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Pathogenicity of the Reniform Nematode on Various Hosts

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INTRODUCTION

The reniform nematode *Rotylenchulus* Linford and Oliveira, 1940, is one of the most common plant-parasitic nematodes in Puerto Rico, as are the spiral, root-lesion, and root-knot nematodes. It is well distributed in our cultivated fields and its populations are usually very high.

New plant hosts of this type of nematodes are constantly being found. At the same time morphological differences have been observed, suggesting that, in Puerto Rico, it is represented by a group of species which, in turn, have their own host preferences. With this in mind an experiment was conducted in the greenhouse. The culture to be studied was obtained from pigeonpea roots, *Cajanus indicus* Spreng., which is one of the most susceptible hosts to the reniform nematode attack. In this experiment six different plant species, and varieties known to be natural hosts of this nematode in the Island, were used. Their susceptibility and suitability as hosts to an isolate of the nematode were tested. At the same time the effect of the nematode on the plant was studied and will be discussed here.

The reniform nematode has been one of the least-studied genera. It was originally described in Hawaii as an obligate parasite of cowpeas (*6*).[2] The same year Linford and Yap (*7*) published a list of 65 host plants; the susceptibility of some of them was determined by field observations and of most of them by experiments conducted under controlled conditions.

One year later its occurrence in the United States was reported by Smith and Taylor (*15*). Steiner (*16*, *17*) also reported its presence in Georgia on two different hosts. It was not until 1954, when Neal (*11*) found it associated with *Fusarium* wilt in cotton in Louisiana, that this nematode was considered of economic importance. Furthermore, Martin in 1960 (*9*) found that it was an important factor in production of the sweetpotato.

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 81-2.

Further publications revealed its presence in Rhodesia and Nyassaland (*8*), Alabama (*10*), Florida (*19*), Texas (*1*), West Africa (*11*), and Guam (*14*). Steiner (*18*) reported it attacking many plants in Puerto Rico, specifically pigeonpeas.

MATERIALS AND METHODS

Six different plant species, including two varieties of pineapple were used, *i.e.* pigeonpeas, *Cajanus indicus* Spreng.; tobacco, *Nicotiana tabacum* L.; coffee, *Coffea arabica* L. var. Puerto Rico; tomato, *Lycopersicon esculentum* Mill. var. Rutgers; pineapple, *Ananas sativus* Schultes vars. Red Spanish and Smooth Cayenne, and sugarcane, *Saccharum officinarum* L. var. B. 37–161. These were set in 10- and 12-inch pots which were filled with fumigated soil. Eight plants of each variety were planted. After 15 days when all plants had rooted, 10,000 preparasitic females, juveniles and males of *Rotylenchulus* sp. from pigeonpeas, were inoculated to four plants of each variety. The other four plants were left as controls.

The pots were distributed at random on a greenhouse bench and the plants allowed to grow for 4 months. Fertilizer was applied to each plant once only, and sprayings with Malathion were made every month for the control of aphids and mites.

After 4 months all the plants were dug out and their roots washed carefully to prevent the loss of females and egg masses. The plants and roots were weighed separately and countings were made of the number of leaves and side branches when present. Observations on the appearance of the plants and their roots were also recorded, but no data were obtained on yield.

Soil samples were taken for the determination of populations of juveniles, preparasitic females, and males in the soil. Three hundred cubic centimeters of soil were processed for each sample. One gram of roots from each plant was examined directly under the microscope to determine the presence of immature females and mature females producing and not producing eggs, as well as their size. Observations were made on location of the female heads in the tissue and the gross damage they did to the roots. Conclusions as to suitability of a host were mainly based on the nematode populations in the soil and in the roots. Damage caused to the roots and plants was taken also as a criterion of susceptibility of the plant to the attack of the nematode.

RESULTS AND OBSERVATIONS

Six of the seven kinds of plants tested proved to be susceptible to this species of the reniform nematode, *Rotylenchulus* sp. Only sugarcane was found to be completely resistant since no females or juveniles were obtained

from its roots after 4 months (table 1). Marked differences were observed among the other plants as suitable hosts for this parasite.

