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Host-range Characterization of Two *Pratylenchus coffeae* Isolates from Brazil[1]

R. A. Silva and M. M. Inomoto[2]

Abstract: Two isolates of *Pratylenchus coffeae* were collected from coffee roots (in Marília, São Paulo State, Brazil) and *Aglaonema* (in Rio de Janeiro City, Rio de Janeiro State, Brazil) and maintained in the laboratory on alfalfa callus. Twenty-four plants were tested in the greenhouse to characterize the host preference of these isolates. The host ranges of the isolates differed from each other and, interestingly, coffee, banana, and citrus were not among the better hosts of either isolate. Rather, sorghum, maize, rice, millet, okra, melon, eggplant, and lettuce were the best hosts of the Marília isolate. Poor hosts included French marigold, Rangpur lime, banana, sesame, peanut, sunflower, cotton, French bean, onion, and small onion. The best hosts of the Rio de Janeiro isolate were sesame, soybean, sorghum, castor oil plant, watermelon, squash, eggplant, and melon; the poorest hosts were French marigold, coffee, Rangpur lime, banana, sunflower, peanut, maize, millet, French bean, cotton, onion, sweet pepper, lettuce, okra, and small onion. These isolates have important molecular and morphological differences, suggesting host preference is linked to these characteristics.

Key words: behavior, Brazil, coffee, host-parasite relationships, host reaction, lesion nematodes, *Pratylenchus coffeae*, variability.

The coffee-lesion nematode *Pratylenchus coffeae* (Zimmermann, 1898) was reported for the first time in Indonesia on roots of *Coffea arabica* L. (Whitehead, 1968) and is now a major pest of coffee in Barbados, Brazil, Congo, Costa Rica, El Salvador, Guatemala, India, Jamaica, Madagascar, Malaysia, Martinique, and the Philippines (Campos et al., 1990; Kumar and Samuel, 1990; Schieber and Grullon, 1969). This nematode is also pathogenic to many other crops such as banana, citrus, yam, soursop, and potato in tropical and subtropical countries (Acosta and Ayala, 1975; Curi et al., 1990; Duncan and Cohn, 1990; Gowen and Quénéhervé, 1990; Moura et al., 1998; Prates and Lordello, 1980).

Studies with *P. coffeae* have shown that populations vary with respect to host preference. Edwards and Wehunt (1973) demonstrated that maize was a host for an isolate of *P. coffeae* from Panama but not for one from Honduras. Eight other plants (*Crotalaria juncea* L., *Crotalaria striata* Schrank, *Flemingia congesta* (Roxb.) Benth., *Mimosa invisa* Mart., *Sesbania* sp., *Stylosanthes gracilis* HBK., *Tephrosia candida* DC., and *Tephrosia vogelii* Hook. f.) revealed an inverse behavior for those isolates. Recent studies demonstrated that significant morphological and molecular differences exist among *P. coffeae* isolates and that more detailed studies might detect new species among these isolates (Duncan et al., 1999).

Although *P. coffeae* is common in Brazil, its pathogenicity and host range have not been adequately studied. For example, a Brazilian *P. coffeae* isolate from cocoyam (*Colocasia esculenta* Schott) did not increase on banana (Oliveira et al., 1995). Because *P. coffeae* is important in tropical countries, this research was conducted to characterize the host range of two *P. coffeae* isolates from Brazil.

Materials and Methods

Inoculum preparation: Two isolates of *P. coffeae* were collected from different hosts in two states of Brazil. One isolate was collected from coffee roots in Marília, São Paulo, from where the species was first reported in Brazil (Monteiro and Lordello, 1974). Another isolate was collected by J. P. Pimentel from roots of ornamental *Aglaonema* sp. in Rio de Janeiro City, Rio de Janeiro. This isolate was previously reported as being recovered from *Dieffenbachia* sp. (Duncan et al., 1999), another ornamental in the family Araceae, but it was actually recovered from *Aglaonema* (Pimentel, pers. comm.). The roots of both the coffee and the *Aglaonema* showed the necrosis of cortical tissue typical of *Pratylenchus* infection.

