptoms.

Table 3. *Pythium* species isolated from sugarcane cv. CP 70-321 grown in pasteurized soil infested with combinations of *Pythium* species

Pythium species	Isolates	Percent isolated	Percentage of isolates composed by each species			
			P. arrhenomanes	*P. spinosum*	*P. irregulare*	Unidentified *Pythium* species
P. arrhenomanes	147 + 11-2	95	92	8	...	...
+ *P. spinosum*	1031 + 1027	96	83	17	...	...
P. arrhenomanes	147 + 7-4	93	83	...	17	...
+ *P. irregulare*	1031 + 1028	95	58	...	42	...
P. spinosum	11-2 + 7-4	80	...	38	62	...
+ *P. irregulare*	1027 + 1028	83	...	50	50	...
P. arrhenomanes	147 + 11-2 + 7-4	85	75	21	4	...
+ *P. spinosum*	156 + 11-2 + 7-4	97	83	4	13	...
+ *P. irregulare*	1031 + 1027 + 1028	87	58	38	4	...
P. arrhenomanes	156 + 29-57	100	50	...	...	50
+ unidentified	156 + 29-58	90	78	...	...	22
Pythium spp.	1031 + 29-57	100	48	...	...	52
	1031 + 29-58	84	71	...	...	29

arrhenomanes was not significantly correlated with the total *Pythium* isolation frequency (Table 6).

DISCUSSION

The results of the pathogenicity tests with single isolates of *P. arrhenomanes*, *P. irregulare*, and *P. spinosum* were similar to previous results (6). All isolates of *P. arrhenomanes* caused severe root rot and reductions in growth. *P. irregulare* and *P. spinosum* are frequently isolated from sugarcane roots (J. W. Hoy, *unpublished*). Previously, a single isolate of *P. spinosum* was shown to cause significant reductions in growth without obvious root rot (6). The results of these tests with additional isolates were similar, suggesting that *P. spinosum* has the potential to be pathogenic under field conditions. Isolates of *P. irregulare* were more variable in pathogenicity, but this species could probably contribute to root rot as part of a complex of minor pathogens (13). In contrast, *P. catenulatum* and the unidentified *Pythium* species were shown to be nonpathogenic, as were other *Pythium* species, including *P. dissotocum* Drechs., *P. heterothallicum* W.A. Campbell & J.W. Hendrix, and *P. torulosum* Coker & F. Patterson (4) and *P. aphanidermatum* (Edson) Fitzp., *P. mamillatum* Meurs, *P. monospermum* Pringsh., *P. periilum* Drechs., *P. ultimum* Trow, and *P. vexans* de Bary in earlier tests (11).

Pathogenicity tests with combinations of *Pythium* species were used as a simplified approach to studying possible interactions affecting sugarcane root rot. When this approach was used, other *Pythium* species had no major effect on root rot caused by *P. arrhenomanes*. However, the inoculum forms, ratios, or amounts used in these experiments might differ from those in field soil. The form, amounts, and sources of inoculum of *P. arrhenomanes* in field soil are uncertain. Mostly aborted oospores are produced in culture (17), and isolating *P. arrhenomanes* from soil is difficult (J. W. Hoy, *unpublished*). However, sugarcane roots become infected when planted in sieved field soil diluted 1:4 with pasteurized soil. In the pathogenicity tests, roots were exposed to equal measured amounts of inoculum of each *Pythium* species. In addition, the amounts of actively growing mycelium and also other types of propagules added to the soil were probably higher than those occurring naturally in field soils. An indication that this experimental design was not analogous to natural conditions was that total *Pythium* and *P. arrhenomanes* infection levels were higher and disease was more severe in the pathogenicity tests than in the field soil tests.

Comparisons of root infection by total *Pythium* species or *P. arrhenomanes* with root rot severity in different field soils were used as an alternative approach to studying interactions of *Pythium* species affecting root rot. The potential for apple replant disease was shown to vary among different orchard soils, and plant growth reductions were associated with the level of infection by *Pythium* species, particularly *P. sylvaticum* W.A.

Table 4. Growth of sugarcane cv. CP 70-321 and root rot severity in soil amended with nonsterile or pasteurized field soils

Field[x]		Plant growth means[y]			Root rot rating means[z]	
No.	Soil	Shoot no.	Shoot wt (g)	Root wt (g)	Lateral roots	Discoloration
1	NS	3.0 a	5.0 b	1.2 a	3.0 a	2.9 a
	S	2.4 a	7.8 a	1.4 a	1.3 b	1.3 b
2	NS	3.0 a	7.7 a	1.6 a	1.4 a	2.1 a
	S	3.7 a	8.6 a	1.8 a	1.1 a	1.1 a
3	NS	4.2 b	14.9 a	6.4 a	1.7 a	2.1 a
	S	5.4 a	19.7 a	8.0 a	1.0 b	1.0 b
4	NS	3.8 b	10.4 b	5.6 a	2.2 a	2.3 a
	S	5.8 a	17.2 a	6.8 a	1.0 b	1.0 b
5	NS	2.8 b	9.7 b	3.2 b	3.3 a	3.4 a
	S	4.8 a	15.1 a	5.6 a	1.1 b	1.0 b
6	NS	3.0 b	9.6 b	3.6 b	3.0 a	3.1 a
	S	4.8 a	16.7 a	5.7 a	1.0 b	1.0 b
7	NS	5.0 a	14.2 b	4.1 b	2.7 a	2.9 a
	S	3.8 a	17.5 a	6.6 a	1.2 b	1.0 b
8	NS	3.8 b	13.6 b	5.5 b	3.0 a	3.5 a
	S	6.0 a	20.9 a	9.1 a	1.0 b	1.0 b
9	NS	5.4 a	15.2 a	6.4 a	2.1 a	2.5 a
	S	4.6 a	19.2 a	6.6 a	1.0 b	1.0 b
10	NS	3.6 b	13.1 b	4.3 b	2.9 a	3.2 a
	S	5.2 a	16.6 a	7.3 a	1.0 b	1.0 b

