Journal of Nematology 30(3):362–367. 1998.

Effects of *Pratylenchus brachyurus* and *P. coffeae* on Seedlings of *Coffea arabica*

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Abstract: Two experiments were carried out to evaluate the effects of *Pratylenchus brachyurus* and *P. coffeae* on *Coffea arabica*. The first experiment was conducted in a greenhouse to determine the effects of *P. brachyurus* and *P. coffeae* on seedlings of *Coffea arabica* cv. Mundo Novo. Both *Pratylenchus* spp. reduced the growth of coffee seedlings. Higher contents of soluble sugars were detected in the leaves of infected plants. The reproduction rate of *P. brachyurus* was very low on cv. Mundo Novo, indicating an intolerance to this nematode. In a second experiment, *C. arabica* cultivars Mundo Novo and Catuaí both were intolerant hosts of *P. brachyurus*.

Key words: caffeine, carbohydrates, chlorophyll, coffee, intolerance, *Pratylenchus brachyurus, Pratylenchus coffeae,* protein susceptibility.

Pratylenchus brachyurus (Godfrey) Filipjev and Schuurmans Stekhoven and *P. coffeae* (Zimmermann) Filipjev and Schurmanns Stekhoven are parasitic on coffee, and, although less harmful than *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. exigua* Goeldi, they can cause yield losses (Gonçalves and Martins, 1993; Gonçalves et al., 1978; Kumar and Samuel, 1990; Lordello et al., 1968; Monteiro and Lordello, 1974; Salas and Echandi, 1961; Schieber, 1966; Schieber and Grullon, 1969; Whitehead, 1968). However, more detailed information on the damage of *P. brachyurus* and *P. coffeae* in coffee plants is needed. Most of the knowledge on these nematodes comes from investigations conducted under field conditions, where many factors may act on the plant.

In the present study, a greenhouse trial was conducted to measure the effects of these lesion nematodes on the growth and physiology of seedlings of *Coffea arabica.*

Received for publication 1 December 1997.

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The authors thank A. R. Monteiro and H. M. C. P. Chamma for their assistance, and FAPESP for financial support (protocol no. 1997/1537-3).

MATERIALS AND METHODS

Experiment 1—inoculum preparation: Pratylenchus brachyurus (Pb) and *P. coffeae* (Pc) were isolated, respectively, from maize (*Zea mays* L.) and cocoyam (*Colocasia esculenta* (L.) Schott.) roots and multiplied in the laboratory on alfalfa callus culture (Riedel et al., 1973). For each nematode, 5,000-cm^3 clay pots were filled with soil that had been previously sterilized and mixed with infected alfalfa callus. Maize and tomato (*Lycopersicon esculentum* Mill.), which are good hosts of Pb and Pc, were grown in the potted soil for 11 months. As uninfested controls, maize and tomato were grown in sterilized soil without alfalfa callus. After 11 months, the tops of the plants were removed and the roots were cut into small pieces and mixed with the soil of the same pots. After incubation for 54 days in a shady room, the roots were completely decomposed and three 250-cm^3 subsamples of each infested soil were processed for nematode extraction by means of a centrifugal-flotation technique (Jenkins, 1964). The average numbers of *Pratylenchus* per cm^3 of soil were 4.23 for Pb and 2.83 for Pc.

Growth determinations: The experiment was set in a completely randomized design, with 3 treatments (Pb, Pc, and control) and 19 replicates of each treatment. Coffee (*Coffea arabica* L. cv. Mundo Novo) seedlings at the "little soldier" stage (also called "beetle," the stage soon after emergence and before the seed-coat is cast off [Wellman, 1961]),

obtained from seeds germinated in sand, were transplanted to plastic pots containing 150 cm^3 of infested or uninfested soil. The remaining soil was stored in plastic containers maintained in a shady room. The initial inoculum level per pot (Pi) was 634 in the Pb treatment, 424 in the Pc, and 0 in the control. The seedlings were maintained in a greenhouse without temperature control and were fertilized every 30 days with 25 ml of an ammonium sulfate solution (3 mg ammonium sulfate/ml of water) per plant.

After 70 days, plant height was measured and the shoot and root wet masses of 10 replicates were determined. In the same plants, the nematodes were extracted with a blender-centrifugal-flotation technique (Coolen and D'Herde, 1972) and the final population data (Pf) were obtained by counting nematodes under a light microscope.

