

# Pythium Root Rot of Sugarcane in Puerto Rico.

## I. Pathogenicity and Identification<sup>1</sup>

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### ABSTRACT

*Pythium aphanidermatum*, a previously unreported species for Puerto Rico, and *P. deliense* were frequently isolated from sugarcane fields in the southern coast of Puerto Rico where symptoms of ratoon stunting disease were also observed. *P. acanthicum*, *P. butleri*, *P. echinocarpum*, *P. graminicola*, *P. oligandrum* and *P. periplocum* were also isolated. The pathogenicity of these *Pythium* species to sugarcane variety PR 1152 was demonstrated in replicated laboratory and greenhouse tests. *P. aphanidermatum* alone significantly reduced dry weights of top and root growth of sugarcane variety PR 1085. Reductions in dry weights of top and root growth incited by the combination of *P. deliense* and *P. aphanidermatum* were the same as those caused by *P. aphanidermatum* alone. However, the reductions in dry weights of top by inoculation of sugarcane with both *P. aphanidermatum* and the ratoon stunting disease causal agent were greater than those caused by inoculation with either one alone.

### INTRODUCTION

Root diseases caused by pythiaceous and non-pythiaceous fungi have long been considered among the most serious diseases affecting sugarcane in Puerto Rico (1). Matz in 1920 (6) isolated a *Pythium* sp. from sugarcane roots in Puerto Rico and proved its pathogenicity, but failed to identify the species. Koike in 1969 (2) isolated several species of *Pythium* from sugarcane roots and soil in Puerto Rico and reported on the pathogenicity of *P. graminicola* to commercial variety PR 980. Pathogenicity of *P. tardicrescens* to sugarcane has also been reported (3). Recently Liu (5) reported on the pathogenicity of several *Pythium* species isolated from sugarcane in Puerto Rico.

In the fall of 1974, the locally developed varieties, PR 62-2314, PR 61-632, PR 1152, PR 64-1791, PR 65-625, M 336 and B 49119 were severely affected by a root disease complex in certain fields of Central Mercedita on the southern coast of the Island. Symptoms of ratoon stunting disease were also observed. More than 50% of the *Pythium* isolates from that area were identified as *P. aphanidermatum* or *P. deliense*. This paper reports on the identification and pathogenicity of *Pythium* spp. isolated from sugarcane in Puerto Rico, and on the interaction between *P. aphanidermatum* and the ratoon stunting disease agent (RSD).

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## MATERIALS AND METHODS

## ISOLATION AND IDENTIFICATION

Roots of sugarcane showing reddish brown lesions resembling *Pythium* root rot were collected from 25 fields at Central Mercedita, Fortuna, Lajas, Central Plata, Fajardo and Humacao in the southern, central and eastern parts of Puerto Rico, respectively (table 1). A selective cultural medium consisting of corn meal agar, 18 g; pimaricin, 2 ml; streptomycin sulfate, 0.1 g; rose bengal, 0.12 g; and benomyl, 0.0025 g per 1000 ml of

