Role of *Pythium* in Sugarcane Stubble Decline: Pathogenicity and Virulence of *Pythium* Species

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ABSTRACT

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Ten isolates of *Pythium arrhenomanes* and a single isolate of *P. spinosum* caused root rot and significant reductions in growth of sugarcane cultivar CP 70-321 in pathogenicity tests. Isolates of six other *Pythium* spp. were tested, and none consistently affected plant growth. Disease severity was greatest in experiments conducted under moderate temperature conditions. In tests conducted at high temperatures, the predominant symptoms were root discoloration and feeder root necrosis, and a range in virulence was apparent among isolates of *P. arrhenomanes*.

Pythium was determined to be the causal agent of a root rot of sugarcane, *Saccharum officinarum* L., in Hawaii in 1919 (2) and in Louisiana in 1929 (11). In Louisiana, *Pythium* spp. isolated from sugarcane roots were tested for pathogenicity to *S. officinarum* and to recently introduced interspecific hybrid cultivars of *Saccharum* (24). *Pythium* spp. tested included the following currently recognized species (30): *P. aphanidermatum* (Edson) Fitz., *P. arrhenomanes* Drechs., *P. dissotocum* Drechs., *P. graminicola* Subra., *P. mamillatum* Meurs, *P. monospermum* Pringsh., *P. periilum* Drechs., *P. ultimum* Trow, and *P. vexans* de Bary. Results indicated that *P. arrhenomanes* was the only species capable of causing severe root rot symptoms and significant growth reductions. Differences in virulence were demonstrated among isolates of *P. arrhenomanes* (23,24).

Results of pathogenicity tests conducted at controlled temperatures indicated that temperature had an indirect effect on disease severity by affecting the rate of plant growth (24). Severe rotting of primary roots occurred at lower temperatures (15–20 C), whereas lateral root pruning was the predominant symptom at higher temperatures (26–30 C). The severity of symptoms varied, particularly at intermediate and higher temperatures, according to the virulence of the isolates of *P. arrhenomanes* tested.

Sugarcane is vegetatively propagated, and successive annual cuttings are obtained from one planting. The ratoon or stubble crops develop from buds on the basal portions of plants left in the soil after harvest. In the tropics, the ratoon crops begin to develop immediately following harvest, and up to 20 ratoon crops may be obtained from one planting (1). In Louisiana, however, sugarcane is harvested at the onset of winter, and buds and young shoots on the remaining stubble must persist through a period of inactivity that lasts several months (10,12).

A complex problem now known as stubble decline, in which stubble buds fail to produce adequate numbers of shoots in the spring, was described and investigated during the 1930s (10,12). Factors thought to be associated with stubble decline included cultivars, low winter temperatures and freezes, soil aeration and drainage, the physiological maturity of cane plants at the time of harvest, the condition of the stubble root system (affected by cultural practices and root rot), and a stalk rot caused by *Glomerella tucumanensis*.

Stubble decline is still an important limiting factor for sugarcane production in Louisiana. In contrast to the tropics, the crop cycle is limited to the plant cane crop and two stubble crops. The environmental and physiological factors thought to influence stubble yields in the 1930s are still considered to be important. In addition, diseases, such as ratoon stunting disease (28), caused by a xylem-limited bacterium (8), and an interaction between ratoon stunting disease and sugarcane mosaic virus (27) are recognized as important factors.

Pythium root rot currently is considered to be a disease capable of causing injury to plants mainly during cold, wet winters (20). The importance of the disease is thought to have been reduced because of increased levels of resistance in modern interspecific hybrid cultivars (24), the antagonism exhibited by numerous soil microorganisms to *P. arrhenomanes* (3,6,18), and improvements in field drainage systems. However, *Pythium* was demonstrated to cause significant reductions in plant top growth in four cultivars (19), and additive growth reductions occurred when plants were infected by both sugarcane mosaic virus and *Pythium*. In addition, root rot caused by *Pythium* spp. has been demonstrated to be a factor in a serious disease complex of sugarcane in Australia known as poor root syndrome (7,13).

