Pathogenicity of Pratylenchus brachyurus and Pythium graminicola to Sugarcane

Hideo Koike and Jesse Roman

Research Microbiologist, Crops Research Division, ARS, USDA, Gurabo, Puerto Rico, and Associate Nematologist, Department of Entomology and Nematology, Agricultural Experiment Station, University of Puerto Rico, Rio Piedras, Puerto Rico 00928, respectively. Present address of senior author: Research Plant Pathologist, Crops Research Division, ARS, USDA, Houma, Louisiana 70360.

Cooperative investigations of the Crops Research Division, ARS, USDA, and the Agricultural Experiment Station, University of Puerto Rico, Rio Piedras, Puerto Rico.

Appreciation is extended to A. F. Schmitthenner, Professor, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, Ohio, for identification of the *Pythium* isolate used in these investigations.

Accepted for publication 1 June 1970.

ABSTRACT

The root lesion nematode *Pratylenchus brachyurus* produced a significant reduction in top growth of sugarcane in greenhouse experiments. The root-rotting fungus *Pythium graminicola* significantly reduced top and root growth. When plants were grown in the presence of both pathogens, reduction in dry wt were the same as those caused by the root-rotting fungus alone. This is the first report of the pathogenicity of *P. brachyurus* to sugarcane, and of the isolation of and pathogenicity of *P. graminicola* to sugarcane in Puerto Rico. Phytopathology 60:1562-1565.

Several species of nematodes have been implied or proven to be pathogenic to sugarcane (species and interspecific hybrids of *Saccharum*). These parasitic nematodes are widely distributed throughout the sugarcane-growing areas of the world (4), including Puerto Rico (7, 8). The root lesion nematode, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941, is commonly associated with sugarcane in the field in Puerto Rico and other sugarcane-growing areas of the world (4). The authors, however, know of no previous published report concerning the pathogenicity of *P. brachyurus* to sugarcane.

Root rot of sugarcane, caused by species of the fungus *Pythium,* was at one time a major disease of sugarcane throughout the world. Due to the use today of improved hybrid varieties and cultural practices, the disease is not considered of major importance in most sugarcane-growing countries (6). Koike (*unpublished data*) recently demonstrated that several *Pythium* species are associated with sugarcane roots and soils in Puerto Rico. *Pythium graminicola* Subr. was found the most pathogenic of these *Pythium* species to sugarcane.

Apt & Koike (2) presented evidence indicating a positive interaction between the root knot nematode *Meloidogyne incognita acrita* Chitwood, 1949, and *P. graminicola* on the growth of sugarcane. Thus, although the *Pythium* disease is not considered of major importance in most sugarcane-growing countries, it is possible that the disease may be of significance in the presence of certain parasitic nematodes. In a related study, Apt & Koike (1) did not find an interaction between *Helicotylenchus nannus* Steiner, 1945 (*H. dihystera* [Cobb, 1893], Sher, 1961) and *P. graminicola.*

The present study was undertaken to test whether or not *P. brachyurus* is pathogenic on sugarcane, and to evaluate the possibility of an interaction between *P. brachyurus* and *P. graminicola.*

MATERIALS AND METHODS.—Stalks of the sugarcane variety P.R. 980 were grown from cuttings that had been treated with hot water (50 C for 2 hr) to control viruses and insects. Single-bud cuttings were taken from these stalks and were germinated in flats containing methyl bromide-fumigated cachaza (screened sugarmill filter press cake). When the plants were about 5 weeks old, the roots were washed free of cachaza and a single plant was transplanted to each of forty 8-inch clay pots in each of two tests. Each pot contained 0.04 m^3 of a sandy loam amended with 10% cachaza (v/v) which had been fumigated with methyl bromide at 1.4 kg/2.8 m^3.

Two tests were conducted. Each test consisted of four treatments which were replicated 10 times. Each replication consisted of a single plant in a pot. Treatments were: (i) uninfested control; (ii) *P. brachyurus* alone; (iii) *P. graminicola* alone; and (iv) *P. brachyurus* + *P. graminicola.*

The two tests differed in the following respects: *Test 1.*—The shoots were separated from parent cuttings prior to transplanting. Upon transplanting, ca. 2,800 nematodes were pipetted on and around the roots of each of 20 plants. The test was initiated on 18 December 1968 and terminated on 24 March 1969. The max and min temp in the greenhouse were 36.7 C and 15.0 C, respectively. Mean max and min temp were 31.8 C and 19.3 C, respectively, and the mean temp was 25.7 C.