TABLE 1.—Sources of inoculum of *Pythium* spp. from sugarcane

Species	Crop	Location	Year	Variety
<i>P. aphanidermatum</i>	plant cane	Central Mercedita	1975	P.R. 66-2314
	1st ratoon	Central Mercedita	1975	P.R. 61-632
	1st ratoon	Central Mercedita	1975	P.R. 61-632
	ratoon cane	Central Mercedita	1975	P.R. 1152
	ratoon cane	Central Mercedita	1975	P.R. 64-1791
	plant cane	Central Mercedita	1975	P.R. 65-625
	ratoon cane	Central Mercedita	1975	P.R. 65-625
	ratoon cane	Central Mercedita	1975	P.R. 64-1791
	ratoon cane	Central Mercedita	1975	P.R. 1152
	ratoon cane	Central Mercedita	1975	M. 336
<i>P. deliense</i>	plant cane	Central Mercedita	1975	P.R. 66-2314
	1st ratoon	Central Mercedita	1975	P.R. 61-632
	ratoon cane	Central Mercedita	1975	P.R. 65-625
<i>P. butleri</i>	ratoon cane	Central Mercedita	1975	M. 336
	ratoon cane	Central Mercedita	1975	P.R. 64-1791
<i>P. oligandrum</i>	ratoon cane	Central Mercedita	1975	P.R. 65-625
	ratoon cane	Central Mercedita	1975	P.R. 1152
<i>P. periplocum</i>	ratoon cane	Central Mercedita	1975	M. 336
	1st ratoon	Lajas	1975	B. 49119
<i>P. graminicola</i>	plant cane	Fortuna	1974	P.R. 980
	plant cane	Central Mercedita	1974	P.R. 980
<i>P. acanthicum</i>	1st ratoon	Central Mercedita	1974	P.R. 980
	1st ratoon	Fajardo		P.R. 980
<i>P. echinocarpum</i>	1st ratoon	Humacao		P.R. 1059

water was used to isolate the species of *Pythium*. Five representative *Pythium* isolates were sent to the Commonwealth Mycological Institute, Kew, Surrey, England for identification.

## PATHOGENICITY TESTS

Pathogenicity tests with the *Pythium* isolates were conducted. Single-eyed seedpieces of sugarcane variety PR 1152 were obtained from a nursery planted with hot-water-treated stock which was planted in steam-sterilized soil. Three weeks after germination, plants were transplanted

to test tubes (100 ml) containing liquid cultures (Czapex dox broth 35 g + 100 ml water) of the various *Pythium* isolates. Ten tubes were used for each species of *Pythium*. Tubes containing only Czapek dox broth were used as controls.

#### EFFECT ON STEM AND ROOT GROWTH

The effect of various species of *Pythium* on root growth of sugarcane was tested in two replicated trials. PR 1152, a promising variety, was used in both trials. Koike's method (4) for testing the effect of *Pythium* on growth and root development of sugarcane was used. This consisted of inoculating roots of sugarcane with *Pythium* grown in a corn meal and sand medium. Seedpieces of sugarcane variety PR 1152 were derived from disease-free, hot water treated nursery stock (50° C for 2 h). Two cuttings were planted in 25-cm clay pots, each containing 65 cm<sup>3</sup> of a steam-sterilized 2:1 soil-cachaza mixture. The mixture used for these experiments was steam sterilized.

The first experiment was planted December 22, 1975, and harvested April 22, 1976. The following treatments were used: 1) soil infested with *P. aphanidermatum*; 2) soil infested with *P. deliense*; 3) soil infested with *P. periplocum*; and 4) uninfested controls. The pots were placed on two greenhouse benches with the control and *Pythium*-infested pots arranged in a complete randomized block design. The experiment was replicated eight times. The mean maximum temperatures were 31.2 and 17.2° C, respectively with an average of 22.4° C.

The second experiment was planted June 29, 1976, and harvested October 29, 1976. The treatments consisted of infesting the soil with the following species of *Pythium*: *P. oligandrum*, *P. periplocum*, *P. graminicola*, *P. echinocarpum*, *P. aphanidermatum*, *P. butleri*, *P. deliense*, and uninfested controls. The pots were arranged on greenhouse benches in a complete randomized block design with five replications. The mean maximum and minimum temperature were 35.2° and 21.4° C, respectively with an average of 28° C.

#### EFFECT OF *Pythium* SPP. ON GROWTH

The variety PR 1085, which has shown a great deal of deterioration in ratoons under field conditions, was chosen for these studies. The experiment with a complete randomized design was initiated August 2, 1975 and harvested November 2, 1975. The treatments consisted of infesting the soil with species of *Pythium*: *P. aphanidermatum*, *P. deliense*, *P. aphanidermatum*, and *P. deliense*, and uninfested controls. The mean maximum and minimum temperature in the greenhouse during the period was 32.4° and 18.2° C, respectively, with a mean of 23.5° C.