Root rot caused by *Pythium* spp., including *P. arrhenomanes*, has long been recognized as a potentially important disease of graminaceous crops, such as wheat, corn, and sorghum (9). However, the involvement of *Pythium* spp. in yield decline or replant diseases of a variety of crops, including wheat (4,5) and corn (29), recently has been demonstrated (4,5,15,16,22,25,26,29). These are subclinical or cryptic diseases in which obvious symptoms are not apparent.

All of the evidence presented above prompted a reinvestigation of the effects of *Pythium* spp. on the growth of sugarcane to determine if they function as cryptic pathogens and adversely affect sugarcane stubble yields in Louisiana. The results of greenhouse experiments to determine the pathogenicity of *Pythium* spp. to a modern sugarcane cultivar are presented here. A portion of these results was presented previously (17).

MATERIALS AND METHODS

Recovery and identification of *Pythium* species. Isolates were obtained from sugarcane roots collected from plants growing in the field or from sugarcane field soil. Sections of roots with lesions were washed in deionized water, dipped in 70% ethanol, dried on a paper towel, and then pressed into plates of pimaricin-vancomycin-pentachloronitrobenzene (PVP) medium (21). Isolates were obtained from field soil by floating sugarcane leaf

disks or sorghum (*Sorghum bicolor* (L.) Moench) seedlings over soil flooded with deionized water for 24 hr and then plating the plant material on PVP medium.

Isolates were identified with the key and species descriptions contained in a recent monograph on the genus *Pythium* (30). Sporangia, vesicles, and zoospore release were observed on hyphae extending into filter-sterilized soil extract from disks of V-8 agar (200 ml of V-8 juice, 2 g of $CaCO_3$, 17 g of agar, and 800 ml of 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 reproductive 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 (14). The agar slant was composed of V-8 agar amended with β-sitosterol (Sigma Chemical Co., St. Louis, MO) (20 mg/L) (21), and the bottom of the petri dish was flooded with 7 ml of sterile deionized water.

Pathogenicity tests. Ten isolates of *P. arrhenomanes*, three isolates of *P. dissotocum*, three isolates of *P. heterothallicum* Campbell and Hendrix, two isolates of *P. irregulare* Buisman, one isolate of *P. spinosum* Sawada, two isolates of *P. torulosum* Coker and Patterson, and four isolates of unidentified heterothallic *Pythium* spp. were tested for pathogenicity on sugarcane cultivar CP 70-321 in three experiments conducted under moderate temperature conditions. Each isolate was included in at least two experiments. In addition, four isolates of *P. arrhenomanes*, two isolates of *P. irregulare*, and one isolate of *P. spinosum* were tested for pathogenicity under high temperature conditions in two experiments. The mean daily soil temperatures ranged from 20 to 26 C and 27 to 34 C for experiments conducted during cool seasons or summer, respectively.

Inoculum of each isolate was prepared by a modification of a method described previously (31) in which 250 cc of vermiculite and 20 cc of whole oat kernels moistened with 175 ml of V-8 juice in a 473-ml canning jar were used. The mixture was autoclaved on two successive days, and each jar was inoculated with agar disks from a single colony of each isolate. The fungus was allowed to colonize the substrate for 4 wk at 20 C. The inoculum then was placed in a cheesecloth bag, thoroughly rinsed with water, and mixed with a sterile silt loam soil:sand mix (1:1, v/v) at a rate of 20 cc per 1,000 cc of potting medium. Controls consisted of plants grown in sterile unamended soil/sand mix.

Plants of sugarcane cultivar CP 70-321 were obtained from single-bud cuttings taken from the middle portions of cane stalks. Cuttings were trimmed to leave 1–2 cm of internode tissue on each side of the node, dipped in a benomyl (0.1 g of a.i./L)-captan (0.7 g of a.i./L) mixture, and planted in a 1:1 sterile sand:silt loam soil mixture in styrofoam trays (Speedling, Inc., Sun City, FL) with 7.5 cm × 7.5 cm cells. Plants were selected for uniformity after 3 wk, and one plant each was transplanted into infested soil contained in clay 15-cm-diameter pots.