The remaining nine seedlings per treatment were transferred to 1,650-cm^3 plastic pots containing 1,250 cm^3 of the correspondingly treated soils. However, at the transfer time the average number of *Pratylenchus* per cm^3 of soil had dropped to 1.13 in soil inoculated with Pb and to 0.18 with Pc. Thus, after both inoculations, the total inoculum level per pot was 2,051 in the Pb treatment (634 from initial inoculum and 1,417 from new inoculum), 649 in the Pc (424 from initial inoculum and 225 from new inoculum), and 0 in the control. The coffee seedlings were maintained in a greenhouse for an additional 280 days, and during this period the plant height was measured every 70 days. The plants were fertilized every 50 days with 50 ml of a nutritive solution (4 mg of ammonium sulfate + 8 mg of potassium nitrate + 4 mg of urea/ml of water) per plant. At the end of the experimental period, small portions of the third pair of leaves were sampled for chemical analyses. The fresh mass was determined, and 10 g of the roots was sampled for analyses of *Pratylenchus* populations. The final population (Pf) was estimated for the total root system. Shoot and root dry masses were obtained after drying at 80 °C for 1 week.

Chemical analyses: The leaf samples were cut into small pieces and disrupted in a Polytron mixer (Kinematica AG, Lucerne, Switzerland) with 15 ml of a methanol/chloroform/water solution (12:5:3, v/v/v/) (Bielesk and Turner, 1966). After one week at 4 °C, aliquots of 0.1 ml were taken and diluted to 1 ml with ethanol, and total chlorophyll (a + b), carotenoids, and xanthophylls determined spectrophotometrically (Lichtenthaler and Wellburn, 1983). Chloroform (3.75 ml) and distilled water (5.7 ml) were added to the remaining solution and aliquots taken from the upper phase (methanolic-aqueous phase) for quantitative determination of soluble sugars and caffeine. Soluble sugars were estimated by the phenol-sulfuric acid method (Dubois et al., 1956) using sucrose as the standard. Caffeine was analyzed by reversed phase-high performance liquid chromatography (Mazzafera et al., 1991). The remaining extracts were filtered and the remaining tissue washed several times with 80% cold ethanol to eliminate any residue of soluble sugars. After quick-drying at 80 °C, part of the dried material was digested with 5 ml of 70% perchloric acid overnight for hydrolysis of the starch, and part treated with 5 ml of 1N NaOH for protein solubilization. Soluble sugars from the perchloric acid starch digestion were determined by the phenol-sulfuric acid method (Dubois et al., 1956), using glucose as the standard. The protein content was estimated by the protein dye-binding method (Bradford, 1976), using bovine serum-albumin as the standard.

Experiment 2: Given the nature of the results of experiment 1 about *P. brachyurus* (Pb), this experiment was carried out to confirm these results on *C. arabica* cv. Mundo Novo and to determine the growth effects of Pb on *C. arabica* cv. Catuaí.

Coffee seedlings at the "little soldier" stage, obtained from seeds germinated in sand, were transplanted into plastic pots containing 500 cm^3 of sterilized soil. The seedlings were maintained in a screenhouse until the "butterfly" stage (also called "cup" or "cotyledonary," the stage when the two cotyledons are expanded and arranged as a cup around the central bud of

the coffee seedling [Wellman, 1961]) was reached, when they were transferred to a greenhouse, where the temperature was kept at 17 °C to 32 °C, and inoculated with Pb. Inoculum was obtained from alfalfa calli infected with the same Pb isolate used in experiment 1. Motile stages of Pb were extracted from calli by a modification of the Baermann method (Southey, 1970). The inoculation was done with a suspension of Pb and pipetted in two holes in the soil near the seedlings. Seedlings of each cultivar were kept free or inoculated with 1,000 motile stages of Pb. The experiment utilized a factorial design (2 × 2), with 2 Pb doses and 2 coffee cultivars, and 10 replicates. Maize seedlings were also inoculated to confirm the infectiveness of the nematode suspension used. The seedlings were maintained in the greenhouse during the experimental period (90 days).

After 90 days, plant height was measured. Roots were weighed (wet mass) and processed as in experiment 1 for nematode extraction. Soil nematodes were extracted from the total volume of each pot (500 cm^3) by the centrifugal-flotation technique (Jenkins, 1964). The final population (Pf) was estimated by counting Pb in the roots and in the soil of each replicate. Shoot dry mass was evaluated after drying at 60 °C for 4 days.