Nematodes were extracted from the roots by the blender-centrifugation method (Coolen and D'Herde, 1972), and live nematodes were separated from dead ones by a modification of the Baermann method (Southey, 1986). Light-microscopy observations were conducted on heat-killed specimens mounted in 2% formaldehyde on temporary slides. The diagnostic morphological characters of *Pratylenchus* (Handoo and Golden, 1989; Loof, 1978; Román and Hirschmann, 1969) were used to identify *P. coffeae*. Nematodes were surface-sterilized in 0.1% ampicillin and maintained on alfalfa callus produced according to Riedel et al. (1973). Each culture was initiated from a single female. The isolate from Marília (coffee roots) was increased from cultures of ca. 30 females, and the isolate from Rio de Janeiro (roots) was increased from cultures of ca. 15 females. These isolates are named K_5 (Marília) and M_2 (Rio de Janeiro), respectively, and placed in two distinct groups based on the morphological characteristics by Duncan et al. (1999).

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[2] Graduate Student and Assistant Professor, respectively, Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura "Luiz de Queiroz," Universidade de São Paulo, Cx. Postal 9, 13418-900 Piracicaba, SP, Brazil. Present address of first author: Ciências Agrárias/UNIVAG, Av. Dom Orlando Chaves, 2655, 78118-000 Várzea Grande, MT, Brazil.

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E-mail: mminomot@carpa.ciagri.usp.br

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The *P. coffeae* isolates were maintained on alfalfa callus by periodic subculturing. The nematodes were extracted from cultures 45 to 90 days after infection by a modification of the Baermann method (Southey, 1986). The resulting suspension of all motile stages of the nematode was used as inoculum for each experiment.

Experiments 1, 2, and 3: These experiments were conducted to identify one good and one poor host of *P. coffeae* to be used as standards for comparison in subsequent experiments. Coffee (*C. arabica* cv. Catuaí Vermelho) and banana (*Musa acuminata* Colla AAA cv. Giant Cavendish) were tested as possible susceptible hosts, and sesame (*Sesamum indicum* L.) and French marigold (*Tagetes patula* L.) as poor hosts (Almeida et al., 1978; Das and Das, 1986; Edwards and Wehunt, 1973; Gowen and Quénéhervé, 1990) in experiment 1. Seeds of coffee were sown in a box containing sand. When the coffee seedlings had two cotyledons expanded, they were transplanted to plastic pots containing 450 ml of soil sterilized with methyl bromide (150 ml of CH_4Br/1,000 liters of soil). Banana plants were obtained by tissue culture and transplanted to pots when they were able to tolerate greenhouse conditions. Sesame and marigold seeds were sown into plastic pots. Inoculation was 16 and 14 weeks after transplanting coffee and banana plants, respectively, and 1 week after emergence of sesame and marigold seedlings.

Only the Marília isolate of *P. coffeae* (K_5) was used in experiment 1. The experiment was a completely randomized design, with four treatments (coffee, banana, sesame, and marigold) and five replicate-pots per treatment. Each replicate received 120 K_5 in 1 ml water pipeted into two holes in the soil near the plants. The inoculated plants were maintained in a shaded room for 12 hours after inoculation to avoid damage to nematodes and subsequently in a greenhouse for 63 days. Nutrient solution (15N:15P:20K:1.1Ca:4Mg:0.4S + micronutrients), insecticide, and acaricide were used as needed.

The host preference of K_5 for the tested plants was evaluated at the end of the experimental period on the basis of increased or decreased *P. coffeae* populations, both in the soil and in the roots. The nematodes were extracted from the soil by the centrifugal-flotation method (Jenkins, 1964) and from the roots by the blender-centrifugal-flotation method (Coolen and D'Herde, 1972). The final population (Pf) of *P. coffeae* was estimated by counting the nematodes extracted from the soil and roots of each replicate, and the population growth (Pf/Pi) was calculated.

