Pathogenicity of Pratylenchus coffeae, Scutellonema bradys, Meloidogyne incognita, and Rotylenchulus reniformis on Dioscorea rotundata

NELIA ACOSTA and ALEJANDRO AYALA

Abstract: Low populations (200 specimens per plant) of Pratylenchus coffeae, Scutellonema bradys, Meloidogyne incognita, and Rotylenchulus reniformis stimulated the development of tops, roots, and tubers of Dioscorea rotundata 'Guinea' yam. We demonstrated experimentally that P. coffeae was responsible for the deterioration in quality of the yam tuber in Puerto Rico, a condition known as a dry rot of yam. Initial populations of 600 P. coffeae, S. bradys, or M. incognita, and populations of 1,000 P. coffeae or S. bradys per plant were high enough to induce dry rot of the yam tubers. P. coffeae and S. bradys were pathogenic to yam cultivar Guinea, but M. incognita and R. reniformis did not cause necrosis or cracking of the tuber cortex in our experiments. Key Words: dry rot of yams, lesion nematode, yam nematode, root-knot nematode, reniform nematode, yam.

In Puerto Rico, the edible yam Dioscorea rotundata Poir. (6), is affected by a disease known as dry rot which reduces production and quality of the crop. Symptoms of the disease are chlorosis, reduction in top growth and root development, cracks, necrosis of tuber tissue, and a loosened cortex. This condition is associated with nematode attack. The first report on nematodes associated with the yam tuber was by Steiner (17) who described a new species, Hoplolaimus bradys Steiner and LeHew from yam (19). Later Andrassy (1) transferred the species to the genus Scutellonema. S. bradys was observed on yam from Nigeria by West (22), who described the symptoms of loosened tuber cortex and named the disease “dry rot”. Similar symptoms were observed by Steiner and Buhrer (18) on yam from Puerto Rico. The new nematode species was associated with several species of yams including D. alata L., D. cayenensis Lam., and D. rotundata Poir.

Other nematodes have been reported associated with yam tubers. Schieber (12) and Schieber and Lassmann (13) observed tubers of D. spiculiflora Hemsl. and D. floribunda Mart. et. Gal. severely damaged by Meloidogyne sp. in Guatemala. Jenkins and Bird (8) found Meloidogyne incognita (Kofoid and White) Chitwood, Pratylenchus brachyurus (Godfrey) Filip. & Schuur.-Stekh., and Criconemonoides sp. associated with wild yam. Ayala (2, 3) and Ayala and Acosta (4) reported the association of Pratylenchus coffeae (Zimmermann) Filip. & Schuur.-Stekh., M. incognita, Rotylenchulus reniformis Linford and Oliveira, Helicotylenchus sp., Aphelenchoides sp., and Aphelenchus sp. with D. rotundata in Puerto Rico.

These are reports of association, however, and not of pathogenicity. The purpose of our studies was to determine experimentally what effect certain of these nematodes might have on Dioscorea rotundata. We report here the results of three experiments on the pathogenicity of Pratylenchus coffeae, Scutellonema bradys, Meloidogyne incognita, and Rotylenchulus reniformis on yam cultivar Guinea.

MATERIALS AND METHODS

Small yam (D. rotundata ‘Guinea’) plants established from stem cuttings in sterile soil and 60 days old were transplanted into a steam-sterilized soil mix in 20 cm diam pots. The mixture was composed of three parts sandy loam from an alluvial soil, two parts organic soil and one part sand, giving a sandy loam texture. It had the following characteristics: pH 7.6, organic matter 10.6%, total cation exchange capacity 24.4 milliequivalents/100 g of soil, and calcium, potassium, magnesium, and phosphorus content of 18.8, 1.8, 0.8, and 3.1 milliequivalents/100 g of soil, respectively. This soil mix was appropriate for Guinea yam culture as shown by investigators at the Federal Agricultural Experiment Station, Mayaguez.

The four species of nematodes we used for the inoculation experiments are commonly associated with Guinea yam in Puerto Rico.
This was based on results of a survey conducted in the principal regions where this crop is planted commercially on the Island. The nematode species were: *Pratylenchus coffeae*, *Scutellonema bradys*, *Meloidogyne incognita*, and *Rotylenchulus reniformis*. The *S. bradys* culture was obtained from yam acquired at a market place in Mayaguez; the *P. coffeae* culture came from commercial plantings of yam in the Laguna section in Aguada, Puerto Rico, and the *M. incognita* and *R. reniformis* cultures from tomato plantings at the Agricultural Experiment Substation, Isabela.