Data analysis: Data from growth (experiments 1 and 2) and chemical determinations were analyzed without transformation using the ANOVA procedure (SAS Institute, Cary, NC), and differences among means were compared at $P \leq 0.05$ using the Tukey's multiple-comparison test.

Results

Experiment 1: Pratylenchus brachyurus and *P. coffeae* reduced the height of plants of *Coffea arabica* cv. Mundo Novo, this being evident from the first measurement at 70 days (Table 1). The differences in height between the infected and the control plants seem to be greater at 70, 140, and 210 days, decreasing at the subsequent measurements, probably because the pots were not sufficiently large to allow the normal development of the roots of the control plants. Indeed, at 350 days, the root system of the control plants had spread throughout the whole pot, but there was only partial occupation by the root system of plants of the Pb and Pc treatments.

A significant reduction in the shoot and root mass of coffee plants infected by both nematodes also was observed (Table 2); however, necrotic tissues were not detected. It is not possible to determine which nematode had the greater effect, since the density of Pb in the soil was higher than that for Pc.

Despite the clear depressive effect of Pb on plant growth, its reproduction rate (Pf/Pi) was very low, being less than one (Table 2). On the other hand, Pc population increased 14-fold during the experimental period.

Among the compounds analyzed in the leaves, significant differences were found only for soluble sugars and starch. Coffee seedlings infected with both nematodes showed higher soluble sugar content than the control, and those infected with Pb had higher starch than those infected with Pc (Table 3).

TABLE 1. Influence of *Pratylenchus brachyurus* (Pb) and *P. coffeae* (Pc) on plant height of *Coffea arabica* cv. Mundo Novo seedlings.

	Plant height (cm)				
Treatment	70 days	140 days	210 days	280 days	350 days
Control	8.3 a	23.6 a	41.0 a	56.7 a	75.2 a
Pc	7.0 b	21.3 b	36.3 b	56.1 a	70.0 b
Pb	6.1 c	11.1 c	19.8 c	39.5 b	64.3 c
C.V. %	10.7	9.6	8.0	7.7	4.8

Data are means of 19 replicates (one plant per replicate) at 70 days and 9 replicates at 140, 210, 280, and 350 days. Different letters indicate statistical difference among treatment using the Tukey test $P \leq 0.05$.

TABLE 2. Influence of *Pratylenchus brachyurus* (Pb) and *P. coffeae* (Pc) on shoot and roots of *Coffea arabica* cv. Mundo Novo seedlings, and reproductive rate (Pf/Pi) of Pb and Pc.

	70 days (fresh mass)			350 days (dry mass)		
Treatment	Shoot (g)	Roots (g)	Pf/Pi	Shoot (g)	Roots (g)	Pf/Pi
Control	1.21 a	0.81 a	—	37.22 a	14.33 a	—
Pc	0.95 b	0.57 b	0.3	32.73 b	10.14 b	14.0
Pb	0.70 c	0.37 c	0.1	24.96 c	7.12 b	0.9
C.V. %	18.7	25.5		9.2	24.8	

Data are means of 10 replicates at 70 days and 9 replicates at 350 days. Different letters indicate statistical difference among treatments using the Tukey test $P \leq 0.05$. Initial population (Pi) to 70 days = 634 Pb and 424 Pc; Pi to 350 days = 2,051 Pb and 649 Pc.

Experiment 2: Pratylenchus brachyurus reduced the height, root mass, and shoot mass of *C. arabica* cultivars Mundo Novo and Catuaí (Table 4). Root necrosis was not observed in any of the cultivars inoculated with Pb.

The reproduction rates (Pf/Pi) of the two inoculated cultivars, after 90 days, were very low (Table 4). In contrast, the reproduction rate on maize was 11.0 (average of eight replicates), confirming the infectiveness of inoculum used.

DISCUSSION

The results about root and shoot growth of Pc-infected seedlings, associated with the values of Pc reproduction rate, show that *Coffea arabica* cv. Mundo Novo is a susceptible host for this nematode, as defined by Dropkin and Nelson (1960).