Pigeonpeas proved to be the preferred host. Their roots harbored the greatest number of nematodes of all stages. A total of 15,901 parasitic females in different stages of development were seen attached to 1 gm. of roots and there were 456,000 juveniles, preparasitic females, and males per 300 cc. of soil. Tobacco seemed to be second in preference, followed by tomato, pineapple, and coffee. Smooth Cayenne was more susceptible than Red Spanish. Coffee roots harbored fewer females, but populations in the soil were almost as high as in Red Spanish variety of pineapple. No females

TABLE 1.—*Populations of* **Rotylenchulus** *sp. in the roots and soil from 7 different host plants*

Host		Eel-shaped females	Females with small secretions	Females with large secretions	Females without secretions	Totals	Nematode populations in the soil
Common name	Scientific name						
Tobacco	*Nicotiana tabacum* L.	525	536	1,027	295	2,383	163,427
Tomato	*Lycopersicon esculentum:* Mill	308	511	64	302	1,185	104,799
Coffee	*Coffea arabica* L.	150	9	339	81	579	9,225
Pineapple (Smooth Cayenne var.)	*Ananas sativus Sch.*	403	43	1,583	176	2,205	32,100
Pineapple (Red Spanish var.)	*A. sativus* Sch.	316	23	1,221	76	1,673	11,200
Pigeonpeas	*Cajanus indicus* Spreng.	3,328	6,409	5,427	727	15,901	456,000
Sugarcane	*Saccharum officinarum* L.	0	0	0	0	0	0

were obtained from roots of sugarcane and only a few males were present in the soil.

EFFECT OF NEMATODE POPULATIONS ON THE PLANTS

Although five of the plant types were found to be good hosts for the isolate of this nematode, only two showed damage on the aerial parts. As may be observed in table 2, a decrease in growth of pigeonpea plants was caused by the nematodes. The weight of stems and roots and number of side branches was lower. The inoculated plants appeared less vigorous and also yellowish compared with the very green and healthy-looking controls.

Differences were observed in the coffee plants, although their nematode populations remained lower than in the other five plant species. As shown in table 2 the length of stem, number of leaves, number of side branches,

and weight of stems and roots were lower in the infected plants. The stems were narrower and the leaves were yellowish.

No significant differences were noticed in the aerial parts of tomato, tobacco, and pineapple between infected and healthy plants.

GROSS OBSERVATIONS ON THE ROOTS

Gross observations showed that, in the susceptible plants, several females were usually concentrated on a small section of the root (fig. 1) where the gelatinous secretions formed a compact covering of the root. The females in different stages of development were seen in various positions on the

TABLE 2.—*Data from experiment with different plant hosts infected with* **Rotylenchulus** *sp.*

Host	Condition	Length of stem	Branches	Weight of stem	Weight of roots	Leaves
		Cm.	*Number*	*Grams*	*Grams*	*Number*
Cajanus indicus Spreng.	Control	195.00	7.75	237.6	66.00	—
	Infected	151.48	7.50	152.5	53.75	—
Coffea arabica L.	Control	66.00	6.50	158.4	24.28	50.00
	Infected	57.20	4.25	139.8	10.50	36.50
Nicotiana tabacum L.	Control	140.64	3.25	149.0	52.13	37.00
	Infected	147.50	4.00	149.0	27.50	36.75
Lycopersicon esculentum Mill.	Control	148.75	—	224.00	49.25	28.75
	Infected	166.25	—	245.00	45.75	32.50
Ananas sativus Sch. (Red Spanish var.)	Control	17.50	—	291.76	4.4	20.80
	Infected	17.75	—	347.76	4.0	20.75
A. sativus Sch. (Smooth Cayenne var.)	Control	21.86	—	499.04	9.17	25.33
	Infected	21.25	—	490.00	8.13	25.25
Saccharum officinarum L.	Control	85.00	—	542.64	5.06	28.00
	Infected	86.88	—	448.00	5.45	27.00

roots. In some instances they were seen either on one side only of the root, or on both opposite sides, but never around it completely. In general, all roots were attacked, but secondary and tertiary roots seemed to be preferred. A quantitative study was not made. Often eel-shaped females were observed in older roots which suggests that they do not depend on tender tissue of the growing region of the roots.

Traces of necrotic tissue were regularly observed around the female nematode head, particularly in tender roots of tomato and pigeonpeas, but necrosis never extended beyond a few millimeters from the head attachment. A brownish discoloration of the root was prevalent in the older roots of many females. This, combined with an abundance of gelatinous secretions covered by adhering soil particles, regularly produced a darker color in infected roots of pigeonpeas, tomatoes, and tobacco.