[x] Plants were grown in soil samples from 10 scattered sugarcane fields; soils were untreated (nonsterile [NS]) or pasteurized (sterile [S]).
[y] Means for plants grown in nonsterile or sterile treatments of the same soil followed by the same letter are not statistically different at $P = 0.05$, as determined by a t test.
[z] Extent of lateral root rot symptoms and root system discoloration was rated on a scale of 1–4, where 1 = normal, healthy appearance and 4 = severe symptoms.

Table 5. Percent growth reductions of sugarcane cv. CP 70-321 in nonsterile compared with pasteurized field soils and isolation frequencies of total *Pythium* species and *P. arrhenomanes*

	Percent growth reductions			*Pythium* isolation frequency[y]		
Soil	Shoot no.	Shoot wt	Root wt	*Pythium* species	Percent *P. arrhenomanes*	*P. arrhenomanes*
1	0	36*[z]	14	34	48	16
2	19	10	11	30	30	9
3	22*	24	20	20	30	6
4	34*	60*	18	56	44	24
5	42*	64*	43*	38	95	36
6	38*	43*	37*	60	94	56
7	0	19*	38*	44	41	20
8	37*	35*	40*	78	92	72
9	0	21	3	44	0	0
10	31*	21	41*	30	26	8

[y] Percent isolation of *Pythium*, percentage of all *Pythium* isolates that were *P. arrhenomanes*, and percent isolation of *P. arrhenomanes* from 210 roots for soils 1 and 2 and 50 roots for soils 3–10.
[z] * = Significant at $P = 0.05$.

Table 6. Correlation coefficients between percent plant growth reductions and frequency of isolation of *Pythium* from roots of sugarcane cv. CP 70-321

	Isolation frequency of all *Pythium* species	Percentage of isolates that were *P. arrhenomanes*	Isolation frequency of *P. arrhenomanes*
Percent reduction in shoot number	0.36	0.67*[z]	0.61
Percent reduction in shoot weight	0.38	0.64*	0.48
Percent reduction in root weight	0.32	0.69*	0.62
Isolation frequency of all *Pythium* species	...	0.57	0.84**
Percentage of isolates that were *P. arrhenomanes*	...	...	0.90**

[z] * = Significant at $P = 0.05$ and ** = significant at $P = 0.01$.

Campbell & J.W. Hendrix (14). In the sugarcane field soil tests, the isolation frequency of *Pythium* from symptomatic tissue, the proportion of isolates composed of *P. arrhenomanes*, the *P. arrhenomanes* isolation frequency, and the severity of root rot varied for plants grown in different soils. The isolation frequency of total *Pythium* species was poorly correlated with disease severity. There was a positive relationship between the amount of *P. arrhenomanes* infection and disease severity, but the only variable significantly correlated with severity was the percentage of *Pythium* isolates that were *P. arrhenomanes*. In addition, the isolation frequency of *P. arrhenomanes* was highly correlated with the total *Pythium* isolation frequency, whereas the percentage of the total that was *P. arrhenomanes* was not. These results suggest that the *P. arrhenomanes* infection level is related to disease severity. However, the composition of the population of *Pythium* species in the roots varies, and this is an important determinant of disease severity. Lower population diversity with a high percentage of *P. arrhenomanes* in the total *Pythium* population results in more severe disease.

The results of the field soil experiments contrast with those of the pathogenicity tests. The conclusion of the pathogenicity tests, i.e., that other *Pythium* species do not affect root rot caused by *P. arrhenomanes*, may have been influenced by the kind or ratios of inoculum types used, as discussed above, or it could be that the inoculum level of *P. arrhenomanes* was high enough to cause severe root rot regardless of the presence of other *Pythium* species.

In the field soil tests, the isolation frequencies of *Pythium* species and resulting reductions in plant growth were lower than in the pathogenicity tests. One additional possibility suggested by these results is that other fungi may affect root rot severity. Previous research on other crops, such as alfalfa (5) and corn (12,16), has shown that interactions of different fungi with *Pythium* species can affect plant growth. These interactions are affected by environmental conditions and seasonal shifts in populations of various fungi. Knowledge about the ecology of *P. arrhenomanes* and other root-infecting fungi during the growing season and over the course of the crop cycle is needed to gain a better understanding of root rot in sugarcane.

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