Three experiments were conducted. The first was established on 13 August, the second on 15 September, and the third on 17 October, 1972. The nematode inoculation level used in the three experiments were 200, 600, and 1,000 nematodes per pot in the first, second, and third experiments, respectively. Plants inoculated with 200 and 1,000 specimens per pot were maintained in a greenhouse and those inoculated with 600 nematodes per pot were maintained outdoors. To obtain inoculum, nematodes were extracted from infected yam tissue by the Baermann funnel method (7). Plants were inoculated by pipetting the desired number of nematodes in water into several holes in the soil around the base of the stem. All treatments were replicated five times in a randomized block design. The duration of each experiment was 3 mo.

All pots in each test were placed on wooden blocks on concrete tables to avoid contamination with nematodes from adjacent pots. To maintain optimum growth, plants received tap water every two days and 100 ml of Hoagland's solution (21) every other week. Other routine cultural practices including mite control were provided during the experimental period. Air and soil temp recorded during the experimental periods ranged from 28.8 to 32.5 C, from 25.6 to 27.6 C from 29.5 to 30.5 C in each experiment, respectively. At the end of the tests, nematode populations extracted from 100 cc of soil, 1 g of roots removed after chopping the whole root system, and 6 g of tuber cortex were counted. Actual values of fresh and dry top wt, fresh wt of roots, wt and number of tubers, and quality index (5), were recorded.

## RESULTS

Data from the first experiment are shown in Table 1. Plants inoculated with *P. coffeae* and *R. reniformis* had greater dry top wt, fresh root wt and fresh tuber wt when compared with the noninoculated control and with those

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**TABLE 1.** Mean wt of fresh and dry tops, fresh roots, and wt and number of yam tubers (*Dioscorea rotundata* 'Guinea') from pathogenicity test in which plants were inoculated with 200 specimens of three nematode species.

<table>
<thead>
<tr>
<th>Nematode species</th>
<th>Top wt (g)</th>
<th>Root wt (g)</th>
<th>Tubers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(fresh)</td>
<td>(dry)</td>
<td>(fresh)</td>
</tr>
<tr>
<td><em>Pratylenchus coffeae</em></td>
<td>20.5 A*</td>
<td>11.2 B</td>
<td>12.8 B</td>
</tr>
<tr>
<td><em>Meloidogyne incognita</em></td>
<td>18.3 A</td>
<td>7.0 A</td>
<td>5.3 A</td>
</tr>
<tr>
<td><em>Rotylenchulus reniformis</em></td>
<td>23.0 A</td>
<td>9.4 B</td>
<td>10.5 B</td>
</tr>
<tr>
<td>Control</td>
<td>16.6 A</td>
<td>5.8 A</td>
<td>5.6 A</td>
</tr>
</tbody>
</table>

*Averages followed by the same letter indicate no significant difference (P= 0.05) according to Duncan's multiple range test.*

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**TABLE 2.** Mean wt of fresh and dry tops, fresh roots, wt and number of tubers, and tuber quality index of 'Guinea' yam from pathogenicity tests in which plants were inoculated with 600 specimens of four nematode species.

<table>
<thead>
<tr>
<th>Nematode species</th>
<th>Top wt (g)</th>
<th>Root wt (g)</th>
<th>Tubers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(fresh)</td>
<td>(dry)</td>
<td>(fresh)</td>
</tr>
<tr>
<td><em>Scutellonema bradys</em></td>
<td>5.4 A*</td>
<td>1.0 A</td>
<td>1.0 A</td>
</tr>
<tr>
<td><em>Pratylenchus coffeae</em></td>
<td>7.5 A</td>
<td>1.3 A</td>
<td>1.0 A</td>
</tr>
<tr>
<td><em>Meloidogyne incognita</em></td>
<td>4.3 A</td>
<td>0.7 A</td>
<td>1.1 A</td>
</tr>
<tr>
<td><em>Rotylenchulus reniformis</em></td>
<td>6.4 A</td>
<td>1.0 A</td>
<td>1.1 A</td>
</tr>
<tr>
<td>Control</td>
<td>2.3 A</td>
<td>0.5 A</td>
<td>0.9 A</td>
</tr>
</tbody>
</table>

*Averages followed by the same letter indicate no significant t-test difference (P = 0.05).*

*Tuber quality index rating scale: 0 = severely damaged tubers; 5 = highest quality tubers.*
inoculated with *M. incognita*. Those inoculated with *R. reniformis* produced more tubers than the other inoculated plants or the noninoculated plants. The general appearance of the tubers from inoculated and noninoculated plants was similar. *S. bradys* was not used in this test. *P. coffeae*, *M. incognita* and *R. reniformis* specimens

FIG. 1. Tubers with symptoms produced by four different species of nematodes; A. Necrotic areas and deep cracks, especially in the apical portion, produced by *Pratylenchus coffeae*; B. Superficial cracks and less pronounced necrosis on tubers infected with *Scutellonema bradys*; C. Nodules on the roots of yam, caused by *Meloidogyne incognita*. "a" shows some of the galled roots; D. Tuber affected by *Rotylenchulus reniformis* showing little necrosis of the roots.
recovered after three months from 100 cc of soil, 1 g of roots and 6 g of tuber cortex were: 34, 27, and 40; 32, 20, and 2; and 89, 9, and 13, respectively. No nematodes were recovered from noninoculated controls.