Frequently, female nematodes were so numerous on thin roots that these appeared completely black or brown and, in some cases, were rotting. A severe curving of young roots was observed where tender roots had been attacked. In other cases, and when females attacked the root tips, proliferation of the roots was marked.

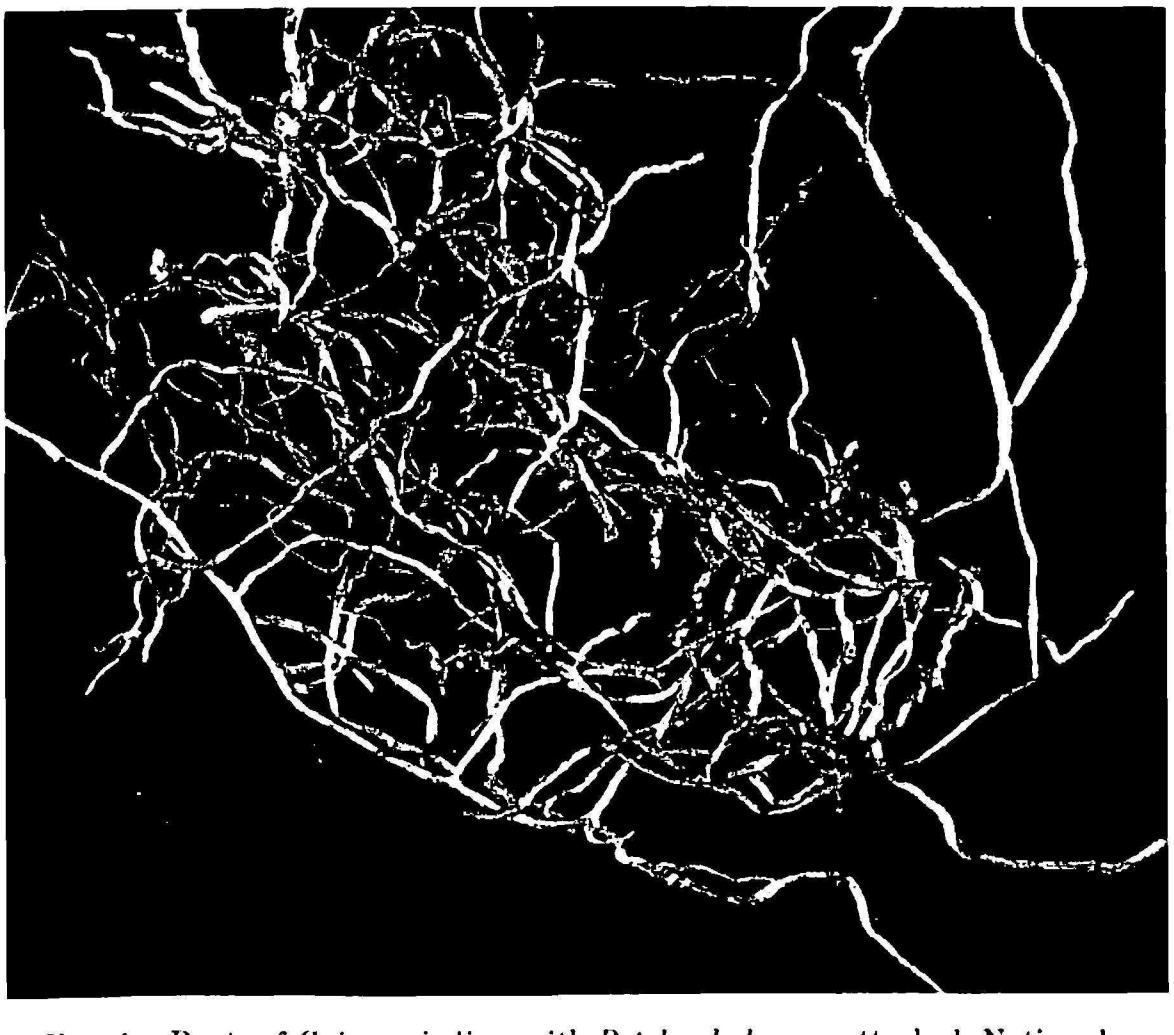

FIG. 1.—Roots of *Cajanus indicus* with *Rotylenchulus* sp. attached. Notice abundance of females with adhering soil particles and gelatinous secretion on tender roots. In the upper left proliferation of the roots may be observed. Immature females may also be noticed.