Individual plants were placed into each of seven pots containing soil infested with a single isolate of *Pythium*, and pots were placed in a greenhouse. The plants were allowed to grow for periods of time ranging from 48 to 59 days; then the plant root systems were gently washed free of soil, and plant growth parameter data were collected from each plant. Root segments were plated on PVP medium to reisolate *Pythium* spp.

Pathogenicity of individual isolates of *Pythium* was determined by comparing control plants with plants grown in infested soil. Data collected for comparison included shoot number, total shoot dry weight, primary root number, and root system dry weight. Two subjective ratings for root rot severity were assigned to each plant based on the extent of lateral root rotting and root system discoloration. Ratings were made on a scale of 1–4, in which 1 = normal appearance and 4 = no lateral roots or severe discoloration.

RESULTS

A portion of the results of one pathogenicity test conducted under moderate temperature conditions are shown in Table 1. Similar results were obtained with other isolates and in two additional experiments. Five isolates of *P. arrhenomanes* were highly pathogenic to cultivar CP 70-321 and caused significant

TABLE 1. Effects of isolates of *Pythium* spp. on the growth of sugarcane cultivar CP 70-321 and evaluation of root rot symptom severity in a pathogenicity test conducted under moderate soil temperatures[a]

Pythium sp. isolate	Growth parameter means				Root rot rating means[b]	
	Shoot no.	Shoot wt. (g)	Root wt. (g)	Root no.	Lateral root	Discoloration
P. arrhenomanes						
147	1.0	1.8	0.4	9.3	3.4	3.4
150	1.2	1.8	0.5	9.2	3.1	2.7
153	1.3	3.6	1.1	21.3	2.6	2.5
198	1.0	2.9	0.8	12.8	3.2	2.8
230	1.2	4.1	1.2	31.5	2.7	2.5
P. dissotocum						
6-3	7.3	19.4	3.0	38.3	1.0	1.0
8-1	6.7	20.9	2.8	37.8	1.0	1.0
P. heterothallicum						
272	6.2	20.5	6.7	51.7	1.2	1.3
285	6.3	19.2	3.5	39.5	1.5	1.5
P. irregulare						
6-1	5.8	16.6	3.9	36.8	1.0	1.0
P. spinosum						
11-2	4.8	13.2	5.2	40.5	1.7	1.7
P. torulosum						
7-2	6.5	21.9	5.2	43.2	1.5	1.5
7-7	5.2	15.5	4.1	39.8	1.9	2.0
Unknown *Pythium* spp.						
299	5.4	17.3	5.8	50.2	1.5	1.4
306	5.3	23.0	5.0	48.2	1.0	1.5
Control	6.2	18.9	6.5	45.4	1.0	1.1
LSD ($P = 0.05$)	1.4	4.6	1.8	9.0	0.4	0.4

[a] Daily soil temperature means ranged from 20 to 26 C.
[b] Ratings of the extent of lateral root symptoms and root system discoloration were assigned to each plant on a scale of 1–4, in which 1 = normal appearance and 4 = no lateral roots or severe discoloration.

reductions in all four growth parameters measured. Some isolates of other *Pythium* spp. caused significant reductions in root weight, but only the single isolate of *P. spinosum* caused significant reductions in shoot number and weight, as well as root weight. Plants inoculated with *P. arrhenomanes* showed severe root rot symptoms, as indicated by lateral root rot and discoloration ratings, which ranged from 2.5–3.4 (Table 1). In comparison, ratings for plants inoculated with isolates of other *Pythium* spp. did not exceed 2.5, except for *P. spinosum* and isolate 7-7 of *P. torulosum*. Growth reductions caused by *P. arrhenomanes* ranged from 82 to 94% and 78 to 90% for root and shoot weights, respectively. *Pythium* was not isolated from 24 root segments collected from the seven plants inoculated with isolates 272, 7-2, 8-1, or control plants. The frequency of isolation from roots of plants inoculated with isolates of *P. arrhenomanes* ranged from 90 to 100%, whereas frequencies for roots collected from plants inoculated with other *Pythium* spp. ranged from 6 to 44% and averaged 10%.