In the second experiment, tubers from plants inoculated with nematodes showed severe necrosis and the quality index was lower than from noninoculated controls (Table 2). The number of cracks and the intensity of tuber necrosis was much greater in plants inoculated with *P. coffeae* than with *S. bradys*. Roots of plants in the presence of *M. incognita* were galled but the tubers were not (Fig. 1).

The highest final population density was obtained from *P. coffeae* inoculations; e.g., 1,701, 912, and 812 from tuber cortex, roots, and soil, respectively. Highest populations from *S. bradys*-inoculated plants were from tubers; e.g., 936. Populations recovered from soil and roots samples were 144 and 261, respectively. The populations recovered from plants inoculated with *R. reniformis* and *M. incognita* were 108, 172, and 41; 38, 20, and 11 nematodes from 100 cc of soil, 1 g of roots, and 6 g of tuber cortex, respectively.

In the third experiment, the effect of nematodes on tubers from inoculated plants was similar to that in Experiment 2. Quality of tubers from plants inoculated with *P. coffeae* and *S. bradys*, respectively, was lower than from those inoculated with *M. incognita*, or *R. reniformis*, or the noninoculated controls. Tubers infected with *P. coffeae* showed severe necrosis and deep cracks along the entire tuber cortex. Cracks were deeper and more pronounced in the region adjacent to the vines. Neither *M. incognita* nor *R. reniformis* caused appreciable damage, except that *M. incognita* caused a slight galling of roots (Fig. 1). Highest nematode populations were recovered from *P. coffeae* inoculated plants; e.g. 384, 350, and 285 from 100 cc of soil, 1 g of roots, and 6 g of tuber cortex, respectively. Only a few specimens of *S. bradys*, *M. incognita*, or *R. reniformis* were recovered; e.g. 8, 1, and 2; 0, 3, and 12; and 4, 0, and 0 from soil, root and tuber cortex samples, respectively. There were no differences between the other values recorded from inoculated and noninoculated plants at harvest time (Table 3).

**DISCUSSION**

Our results in Experiment 1 are similar to those of Wallace (20), who reported that low populations of *Meloidogyne javanica* Treub, increased top development of several different crops. Mountain (10) stated that low nematode populations stimulate a proliferation of root system tissue which is more rapid than nematode population increase, and that the effect is to increase overall rates of plant growth and development.

Final populations of *M. incognita* and *R. reniformis* were low in all the tests, suggesting either that the Guinea yam is not a good host for these species, or that the initial populations used were too low (15). The reproduction of these organisms apparently was affected primarily by the prevailing environmental conditions along with the high organic matter and the calcium or the phosphorus content in the soil (9), although the soil condition was appropriate for Guinea yam culture. The short duration of the test and the fluctuating greenhouse and outside temp may also have affected nematode reproduction. Kirkpatrick et al. (9) found that population fluctuations of different nematode species depended on concn of cations in the soil, on the crop used, and on the affected part of the plant. Other investigators (11, 23)

### TABLE 3. Mean wt of fresh and dry tops, fresh roots, wt and number of tubers, and quality index of 'Guinea' yam from pathogenicity tests where plants were inoculated with 1,000 specimens of four nematode species.

<table>
<thead>
<tr>
<th>Nematode species</th>
<th>Top wt (g)</th>
<th>Root wt (g)</th>
<th>Tubers</th>
<th>Quality index*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(fresh)</td>
<td>(dry)</td>
<td>(fresh)</td>
<td>Fresh wt (g)</td>
</tr>
<tr>
<td><em>Scutellonema bradys</em></td>
<td>13.1 A*</td>
<td>4.0 A</td>
<td>3.7 A</td>
<td>83.1 A</td>
</tr>
<tr>
<td><em>Pratylenchus coffeae</em></td>
<td>16.9 A</td>
<td>3.8 A</td>
<td>3.2 A</td>
<td>82.4 A</td>
</tr>
<tr>
<td><em>Meloidogyne incognita</em></td>
<td>15.8 A</td>
<td>3.8 A</td>
<td>2.5 A</td>
<td>86.5 A</td>
</tr>
<tr>
<td><em>Rotylenchus reniformis</em></td>
<td>16.0 A</td>
<td>4.1 A</td>
<td>2.7 A</td>
<td>78.9 A</td>
</tr>
<tr>
<td>Control</td>
<td>14.5 A</td>
<td>4.1 A</td>
<td>2.7 A</td>
<td>85.7 A</td>
</tr>
</tbody>
</table>

* Averages followed by the same letter indicate no significant t-test difference, P = 0.05.