Test 2.—Plants were not separated from parent cuttings when they were transplanted to the pots. One week after transplanting, ca. 16,000 nematodes were introduced into each pot through five holes made through the root zone. The test was initiated 15 April 1969 and terminated on 14 July 1969. Maximum and min temp were 38.3 C and 18.9 C, respectively. Mean max and min temp were 35 C and 23.3 C, respectively, and the mean temp was 29 C.

The nematode inoculum consisted of aq suspensions of root lesion nematodes obtained from monoxenic cultures on alfalfa callus tissue by the methods of Krusberg (5). The nematode culture was originally obtained from cotton in North Carolina.

The isolate of *P. graminicola* used was obtained from sugarcane roots grown in field soil from Arecibo, Puerto Rico. Each of the pots to be infested with *P. graminicola* received 50 cc of a 10-day-old cornmeal sand inoculum 2 weeks after introduction of the nematodes. The soil from the upper 5 cm near the root zone was carefully removed. The inoculum was then spread on and around the roots and covered with soil.

The pots were spaced on two greenhouse benches in a complete randomized block design and were fertilized with a complete fertilizer and watered as necessary.

At the conclusion of the test, the following determinations were made: (i) height of the primary shoot in each pot; (ii) number of tillers; (iii) oven-dry (85 C for 7 days) wt of shoots; and (iv) oven-dry (85 C for 4 days) wt of roots. Roots from each pot were examined for symptoms. Isolations were made from root pieces showing lesions or discoloration. These pieces were washed under a spray of tap water for several hr, surface-sterilized in 0.5% sodium hypochlorite for 2 min, rinsed 3 times with sterile deionized water, and plated on water agar containing 10 ppm pimaricin. Numbers of lesion nematodes in each pot were determined by separating the nematodes from 150 cc of soil by the method of Christie & Perry (3). Numbers of lesion nematodes in 5 g of roots from each pot were determined using Baermann funnels for 5 days.

RESULTS AND DISCUSSION.—*Test 1.*—Inoculation with *P. graminicola* or *P. graminicola* + *P. brachyurus* brought about a significant reduction (< 0.01) in top and root dry wt, number of tillers, and height of the primary shoot (Table 1). There were no differences in growth between the treatments *P. graminicola* alone or *P. graminicola* + *P. brachyurus*. Plants inoculated with *P. brachyurus* alone did not differ significantly from the noninoculated control plants.

Root systems of plants grown in *Pythium*-infested soil were much smaller and generally darker than roots of plants not inoculated with *Pythium*. They possessed abundant reddish-brown to black lesions and fewer lateral roots. *Pythium graminicola* was reisolated from practically all the root segments of inoculated plants. Lesions suggestive of *Pythium* root rot were observed in two control plants and in three plants initially infested with nematodes alone. *Pythium graminicola* was recovered from these plants. This contamination was very likely due to water splashing from *Pythium*-infested pots during irrigation. The soil was free of pythiaceous fungi soon after fumigation.

Roots of plants inoculated with lesion nematodes alone did not differ appreciably from those of control plants in size or appearance. Approximately equal numbers of *P. brachyurus* individuals were recovered from roots of plants inoculated with nematodes alone and with both microorganisms (Table 1). A few *P. brachyurus* individuals were recovered from roots of noninoculated plants and plants inoculated with fungus alone. This contamination was probably due to water splashing from nematode-infested pots during irrigation. *Pratylenchus brachyurus* individuals were recovered from soils in pots initially infested with nematode alone, but not from soils initially infested with both microorganisms. When nematode individuals are in low numbers and unevenly distributed in soils, they may not be included in aliquot samples.

The reductions in top and root growth of plants inoculated with both microorganisms were evidently due solely to the fungus.

Test 2.—Inoculation with *P. graminicola* gave a significant reduction in top and root dry wt and height of primary shoots (Table 1, Fig. 1). Unlike test 1, *P. graminicola* did not decrease the number of tillers. The fungus was recovered from virtually all plants inoculated with *P. graminicola.*

Plants inoculated with *P. brachyurus* alone showed a slight but significant reduction in top wt and height of

TABLE 1. Effect of *Pratylenchus brachyurus* and *Pythium graminicola,* separately and in combination, on growth of the sugarcane variety P.R. 980[a]