The results of a pathogenicity test conducted under high temperature conditions are shown in Table 2. Similar results were obtained in an additional experiment. Two isolates of *P. arrhenomanes*, 147 and 212, caused significant reductions in all four growth parameters. Isolate 230 caused significant reductions in root weight, shoot weight, and shoot number, and isolate 153 caused a significant reduction in root number. Differences in root and top weights and root number between plants inoculated with isolate 153 and the highly virulent isolates 147 and 212 were significant (Table 2). Growth reductions caused by virulent isolates ranged from 28 to 50% and 27 to 46% for root and shoot weights, respectively. *P. spinosum* caused significant reductions in root weight and shoot number. Both isolates of *P. irregulare* caused a significant reduction in shoot number, and isolate 6-2 caused a significant reduction in root weight. Root symptoms were evident in plants inoculated with all four isolates of *P. arrhenomanes*, as indicated by lateral root system and discoloration ratings that ranged from 2.2 to 2.9 and 2.4 to 3.1, respectively. Ratings for plants inoculated with *P. spinosum* and *P. irregulare* were not significantly different from ratings for control plants. *Pythium* was not isolated from 50 root segments collected from the seven control plants. The isolation frequency for roots collected from plants inoculated with *P. arrhenomanes* ranged from 90 to 100%. In comparison, frequencies for plants inoculated with *P. spinosum* and *P. irregulare* 6-2 and 7-4 were 10, 16, and 12%, respectively.

In experiments conducted under moderate temperature conditions, *P. arrhenomanes* caused extensive rotting of lateral roots and primary roots as compared with uninoculated plants (Fig. 1A). Infected lateral roots and primary root tips were flaccid and water-soaked with a reddish brown lesion margin (Fig. 2A and B). Lateral roots often were completely destroyed, and reddish brown lesions occurred on primary roots where lateral roots were attached (Fig. 2C). In comparison, plants inoculated with *P. arrhenomanes* under high temperature conditions showed similar but less extensive symptom development. Primary root