INTERACTION BETWEEN *Pythium* AND RATOON STUNTING DISEASE

Single-eyed cuttings of PR 1152, derived from seedpieces previously inoculated with juice extracted from RSD-affected plants were planted February 12, 1975 in metal flats containing a steam sterilized soil and cachaza mixture. Cuttings derived from hot water treated seedpieces were planted as controls. The experiment, replicated five times in a complete randomized design, was harvested June 12, 1975. Included were four treatments with seedpieces from the following sources: RSD-affected nursery; hot water treated nursery; RSD-affected nursery, planted in soil

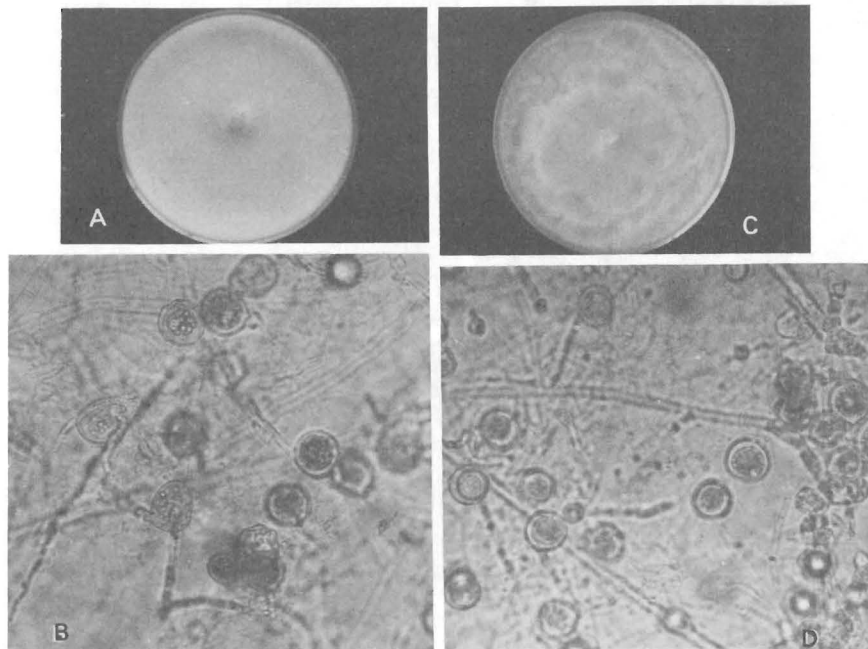


FIG. 1.—Cultural appearance and oogonia or oospores of the two most frequently isolated species of *Pythium* from sugarcane on the southern coast of Puerto Rico: A and B, *P. aphanidermatum*; C and D, *P. deliense*.

infested with *P. aphanidermatum*; and hot water-treated nursery, planted in soil infested with *P. aphanidermatum*. The mean maximum and minimum temperature during the period was 23.8° and 20.6° C, respectively, with a mean of 28.4° C.

In all the replicated greenhouse experiments, both fresh weights and dry weights of top and root growth were recorded. The dry weights were obtained by oven-drying the fresh stem and root at 110° C for 24 h. Roots from all plants were examined for root rot symptoms. *Pythium* spp. were reisolated from root pieces showing lesions or discoloration.

## RESULTS

## ISOLATION AND IDENTIFICATION

*Pythium aphanidermatum*, *P. deliense*, *P. acanthicum*, *P. butleri*, *P. echinocarpum*, *P. oligandrum*, *P. graminicola* and *P. deliense* were isolated from the Central Mercedita area on the southern coast of the Island. *P. butleri*, *P. oligandrum* and *P. periplocum* were isolated from