Experiments 2 and 3 were conducted concurrently—one with the K_5 (experiment 2) and another with the M_2 isolate (experiment 3). The plants tested were the same as in experiment 1, except for the inclusion of Rangpur lime (*Citrus limonia* Osbeck). Rangpur lime seedlings of approximately 16 months were used. They were transplanted to plastic pots containing 450 ml of soil sterilized with methyl bromide. Coffee and banana plants used in these experiments were the same as in experiment 1, and sesame and marigold plants were obtained by direct seeding in plastic pots.

Experiments 2 and 3 were two separate experiments, although they were conducted concurrently. Each one was a completely randomized design, with five treatments (coffee, banana, lime, sesame, and marigold) and six replicate-pots per treatment.

The inoculum was adjusted to 500 nematodes per ml, and each plant received 1,000 nematodes by pouring 2 ml of inoculum into two holes in the soil near the plants. The inoculated plants were maintained in a shaded room for 12 hours and in the greenhouse for 70 days. Cultural practices and evaluations of experiments 2 and 3 were the same as in experiment 1.

Experiments 4 and 5: Experiment 4 was done concomitantly with experiment 5 to evaluate the growth of K_5 (experiment 4) and M_2 (experiment 5) on 10 cash crops, including the following plants commonly cultivated in Brazil: soybean (*Glycines max* Merril cv. Pintado), cotton (*Gossypium hirsutum* L. cv. Antares), millet (*Pennisetum glaucum* R. Br.), French bean (*Phaseolus vulgaris* L. cv. Safira), sorghum (*Sorghum vulgare* Pers. cv. IPA-7301011), rice (*Oryza sativa* L. cv. Primavera), maize (*Zea mays* L. cv. Br-106), peanut (*Arachis hypogea* L. cv IAC Tatu), sunflower (*Helianthus annuus* L. cv. Morgal-734), and castor oil plant (*Ricinus communis* L. cv. Guarani). The plants used as standards for comparison were defined from the results of experiments 1, 2, and 3: coffee as a good host and French marigold as a poor host to K_5, and sesame as a good host and French marigold as a poor host to M_2. However, coffee was tested again in relation to M_2. Young seedlings of Rangpur lime (7 weeks old) were included to determine if the host reaction of young seedlings was the same as older plants (the 16-month-old plants used in experiments 2 and 3).

The seedlings of all plants, except lime and coffee, were obtained by seeding in plastic pots containing 450 ml of soil sterilized with methyl bromide. Seedlings were thinned 2 weeks after sowing to two seedlings per pot. Lime and coffee were sown earlier than the other plants (7 and 13 weeks prior to inoculation, respectively) because of their slow growth and greater time required to reach the appropriate size for inoculation.

The inoculation was the same as in experiments 2 and 3—1 week after thinning—and the plants were maintained in the greenhouse for 70 days. Cultural practices and evaluations in experiments 4 and 5 were the same as in the experiment 1.

Experiment 4 was set in a completely randomized design, with 12 treatments (soybean, cotton, millet, sorghum, rice, maize, peanut, sunflower, castor oil plant, coffee, lime, and marigold) and four replicates (each pot constituted one replicate). The experimental de-

sign for experiment 5 was similar to experiment 4, except for the additional treatment sesame.

Experiments 6 and 7: Experiments 6 and 7 were conducted simultaneously to evaluate the growth of K_5 (experiment 6) and M_2 (experiment 7) on 10 vegetables: squash (*Curcubita moschata* Duchesne cv. Menina Brasileira), lettuce (*Lactuca sativa* L. cv. Grandes Lagos), eggplant (*Solanum melongena* L. cv. Embu), onion (*Allium cepa* L. cv. Baia Periforme), small onion (*Allium fistulosum* L. cv. Todo Ano), carrot (*Daucus carota* L. cv. Brasília), sweet pepper (*Capsicum annuum* L. cv. Cascadura Ikeda), okra (*Abelmoschus esculentus* Moench. cv. Santa Cruz IAC-47), watermelon (*Citrullus lanatus* Schrad. cv. Rajada), and melon (*Cucumis melon* L. cv. Valenciano). The standard hosts of two *P. coffeae* isolates were the same as experiments 4 and 5, and coffee was included in these experiments.