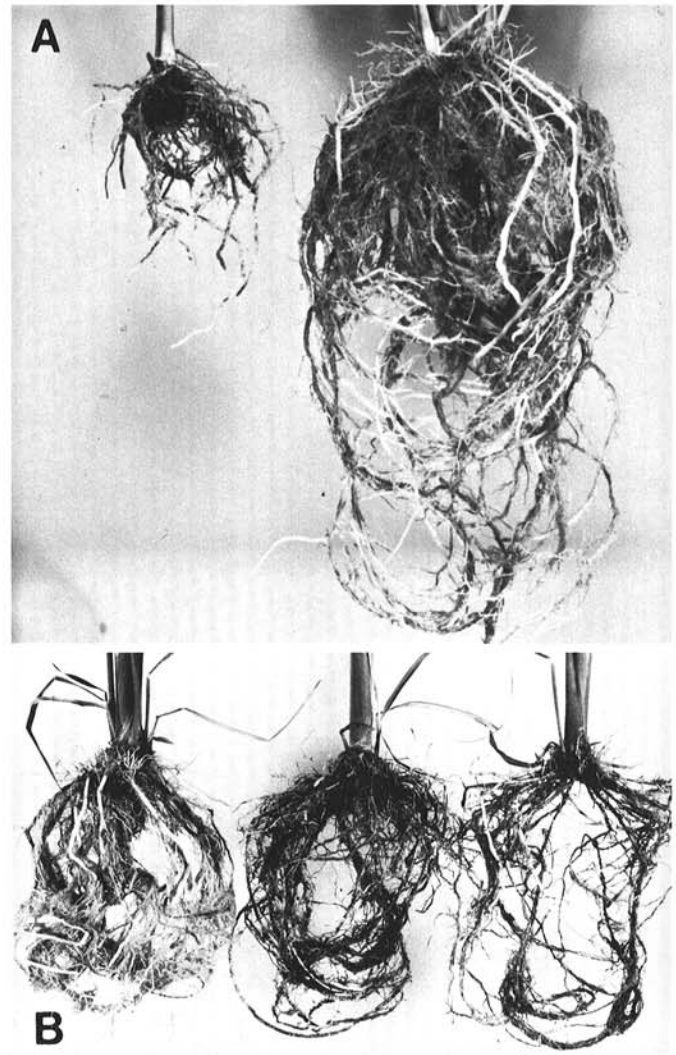

Fig. 1. Comparison of root systems of plants of sugarcane cultivar CP 70-321 grown in soil infested with *Pythium arrhenomanes* or uninfested, sterile soil. **A**, Inoculated plant (left) compared with control plant (right) in pathogenicity test conducted under moderate soil temperature conditions. **B**, Control plant (left) compared with plants inoculated with two isolates of *P. arrhenomanes* (middle and right) in a pathogenicity test conducted under high soil temperature conditions.

TABLE 2. Effects of isolates of *Pythium* spp. on the growth of sugarcane cultivar CP 70-321 and evaluation of root rot symptom severity in a pathogenicity test conducted under high soil temperatures[a]

Pythium sp. isolate	Growth parameters means				Root rot rating means[b]	
	Shoot no.	Shoot wt. (g)	Root wt. (g)	Root no.	Lateral root	Discoloration
P. arrhenomanes						
147	1.0	9.8	1.8	24.7	2.5	3.1
153	1.3	14.3	3.3	32.4	2.9	3.0
212	1.0	8.2	1.6	24.0	2.9	3.0
230	1.6	11.0	2.3	29.3	2.4	2.5
P. irregulare						
6-2	1.3	13.2	2.1	31.3	1.0	1.0
7-4	1.3	12.2	2.6	34.1	1.0	1.0
P. spinosum						
11-2	1.0	12.5	2.2	29.1	1.0	1.1
Control	2.6	15.1	3.2	33.0	1.0	1.0
LSD ($P = 0.05$)	0.8	3.2	0.7	5.2	0.1	0.1

[a] Daily soil temperature means ranged from 27 to 34 C.
[b] Ratings of the extent of lateral root symptoms and root system discoloration were assigned to each plant on a scale of 1–4, in which 1 = normal appearance and 4 = no lateral roots or severe discoloration.