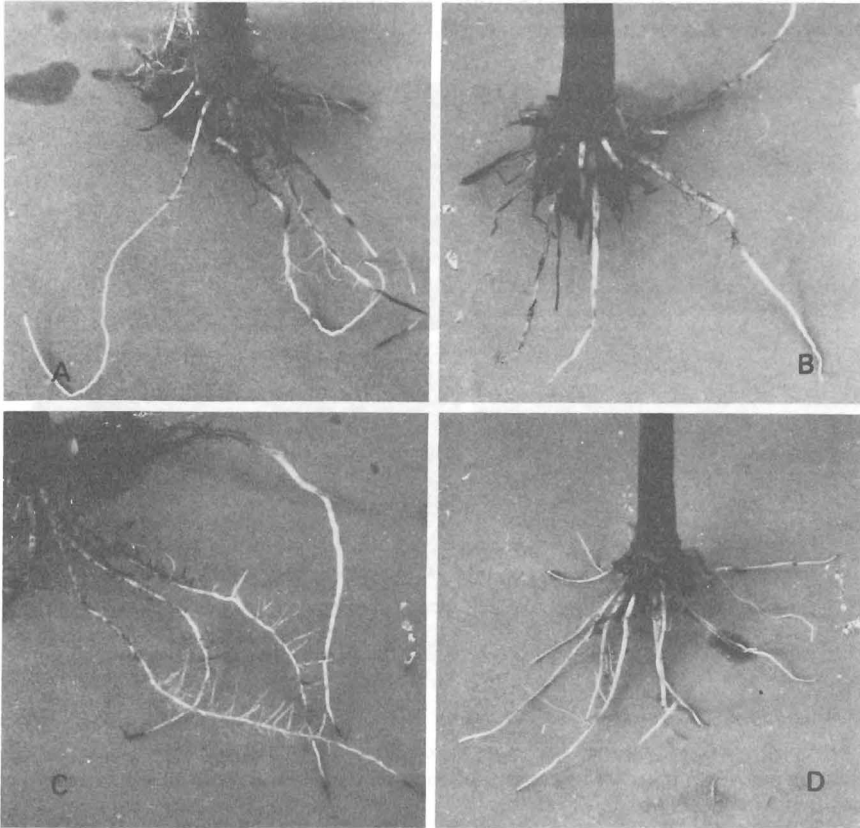


FIG. 2.—Lesions on roots of 6-week-old plants of variety PR 1152 growing in liquid cultures of four species of *Pythium*. A, *P. graminicola*; B, *P. aphanidermatum*; C, *P. deliense*; D, control-no *Pythium*.

roots of varieties PR 64-1791, PR 65-625 and M 336 from the same general area. *P. graminicola*, *P. acanthicum* and *P. echinocarpum* were isolated from roots of PR 980 and PR 1059 from Central Plata, Fajardo and Humacao (table 1).

*Pythium aphanidermatum*, *P. deliense*, *P. butleri*, *P. acanthicum* and *P. echinocarpum* were identified by the Commonwealth Mycological

Institute, Kew, Surrey, England. *P. oligandrum*, *P. periplocum* and *P. graminicola* were identified by the author by comparison with the known *Pythium* cultures supplied by Dr. H. Koike (3). Figure 1 shows the cultural appearance and oogonia of the two most frequently isolated *Pythium* species (*P. aphanidermatum* and *P. deliense*).

#### PATHOGENICITY TESTS

The results from inoculation studies on sugarcane variety PR 1152 with *P. aphanidermatum*, *P. deliense*, *P. acanthicum*, *P. butleri*, *P.*

TABLE 2.—Effect of three species of *Pythium* on stem growth and root development of sugar cane variety PR 1152

Treatment	Dry weight (grams)	
	Stem	Root
<i>Pythium aphanidermatum</i>	236 <sup>1*</sup>	43*
<i>Pythium deliense</i>	214*	46*
<i>Pythium periplocum</i>	321	68
Control	322	79

<sup>1</sup> Average of 8 replications.

\* Significantly different from the control at the 5% level.

TABLE 3.—Effect of *Pythium* spp. on stem and root growth of sugarcane variety PR 1152

Treatment	Dry weight (grams)	
	Stem	Root
<i>P. oligandrum</i>	72 <sup>1</sup>	41
<i>P. periplocum</i>	70	41
<i>P. graminicola</i>	53 <sup>2</sup>	31 <sup>2</sup>
<i>P. echinocarpum</i>	52 <sup>2</sup>	30 <sup>2</sup>
<i>P. aphanidermatum</i>	53 <sup>2</sup>	31 <sup>2</sup>
<i>P. butleri</i>	52 <sup>2</sup>	31 <sup>2</sup>
<i>P. deliense</i>	60 <sup>2</sup>	31 <sup>2</sup>
Control	77	42

<sup>1</sup> Average of 5 replications.