The vegetables used in experiments 6 and 7, as well as the standard hosts, required different periods of time to germinate and to reach the appropriate size for inoculation. Therefore, they were initiated on different days. Nine plants were sown in a germibox: onion and small onion 7 weeks before inoculation; lettuce, eggplant, carrot, and sweet pepper 5 weeks before inoculation; and French marigold, sesame, and okra 4 weeks before inoculation. The plants were transplanted to plastic pots with 450 ml of sterilized soil (two seedlings per pot) 2 weeks after germination. Watermelon, melon, and squash were sown in plastic pots, 1 week before inoculation, and thinned to two seedlings per pot 4 days before inoculation. Coffee plants were transplanted 6 days before inoculation. Inoculation was as in experiments 4 and 5, and the data were collected after 75 days. Cultural practices and evaluations of experiments 6 and 7 were the same as experiment 1.

Experiment 6 was set in a completely randomized design, with 12 treatments (squash, lettuce, eggplant, onion, small onion, sweet pepper, okra, watermelon, melon, coffee, lime, and marigold) and 5 replicates (each pot constituted one replicate). The experimental design of experiment 7 was the same as experiment 6, except for one additional treatment—sesame.

Data analyses: Data (Pf/Pi) were transformed using log $(x + 1)$ to normalize the data. Differences among treatment means for Pf/Pi were determined with analysis of variance using the software SANEST (developed by Departamento de Matemática e Estatística, ESALQ/USP, Piracicaba, Brazil), mean separations were by Tukey's test, and all statements regarding significance are relative to $P \leq 0.05$.

Voucher specimens: Slides containing females and males of Marília and Rio de Janeiro isolates of *P. coffeae* were labelled K_5/ESALQ/1999 and M_2/ESALQ/1999, respectively, and deposited in the nematology collections of the Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, ESALQ/USP, Piracicaba, Brazil. Both isolates are maintained in vitro (alfalfa callus) and in the greenhouse (coffee plants for K_5 and *Aglaonema* sp. plants for M_2).

Results

The population growth of K_5 was higher on coffee than on banana and French marigold, and the latter hosts did not differ (Table 1). Similar results were obtained in experiment 2, except that lime, which was not tested in experiment 1, did not differ from banana and French marigold (Table 1). The population growth of K_5 was higher on coffee than on sesame in experiment 2 but did not differ in experiment 1. Although the growth of this isolate was low (Pf/Pi = 1.64 and 2.35), coffee was chosen as the standard for comparison (good host) for further experiments with K_5 because it was a better host than the other plants tested in experiments 1 and 2. The Marília isolate did not survive on French marigold, which was chosen as the nonhost standard for the experiments.

Sesame is considered a poor host for *P. coffeae* (Das and Das, 1986), but the M_2 isolate increased 4-fold on sesame in experiment 3. Coffee, banana, and lime, considered good hosts of *P. coffeae,* supported very low numbers of this isolate. Thus, sesame and French marigold were used as good and poor hosts, respectively, for further experiments with isolate M_2.

Population growth of K_5 was low on coffee (Pf/Pi = 1.06) in experiment 4, confirming the results of previous experiments (Table 2). However, sorghum, maize, rice, and millet were better hosts of K_5, which increased 3.5- to 6.3-fold on these plants. Peanut, French marigold, sunflower, lime, cotton, and French bean had low growth rates with no differences among them. Population growth on soybean and castor oil plant did not differ from coffee.