Table 1 shows, for 1 gm. of roots, the number of eel-shaped females, females with small and with large amounts of gelatinous secretions, and females without secretions. The estimated nematode populations of the surrounding soil are also given. As may be seen, there is no apparent relation between the number of females producing secretions and those not producing them in the different hosts. The number of voluminous secretions was greater in tobacco, coffee, and both varieties of pineapple than in tomatoes and pigeonpeas. Even in pigeonpeas, the preferred host, the num-

ber of females not covered by a secretion was high. These were even females which might never produce eggs or females which had not reached the egg-laying stage.

FIG. 2.—Females of *Rotylenchulus* sp. with their heads inserted in a pigeonpea root. Notice vulva in the upper side of first specimen.

In the pigeonpea roots many of the abundant bacterial nodules were attacked by females as shown in fig. 3. It is not yet known whether they were feeding in the cortex of the plant around the nodule, or on the bacteria,

but it was evident that the nematodes were feeding since development was achieved and they produced gelatinous secretions and eggs, the same as the ones present in the roots.

In general, all the plants infected with nematodes had weaker root systems. Table 2 summarizes the differences on pigeonpea and coffee roots, and the data from all the other plants. Tobacco roots were very much affected, as evidenced by the weight of the roots which was: 27.5 gm. in infected *versus* 52.1 in healthy roots. These differences were not as pronounced, but also existed in pineapples and tomatoes.

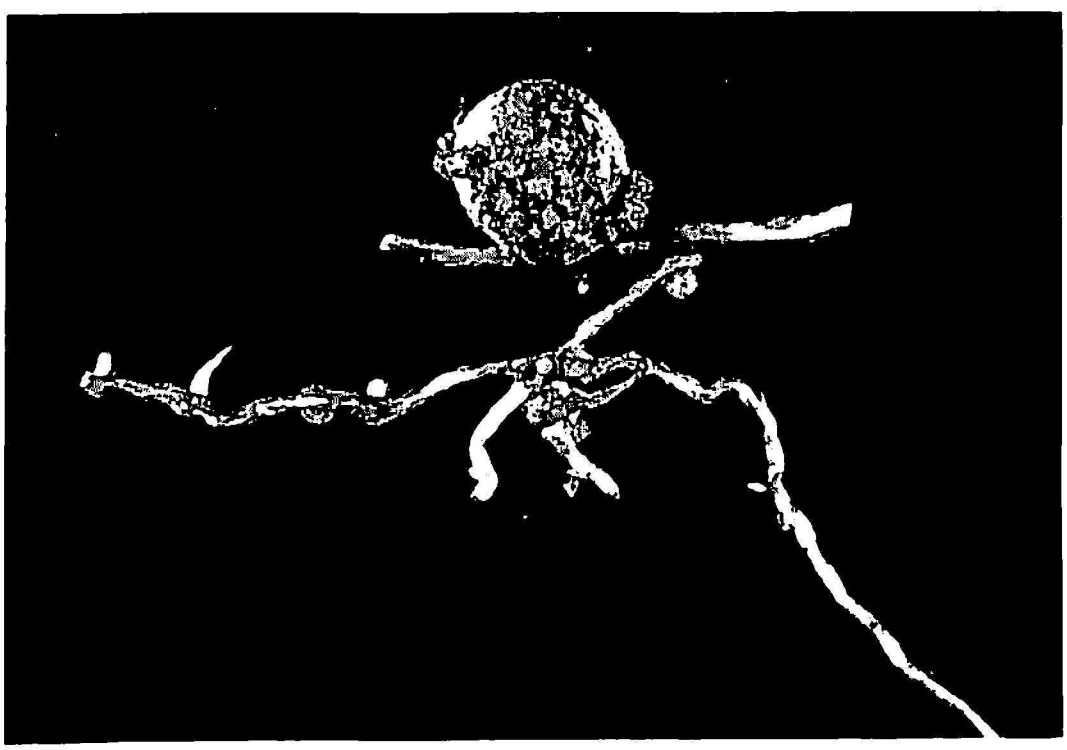

FIG. 3.—Pigeonpea roots with females of the reniform nematode attached, and a bacterial nodule with females with gelatinous secretions containing eggs and with adhering soil particles.