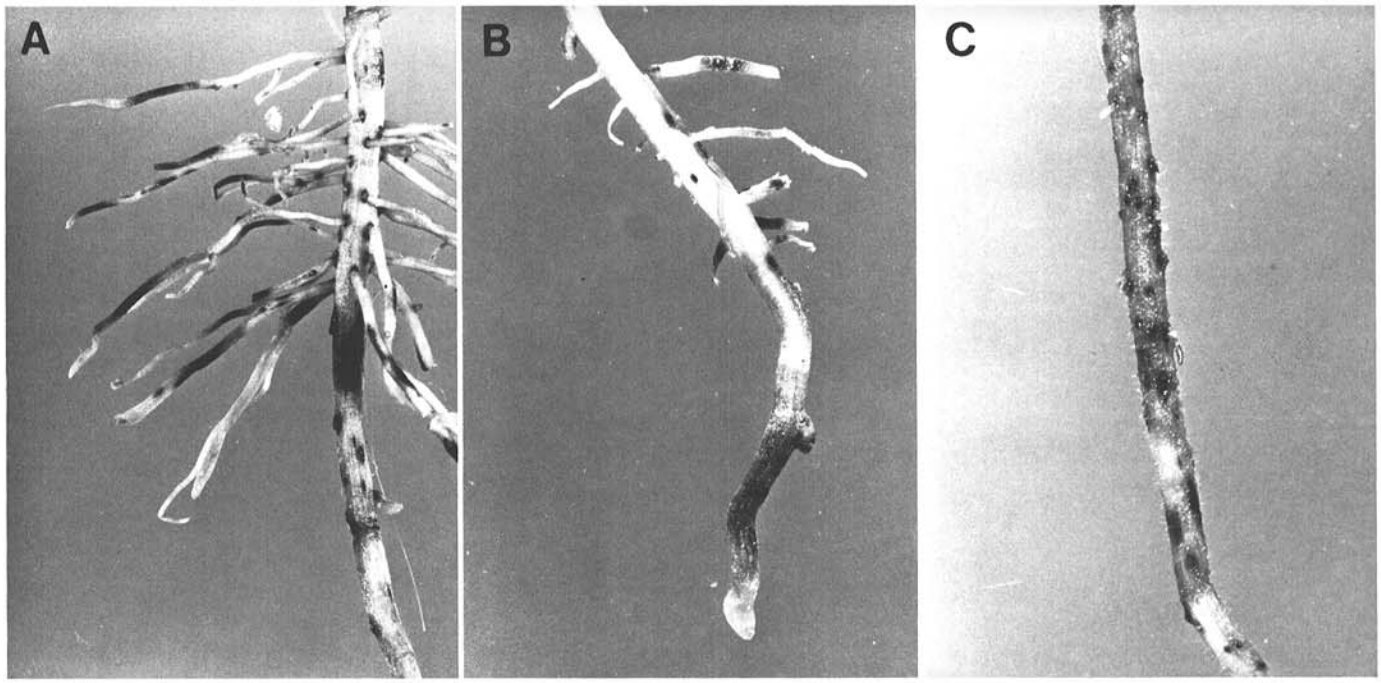

Fig. 2. Symptoms caused by *Pythium arrhenomanes* in roots from a plant of sugarcane cultivar CP 70-321. **A**, Lateral root rot symptoms. Note flaccid, water-soaked root tips, reddish brown lesion margins, and lesions at the base of roots. **B**, Lateral and primary root rot symptoms. Note water-soaked primary root tip with reddish brown lesion margin. **C**, Primary root section on which all lateral roots have been destroyed by *P. arrhenomanes*.

development was similar in inoculated and control plants. The most obvious symptoms were reddish discoloration and a reduction in extent of lateral root development (Fig. 1B). Similar symptoms were observed on field-collected roots from which the original isolations were made.

DISCUSSION

Ten isolates of *P. arrhenomanes* were demonstrated to be pathogenic to the modern sugarcane cultivar CP 70-321. In addition, preliminary results suggest that isolates of *P. arrhenomanes* can cause significant growth reductions in eight other sugarcane cultivars (unpublished). Isolates of four *Pythium* spp. not included in previous studies, *P. heterothallicum*, *P. irregulare*, *P. spinosum*, and *P. torulosum*, also were tested for pathogenicity, and only a single isolate of *P. spinosum* consistently caused significant reductions in plant growth. Isolates of the other three *Pythium* spp., *P. dissotocum*, and unidentified *Pythium* spp., infected sugarcane roots and some caused significant reductions in root and/or shoot weights in some experiments but not in others. Further investigation is needed concerning the pathogenicity of *P. spinosum* and interactions between *Pythium* spp. affecting sugarcane growth. *P. arrhenomanes* caused severe root rot in three experiments conducted under conditions of moderate soil temperatures, and significant variability in virulence was not detected among isolates. In two pathogenicity tests conducted under high soil temperatures, root rot symptoms in plants inoculated with *P. arrhenomanes* were less severe, with the most prominent symptoms being discoloration and rotting of lateral rather than primary roots. In addition, differences in virulence were evident among the isolates tested.

The pathogenicity test results were similar to results from experiments conducted during the 1930s (24), indicating that modern interspecific hybrid sugarcane cultivars also are susceptible to *P. arrhenomanes*. Symptom development and growth reductions were most severe under moderate temperature conditions; however, root rot never resulted in the death of plants. Feeder root necrosis apparently was sufficient to cause significant growth reductions at higher temperatures. Pythium root rot currently is not recognized as an important factor consistently limiting sugarcane stubble crop yields in Louisiana (20); however, results reported here suggest that a cryptic phase of Pythium root rot, in which the only shoot symptoms are reduced growth and tillering, is probably a factor contributing to sugarcane stubble decline in Louisiana.

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biological control of root rot was demonstrated in plants grown in