The greatest increase of M_2 occurred on sesame, soybean, sorghum, and castor oil plants in experiment 5 (Table 2). Population growth on French marigold, lime, sunflower, coffee, maize, cotton, French bean, and millet were the lowest, with no difference among

Table 1. Population growth (Pf/Pi) on Marília isolate (K5) of *Pratylenchus coffeae* 63 days after inoculation (experiment 1), and on Marília (K_5) and Rio de Janeiro (M_2) isolates 70 days after inoculation (experiments 2 and 3, respectively).

Plant	K_5 Experiment 1	K_5 Experiment 2	M_2 Experiment 3
Coffee	2.35 a	1.64 a	0.15 b
Sesame	1.66 ab	0.16 b	4.33 a
Lime	—[a]	0.15 b	0.02 b
Banana	0.65 b	0.01 b	0.11 b
French marigold	0.00 b	0.00 b	0.00 b

Data are means of five (experiment 1) or six (experiments 2 and 3) replicates. Means within a column followed by a common letter are not different according to Tukey test ($P \leq 0.05$).

[a] Not tested.

TABLE 2. Population growth (Pf/Pi) on Marília (K_5) and Rio de Janeiro (M_2) isolates of *Pratylenchus coffeae* (experiments 4 and 5, respectively) 70 days after inoculation.

Plant	K_5 Experiment 4	M_2 Experiment 5
Sesame	—[a]	2.84 a
Sorghum	6.27 a	1.76 ab
Maize	4.05 ab	0.16 cd
Rice	3.52 abc	0.79 bc
Millet	3.50 abc	0.31 cd
Soybean	2.82 bcd	2.06 a
Castor oil plant	1.60 cde	1.46 ab
Coffee	1.06 def	0.05 d
Bean	0.61 efg	0.25 cd
Cotton	0.41 efg	0.30 cd
Lime	0.35 fg	0.01 d
Sunflower	0.15 fg	0.06 d
Marigold	0.13 fg	0.00 d
Peanut	0.11 g	0.18 cd

Data are means of four replicates. Means within a column followed by a common letter are not different according to Tukey test ($P \leq 0.05$).
[a] Not tested.

them. Rice had an intermediate reaction to M_2—lower than sesame but higher than French marigold.

Okra, melon, eggplant, and lettuce were the best hosts for K_5 in experiment 5, increasing 3.5- to 6.2-fold the initial population of the nematode and supporting populations higher than coffee ($P \leq 0.05$) (Table 3). As in the previous experiments, population growth on coffee was low (Pf/Pi = 1.02). Squash and sweet pepper did not differ statistically from okra and coffee but were higher than French marigold. The lowest Pf/Pi for K_5 occurred on French marigold, onion, small onion, coffee, and watermelon, and no statistical difference was found among them.

Watermelon was the only plant that supported M_2 population growth greater than sesame (Table 3). Other good hosts for M_2 were squash, eggplant, and melon, which had Pf/Pi similar to watermelon and sesame. The population growth of M_2 on French marigold, onion, sweet pepper, coffee, lettuce, small onion, and okra were low, with no difference among them. In experiments 6 and 7, okra, lettuce, and sweet pepper were good hosts for K_5 and poor hosts for M_2. Watermelon was the best host of M_2 but one of the poorest hosts for K_5.

TABLE 3. Population growth (Pf/Pi) on Marília (K_5) and Rio de Janeiro (M_2) isolates of *Pratylenchus coffeae* (experiments 6 and 7, respectively) 75 days after inoculation.

Plant	K_5 Experiment 4	M_2 Experiment 5
Sesame	—[a]	1.71 b
Okra	6.16 a	0.64 c
Melon	4.64 ab	2.05 ab
Eggplant	3.72 ab	2.45 ab
Lettuce	3.47 ab	0.18 c
Sweet pepper	2.21 abc	0.04 c
Squash	2.12 abc	2.90 ab
Watermelon	2.00 bcd	3.87 a
Coffee	1.02 cd	0.08 c
Small onion	0.66 cd	0.51 c
Onion	0.20 cd	0.28 c
French marigold	0.00 d	0.02 c

Data are means of five replicates. Means within a column followed by a common letter are not different according to Tukey test ($P \leq 0.05$).
[a] Not tested.