DISCUSSION AND CONCLUSIONS

The reniform nematode, although generally considered of little importance as a plant parasite, may cause much damage. Conforming with other nematode types, modes and degrees of pathogenicity differ according to host. Pigeonpeas, the preferred host, suffered as much damage in the aerial parts and roots as did coffee, in which the nematode populations remained very low.

Sugarcane, which is regularly found affected in the field, was completely resistant to the present isolate from pigeonpeas, a fact that suggests that the reniform nematode is represented by several species in Puerto Rico. One of these is able to infest and reproduce in sugarcane roots, although

usually not in great numbers, but the form found in pigeonpeas will not transfer to cane roots. It appears that the present species is unable to penetrate or to establish itself on cane roots.

Coffee did not seem to be a good host for this isolate because the nematode populations remained low both in the roots and in the soil. This might be the result of a lower egg production, a slower development, or a lower propagation.

Tomato, tobacco, and pineapple seemed to be good hosts, but they were not affected in an appreciable way. A marked reduction of the root system was observed in tobacco, but no aerial symptoms developed. A higher population might be necessary to develop symptoms in this plant species. Remarkable differences in susceptibility or suitability as a host were observed between the two varieties of pineapple. Smooth Cayenne was more susceptible than Red Spanish. This had already been found by the author (*2*) in a previous study.

In contrast to root-knot nematodes, *Meloidogyne* spp., the young females of the reniform nematode are able to penetrate older tissue. This was demonstrated by the finding of many eel-shaped females in primary and secondary roots.

This nematode usually does not produce necrosis or cracking of the roots; the small necrotic area observed around the feeding head will not extend for more than a few millimeters. Roots appear to die only when an excessive number of females develop on or near the growing point. When this region is affected proliferation of the root is prevalent. A severe curving of the roots is caused presumably by the interruption of growth in the area where the female feeds.

The attack of bacterial nodules by parasitic nematodes has been known for some time, but information is here provided on a new type of nematode and host, and also on the locality (Puerto Rico). Christie in 1958 (*3*) found that root-knot will invade bacterial nodules of soybean plants which were otherwise apparently free from nematode galls. Robinson in 1961 (*13*) found that root-knot would cause the formation of giant cells in bacterial nodules. None of these authors mentioned the production of egg masses by the respective nematode, a phenomenon here established for the reniform nematode. Fred, in 1932, (*5*) reported the attack of bacterial nodules by several factors, *e.g.* insect larvae and fungi, but nematodes were not mentioned.

SUMMARY

The reniform nematode, *Rotylenchulus*, Linford and Oliveira, 1940, is one of the most common parasitic nematodes in Puerto Rico. Its host list includes most of the agricultural crops of economic importance. To study

the effect on different host plants of an isolate of a new species of *Rotylenchulus*, an experiment was conducted in the greenhouse. Six species of plants, *e.g.* sugarcane, coffee, tobacco, tomatoes, pigeonpeas, and two varieties of pineapple, were inoculated with 10,000 juveniles, preparasitic females, and males obtained from pigeonpeas. After 4 months the plants were dug and observations made.

The degrees of pathogenicity and susceptibility of the different hosts were observed and are discussed. Marked differences were observed. Five of the plant species and varieties—pigeonpea, pineapple, tomato, and tobacco, were found to be good hosts, coffee was incompletely resistant, and sugarcane was completely resistant. The attack of bacterial nodules on pigeonpea roots by this nematode is discussed also.

RESUMEN

El nemátodo reniforme, *Rotylenchulus*, Linford y Oliveira, 1940, es uno de los tipos parasíticos más comunes en Puerto Rico. Ataca la mayor parte de las plantas de importancia económica.

Se llevó a cabo un experimento en el invernadero con el propósito de estudiar el efecto de este nemátodo en varias de sus plantas-huéspedes. Se probó la inoculación de 10,000 larvas, machos y hembras filiformes, obtenidas de un cultivo en gandur, en 7 especies y variedades de plantas que incluyeron caña de azúcar, café, tabaco, tomate, gandures y piña. Las observaciones se llevaron a cabo durante cuatro meses.

Aquí se discuten los resultados obtenidos en cuanto al efecto patógeno del nemátodo y la susceptibilidad de las plantas. Se observaron marcadas diferencias. Se determinó que 5 de las plantas, esto es, tabaco, gandur, tomate y piña, fueron buenas huéspedes. El café fué parcialmente bueno y la caña completamente resistente. Se informa también la presencia de este nemátodo en los nódulos bacterianos de las raíces del gandur.

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