<sup>2</sup> Significantly different at the 1% level.

*echinocarpum*, *P. graminicola*, *P. oligandrum* and *P. periplocum* indicate that all the species mentioned above could infect roots of sugarcane variety PR 1152 and induce symptoms similar to those reported for *Pythium* root rot. The first lesions appeared 4 days after transplanting the healthy plants into a test tube containing the liquid cultures of the *Pythium* isolate (fig. 2). No such symptoms occurred in roots of control plants.

EFFECT OF *Pythium* SPP. ON STEM AND ROOT GROWTH

Tables 2 and 3 indicate that dry weights of roots and stems of variety PR 1152 inoculated with *P. aphanidermatum*, *P. deliense*, *P. graminicola*, *P. butleri*, and *P. echinocarpum* were significantly reduced but not those inoculated with *P. oligandrum* and *P. periplocum*.

EFFECT OF *Pythium* SPP. ON TOP AND ROOT GROWTH

Table 4 shows that *P. aphanidermatum* alone significantly reduced dry weights of top and root growth of variety PR 1085. Reductions in dry

TABLE 4.—Effect of three *Pythium* species on stem growth and root development of sugarcane variety PR 1085

Treatment	Dry weight (grams)	
	Stem	Root
<i>Pythium deliense</i>	33 <sup>1, 2</sup>	11 <sup>2</sup>
<i>Pythium aphanidermatum</i>	31 <sup>2</sup>	10 <sup>2</sup>
<i>Pythium aphanidermatum</i> + <i>Pythium deliense</i>	29 <sup>2</sup>	9 <sup>2</sup>
Control	47	16

<sup>1</sup> Average of 7 replications.

<sup>2</sup> Significantly different from the control at the 1% probability level.

TABLE 5.—Interaction between *Pythium aphanidermatum* and ratoon stunting disease agent on sugarcane variety PR 1152

Treatment	Dry weight (grams)	
	Stem	Root
Hot water treated cane (control)	104 <sup>1</sup> a	52 <sup>2</sup> a
Hot water treated cane inoculated with <i>Pythium aphanidermatum</i>	85 b	41 b
Ratoon stunt affected cane	66 c	37 c
Ratoon stunt affected cane inoculated with <i>Pythium aphanidermatum</i>	53	31 c

<sup>1</sup> Average of 5 replications.

<sup>2</sup> Means followed by the same letter do not differ significantly at the 5% probability level.

weights of top and root growth by the combination of *P. deliense*, and *P. aphanidermatum* were similar to those caused by *P. aphanidermatum* alone.

INTERACTION BETWEEN *Pythium aphanidermatum* AND RATOON STUNTING AGENT

Table 5 shows that the reduction in dry weights of top growth by inoculation of sugarcane with both *P. aphanidermatum* and ratoon stunting disease agent was greater than that when inoculated by either