According to the same concept, the results of experiments 1 and 2 show that coffee cv. Mundo Novo is an intolerant host for Pb. In experiment 2, a similar reaction was observed in cv. Catuaí. There are few documented cases of plants that are intolerant to nematodes, and a classic example of intolerance is the reaction of coffee cv. Mundo Novo to *Meloidogyne incognita* races 1, 2, and 3 (Costa et al., 1991; Gonçalves et al., 1996). However, the effects of Pb on growth of coffee plants seems to be quite different from the effects of *M. incognita* on the same host. The reproduction rate of *M. incognita* on coffee cv. Mundo Novo is low, but enough for the nematode to cause a significant effect on plant growth (Costa et al., 1991; Gonçalves et al., 1996). Our results suggest that Pb reproduction rate on coffee is so low that its infection is self-limiting and its effect on growth parameters probably weakens as coffee plants become older. Unfortunately, discussions about the long-term effects of Pb on coffee are very difficult because the reaction of mature coffee plants to Pb is unknown and may be different from that on young plants.

A practical implication of these results is that seedlings of coffee must be produced using disinfected substrate and the areas for coffee plantations must be free or have a low population of Pb when the seedlings are transferred to the field in order to avoid the

TABLE 3. Contents of soluble sugars (SS), starch, protein, total chlorophyll (Chl), and carotenoids and xanthophylls (Car/Xan) in leaves of *Coffea arabica* cv. Mundo Novo seedlings infected and uninfected with *Pratylenchus brachyurus* (Pb) and *P. coffeae* (Pc) at 350 days.

Treatment	SS mg/g	Starch mg/g	Protein mg/g	Caffeine mg/g	Chl mg/g	Car/Xan mg/g
Control	143.5 b	133.4 ab	29.35	1.383	4.078	0.508
Pc	157.0 a	115.6 b	34.19	1.107	3.949	0.479
Pb	162.9 a	141.7 a	31.78	1.198	3.993	0.517
C.V. %	7.4	13.2	23.5	29.8	15.8	15.3

Data are means of 9 replicates. Different letters indicate statistical difference among treatments using the Tukey test $P \leq 0.05$.

TABLE 4. Influence of *Pratylenchus brachyurus* on seedling height, shoot and roots masses of two *Coffea arabica* cultivars and nematode reproduction rate (Pf/Pi).

Cultivar	Doses of Pb	Height (cm)	Shoot dry mass (g)	Roots wet mass (g)	Pf/Pi
Mundo Novo	0	12.83 a	0.708 a	3.44 a	—
	1,000	10.56 b	0.547 b	2.02 b	0.26
C.V. %		11.4	19.8	24.1	
Catuaí	0	9.73 a	0.778 a	4.32 a	—
	1,000	8.10 b	0.593 b	3.38 b	0.25
C.V. %		11.0	26.8	25.8	

Data are means of 10 replicates. Different letters within the cultivars indicate statistical difference among treatments using the Tukey test $P \leq 0.05$.

initial damage caused by Pb infestation, as observed in this study. This might be attained by proper sterilization of the substrate used to produce the seedlings and, at field level, elimination of weeds that may maintain high populations of Pb.

Although our data showed clear effects of Pb and Pc on seedlings and young plants of coffee cv. Mundo Novo, we did not detect root necrosis or severe shoot symptoms, which are normally attributed to Pb and Pc under field conditions (Lordello, 1972; Schieber, 1966). The same effects were observed on roots of cv. Catuaí inoculated with Pb in experiment 2. This confirms a previous study conducted under greenhouse conditions with *C. arabica* cv. Bourbon inoculated with Pc (Salas and Echandi, 1961). So, the extensive root necrosis observed in the fields infested with Pb or Pc are probably long-term symptoms, resulting from a synergism between the nematodes and opportunistic micro-organisms of the soil microflora.

The influence of nematode infection on the growth and biochemical alterations due to nematode infection has been characterized primarily with *Meloidogyne* species. Depending on the plant and nematode, a decrease (Kaushal and Madavi, 1992; Melakeberhan et al., 1985) or increase (Mahmood and Siddiqui, 1993; Prasad et al., 1982) of these compounds and photosynthesis was observed. In some cases, however, there were no changes (Koenning and Barker, 1995). In these aspects, a few studies were carried out with *Pratylenchus;* however, none were conducted with coffee, Pb, or Pc (Anwar, 1995; Haseeb and Shukla, 1995; Sindararay and Mehta, 1991).

The carbohydrate alterations in plants infected with nematodes might be related to the source-sink interactions, with the infected roots representing the sink (McClure, 1977). Depending on the strength of the sink (number of nematode feeding sites in the roots), a higher energy demand might induce an increase of sucrose in the leaves, via photosynthesis and starch hydrolysis, with subsequent transport to the roots. This drain would mean a diversion of sucrose for plant growth, explaining the usual reduction of shoots in plants infected by nematodes, as observed here.

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