Treatment	Dry wt (g)		No. of tillers	Height (cm), primary shoot	*P. brachyurus*[d] in	
	Top growth	Root			150 cc soil	5 g roots
Test 1[b]						
Control	60.4	20.3	6.3	67.6	0	0.8
Nematode	57.7	21.7	6.8	62.2	10.2	81.6
Fungus	26.2	6.0	3.1	45.5	0	0.3
Nematode + fungus	27.4	8.0	2.9	46.9	0	74.1
LSD (.05)	5.6	4.3	1.4	8.3		
(.01)	7.5	5.1	1.8	11.2		
Test 2[c]						
Control	119.4	26.2	3.1	107.5	0	0.1
Nematode	111.8	27.8	2.9	85.4	969.2	157.5
Fungus	83.9	16.3	3.7	83.0	0.1	1.4
Nematode + fungus	90.6	19.4	4.4	118.7	266.1	238.9
LSD (.05)	7.4	4.6	1.1	16.9		
(.01)	9.9	6.1	1.5	22.6		

[a] Figures are means of 10 replicates.
[b] Duration of test, 14 weeks (18 December 1968 to 24 March 1969). Mean temp in greenhouse 25.7 C.
[c] Duration of test, 13 weeks (15 April 1969 to 14 July 1969). Mean temp in greenhouse 29 C.
[d] Inoculum consisted of approx 2,800 nematodes per pot in Test 1 and 16,000 nematodes per pot in Test 2.

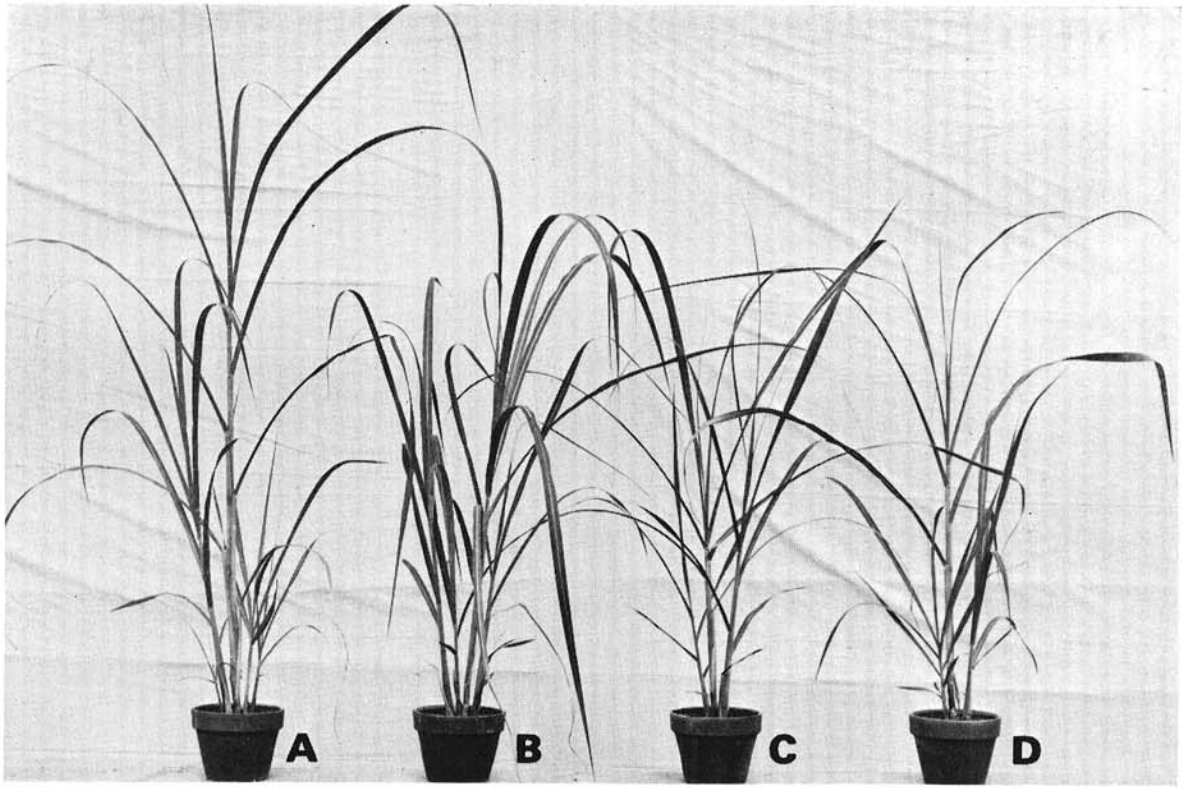

Fig. 1. Sugarcane plants (14.5 weeks old) of var. P.R. 980 grown in fumigated soil artificially infested with approximately 16,000 nematodes/pot of *Pratylenchus brachyurus* (B), *Pythium graminicola* (C), and *P. brachyurus* + *P. graminicola* (D), and in fumigated uninfested soil (A). Test 2.