The initial population (Pi = 120 in experiment 1 and 1,000 in experiments 2, 3, 4, 5, 6, and 7) of both isolates probably did not affect the health of the plants tested, and the appearance of plant roots was normal at the end of the experiments.

DISCUSSION

The host range of K_5 differs from that of M_2. The population growth of the isolates differed substantially in approximately one-third of the plants in the experiments. For example, the low Pf/Pi of M_2 on gramineous crops, except sorghum, is remarkable because these plants were the best hosts for K_5. Differences in the host ranges of K_5 and M_2 are in agreement with Duncan et al. (1999) isolate differentiation based on morphometric characteristics. According to molecular analyses, two isolates collected from citrus roots in São Paulo State (C_1 and C_2 in their work) were unlike the other isolates of *P. coffeae* collected worldwide, perhaps defining one or more undescribed species. Molecular analysis of K_5 was not done, but C_1 and C_2 were included in Group V based on morphometric relationships. The status of K_5 requires further research because this isolate is different from C_1 and C_2 in not reproducing on Rangpur lime (*Citrus limonia*). Duncan et al. (1999) included M_2 in Group III, which comprises five *P. coffeae* isolates collected from coffee in Indonesia and four isolates collected from citrus roots in Florida and Oman. However, our results showed that coffee and citrus (*C. limonia*) did not differ from French marigold as poor hosts of M_2 (experiment 3, 5, and 7). The low final population of K_5 on Rangpur lime and of M_2 on coffee and Rangpur lime suggests the presence of biotypes in the groups defined by Duncan et al. (1999). If the groups are considered as possibly different species, these species probably have races.

Population growth of both isolates was low on coffee. Even the K_5 isolate, obtained from coffee roots, did not increase greatly on coffee (Pf/Pi ranging from 1.02 to 2.35 in four experiments). Greatest population growth of K_5 on coffee occurred in experiment 1, perhaps because only 120 nematodes were inoculated per plant and more root tissue was available to each nematode. Coffee supported fewer K_5 than half of the plants in experiments 4 and 6. It is noteworthy that one isolate collected from cocoyam in São Paulo State (code M_1 in Duncan et al., 1999), and included with M_2 in Group III, reduced the growth of coffee seedlings cv. Mundo Novo (Inomoto et al., 1998). However, nematode num-

bers increased slowly on coffee (Pf/Pi = 0.3 at 70 days after inoculation), reaching high numbers only much later (Pf/Pi = 14.0 at 350 days). So, M_2 and K_5 appear to increase very slowly on coffee; but, because coffee is a perennial crop, sufficient time is available in the field for the nematode to attain damaging population densities.

The low population of K_5 and M_2 on banana (*Musa acuminata*) and the high population of M_2 on sesame were other findings that support the suggestion that both isolates are variants of *P. coffeae,* because banana is typically a host and sesame a nonhost (Almeida et al., 1978; Das and Das, 1986; Gowen and Quénéhervé, 1990). It is remarkable that the *P. coffeae* isolate from cocoyam (M_1) decreased (Pf/Pi = 0.01) on banana (Oliveira et al., 1995).

Duncan et al. (1999) addressed the taxonomic status of *P. coffeae* using morphometric and molecular characteristics. Studies on reproductive compatibility among the isolates of *P. coffeae* and other biological studies are necessary to conclude this work. Our research shows that K_5 and M_2 exhibit conspicuous differences in host range, and they differ from other isolates. Our results demonstrate that K_5 and M_2 may be characterized by host range and that the differences between these isolates noted by Duncan et al. (1999) are linked to host range. In conclusion, K_5 and M_2 are likely separate species, in accord with Duncan et al. (1999), but they probably include host races.

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