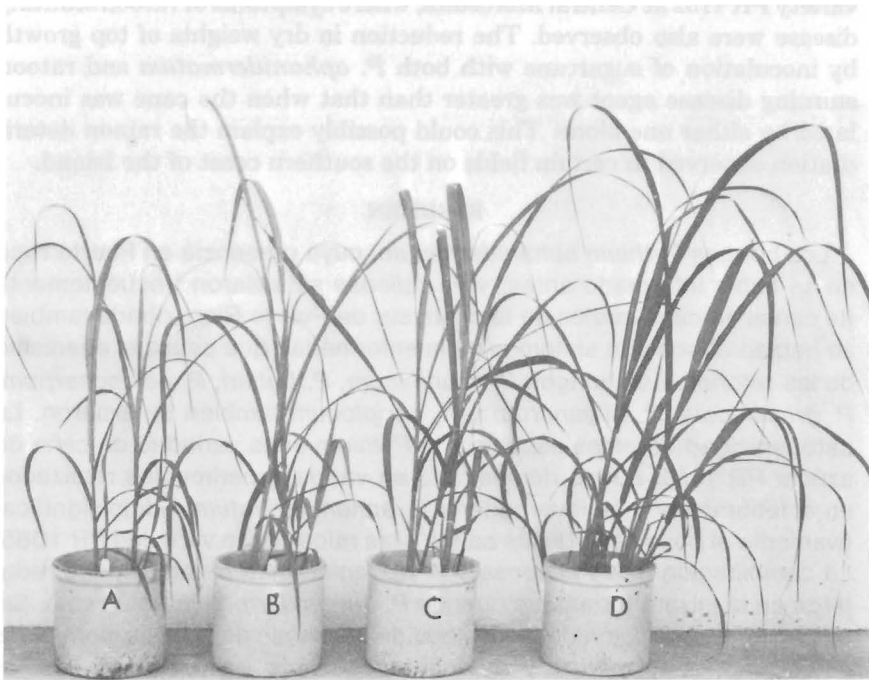


FIG. 3.—Growth differences between plants of variety of PR 1152 affected by *Pythium aphanidermatum* alone and those affected by a combination of *Pythium* and ratoon stunting disease agent. A, ratoon stunt affected canes inoculated with *P. aphanidermatum*; B, ratoon stunt affected cane; C, hot water treated canes inoculated with *P. aphanidermatum*; D, hot water treated canes (control).

one alone. Figure 3 shows the differences in top growth between canes affected by ratoon stunting disease alone and those affected by the combination of *Pythium* and ratoon stunting disease.

#### DISCUSSION

The results obtained agree in general with the findings of Koike (3, 4, 5), who reported that *P. graminicola* reduced significantly the dry weights of top and root growth of sugarcane. In addition, *P. aphanidermatum*, *P. deliense*, *P. butleri*, and *P. echinocarpum* were also demonstrated to reduce dry weights of top and root growth of sugarcane variety PR 1152 in Puerto Rico.

*Pythium echinocarpum*, *P. acanthicum*, and *P. aphanidermatum* had not been reported on sugarcane in Puerto Rico. The first two species were isolated from roots of varieties PR 1152 and PR 980, respectively, in the Fajardo and Humacao areas on the eastern coast of the Island. The third species and *P. deliense* were frequently isolated from roots of



variety PR 1152 at Central Mercedita, where symptoms of ratoon stunting disease were also observed. The reduction in dry weights of top growth by inoculation of sugarcane with both *P. aphanidermatum* and ratoon stunting disease agent was greater than that when the cane was inoculated by either one alone. This could possibly explain the ratoon deterioration observed in certain fields on the southern coast of the Island.

#### RESUMEN

Los hongos *Pythium aphanidermatum*, cuya presencia en Puerto Rico no se había informado antes, y *P. deliense* se aislaron frecuentemente de cañas en cañaverales de la costa sur de Puerto Rico, donde también se habían observado síntomas de la enfermedad que causa el enanismo de los retoños. Los hongos *P. acanthicum*, *P. butleri*, *P. echinocarpum*, *P. graminicola*, *P. oligandrum* y *P. periplocum* también se aislaron. La patogenicidad de estas especies de *Pythium* en la variedad de caña de azúcar PR 1152 quedó demostrada en varios experimentos realizados en el laboratorio y en invernadero. *P. aphanidermatum* redujo significativamente el peso seco de las cañas y las raíces de la variedad PR 1085. La combinación de *P. deliense* y *P. aphanidermatum* también los redujeron en la misma forma que cuando *P. aphanidermatum* actúa solo. Sin embargo, la reducción del peso seco de las cañas de plantas inoculadas con *P. aphanidermatum* y el agente causante del enanismo de los retoños fue más severa que en las plantas inoculadas con el uno o el otro separadamente.

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