the primary shoot. Relatively high numbers of *P. brachyurus* individuals were recovered from roots and soils to which only nematodes were added and where nematode + fungus were added (Table 1). The wide difference in numbers of root lesion nematodes recovered from soil infested with nematodes alone and with nematodes plus fungus is probably due to sampling error. Very low numbers of root lesion nematodes were recovered from roots of noninoculated and fungus-inoculated plants; splashing during irrigation very probably caused this contamination.

Plants inoculated with *P. graminicola* + *P. brachyurus* did not differ from plants inoculated with *P. graminicola* alone in top or root dry wt. Plants inoculated with both microorganisms, however, showed a significantly greater number of tillers than noninoculated control plants. Tillers from plants inoculated with fungus alone and with both microorganisms were much smaller than those from noninoculated control plants and plants inoculated with nematodes alone; tillers from plants inoculated with both microorganisms were slightly smaller than those from plants inoculated with fungus alone. Approximately 11% and 7% of the small tillers from plants inoculated with fungus alone and with both microorganisms were dead. The fungus alone evidently reduced the size of tillers and killed the tillers.

The significant increases in numbers of tillers and height of primary shoots as a result of the presence together in soil of *P. brachyurus* and *P. graminicola* in Test 2, but not in Test 1, is difficult to explain. In Test 2, in contrast to Test 1, (i) shoots were not separated from parent cuttings when transplanting to pots; (ii) many small tillers were already emerged on the shoots at time of transplanting; and (iii) higher temp prevailed in the greenhouse during growth of the shoots.

It is a common observation with sugarcane that, when growth of the tillers are limited (e.g., by root-rotting fungus), the primary shoot will elongate more than the primary shoot in a stool of sugarcane with large tillers. This probably explains the significantly greater height of primary shoots of plants inoculated with both microorganisms. In the treatment with fungus alone, although some tillers were dead, the surviving tillers were larger than those of plants infected with both microorganisms.

Results of our tests showed that the root lesion nematode *P. brachyurus* and the root-rotting fungus *P. graminicola* are each pathogenic to sugarcane. Both pathogens in combination did not result in a greater reduction in growth of the test variety. The reductions in top and root growth as a result of the presence of both agents together were evidently due to the fungus alone.

LITERATURE CITED

1. APT, W. J., & H. KOIKE. 1962. Pathogenicity of Helicotylenchus nannus and its relation with Pythium graminicola on sugarcane in Hawaii. Phytopathology 52:798-802.
2. APT, W. J., & H. KOIKE. 1962. Pathogenicity of Meloidogyne incognita acrita and its relation with Pythium graminicola on sugarcane in Hawaii. Phytopathology 52:1180-1184.
3. CHRISTIE, J. R., & V. G. PERRY. 1951. Removing nematodes from soil. Helminthol. Soc. Washington, D.C. Proc. 18:106-108.
4. HOLTZMANN, O. V. 1964. Nematodes and sugar cane, p. 319-341. *In* C. G. Hughes, E. V. Abbott, & C. A. Wismer [ed.] Sugar-cane diseases of the world. Vol. II. Elsevier Publishing Co., Amsterdam, The Netherlands.
5. KRUSBERG, L. R. 1961. Studies on the culturing and parasitism of plant-parasitic nematodes, in particular Ditylenchus dipsaci and Aphelenchoides ritzemabosi on alfalfa tissues. Nematologica 6:181-200.
6. RANDS, R. D. 1961. Root rot, p. 288-309. *In* J. P. Martin, E. V. Abbott, & C. G. Hughes [ed.] Sugarcane diseases of the world. Vol. I. Elsevier Publishing Co., Amsterdam, The Netherlands.
7. ROMAN, J. 1965. Nematodes of Puerto Rico: The genus Helicotylenchus Steiner, 1945 (Nematoda: Hoplolaimidae). Agr. Exp. Sta., Univ. Puerto Rico Tech. Paper 41:1-23.
8. ROMAN, J. 1967. Preliminary investigations on the nematodes associated with sugarcane in Puerto Rico, p. 1401-1407. 12th Congr. Int. Soc. Sugar Cane Technol. Proc. 1965. (P.R.), Elsevier Publishing Co., Amsterdam, The Netherlands.