*Tuber quality index rating scale: 0 = severely damaged tubers; 5 = highest quality tubers.*
observed differences in the reproduction of various nematode species feeding in different crops when exposed to variations in temp, pH, and nutritional content in the soil.

We found the Guinea yam to be a good host for *P. coffeae* and *S. bradys*. *P. coffeae* severely affected tuber quality by causing necrosis and crack formation. This organism presumably attained optimum reproduction because of the predominant high temp (14). *S. bradys* also affected yam quality, without reaching high population levels, which apparently was due to soil temp unfavorable for reproduction. The optimum temp for reproduction of *S. bradys* seems to be lower than temperatures recorded during the experiments (16).

In plants inoculated with 1,000 *P. coffeae* only low nematode numbers were recorded from tuber samples; this probably was the result of migration to deeper levels in the tissue due to the severe deterioration of the surface 3 mm of tissue which comprised the sample (14).

**CONCLUSIONS**

Low initial populations (200 specimens per plant) of *P. coffeae, M. incognita* and *R. reniformis* stimulated top growth of plants of Guinea yam under green-house conditions in Puerto Rico. Populations of 600 *P. coffeae* per plant produced significant damage (severe necrosis and deep cracks) to the tubers, and 1,000 specimens per plant caused complete deterioration and severe reduction in tuber quality. Tuber quality was affected to a lesser degree by *S. bradys* than by *P. coffeae*.

Field, green-house, and laboratory observations seem to justify the conclusion that *P. coffeae*, rather than *S. bradys*, is the nematode reponsible for the severe deterioration of the Guinea yam under Puerto Rican conditions. The main symptoms are cracking and severe necrosis of the cortex, a condition described as “dry rot” by previous authors.

**LITERATURE CITED**

Effect of Meloidogyne incognita on Reproduction of Pratylenchus penetrans in Red Clover and Alfalfa

R. A. CHAPMAN and D. R. TURNER

Abstract: Roots of seedlings of red clover and alfalfa growing on 10^5 Hoagland and Arnon solution agar were inoculated with various combinations of Meloidogyne incognita and Pratylenchus penetrans. Egg-laying by P. penetrans decreased as the number of nematodes, the ratio of entrant M. incognita to entrant P. penetrans, and the priority of invasion of roots by M. incognita increased. Embryogeny and hatching of eggs of P. penetrans, and development of larvae of M. incognita, were not affected. In red clover, the greatest reduction occurred when there were 65 entrant nematodes, the ratio of M. incognita:P. penetrans was 4:1, and M. incognita was inoculated four days prior to P. penetrans. In alfalfa, the less-favorable host for both nematodes, the greatest reduction occurred when there were 45 entrant nematodes, the ratio of M. incognita:P. penetrans was 2:1, and M. incognita was inoculated 4 days prior to P. penetrans. Key Words: Trifolium pratense, Medicago sativa, concomitant nematodes.

When Meloidogyne incognita (Kofoid and White) Chitwood and Pratylenchus penetrans (Cobb) Filipjev and Schuurmans-Stekhoven are concomitant inhabitants of plants that are suitable hosts for both, M. incognita should dominate the population eventually because of its shorter generation time (20-30 days vs. 50-60 days for P. penetrans), greater fecundity (hundreds of eggs per female vs. tens of eggs per female for P. penetrans), and more efficient mode of reproduction (parthenogenesis vs. amphimixis for P. penetrans). This assumes that each nematode functions in the association as it does when alone. We reported (9) that invasion of roots of red clover (Trifolium pratense L.) and alfalfa (Medicago sativa L.) by larvae of M. incognita was reduced significantly when the ratio of entrant P. penetrans to M. incognita in inoculum was 2-3:1 and there were 150-200 nematodes per root. However, invasion of roots by adults of P. penetrans was not reduced in the reciprocal combination. These observations were made 72 hours after inoculation and little was learned about development of the nematodes within roots.

Estores and Chen (4) reported that M. incognita repressed development of populations of P. penetrans in tomato (Lycopersicon esculentum Mill.) in 30 and 60 days, and presented evidence from split-root experiments that translocatable inhibitory substance(s) were involved. Their results indicate a deleterious effect of M. incognita on reproduction of P. penetrans during development of the first generation. In addition, they found that P. penetrans did not invade roots galled by M. incognita as readily as ungalled roots.

We investigated the influence of concomitant M. incognita on reproduction of P. penetrans in roots of red clover and alfalfa. The proportion of P. penetrans was kept low in order to avoid interference with invasion by M. incognita. An abstract which presents some of the results has been published (1).

MATERIALS AND METHODS

Larvae of M. incognita and females of P. penetrans were reared and collected for inoculum as described previously (9). Aseptic seedlings of 'Kenland' red clover and 'Buffalo'...