COFFEA ARABICA CVS. CATURRA AND CATUAI NONHOSTS TO A CALIFORNIA ISOLATE OF *MELODOGYNE JAVANICA*

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ABSTRACT

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Root penetration, nematode egress from roots, development, and reproduction of a California *Meloidogyne javanica* isolate on *Coffea arabica* cvs. Caturra and Catuai was assessed. Tomato, *Lycopersicon esculentum*, was included as a susceptible control. All experiments were conducted either in growth chambers at 25° C or in a lath house at approximately 22-28° C. Plants were inoculated with *M. javanica* second-stage juveniles (J2) and at 10, 40, or 90 days after inoculation, data on plant growth were recorded, and nematodes in roots counted after staining or extraction by mist chamber or Baermann funnel. Caturra and Catuai were nonhosts to the California isolate of *M. javanica*. Nematode penetration of Caturra and Catuai roots was very low 10 days after plants were inoculated with J2. Less than 2% of the initial inoculum (Pi) entered coffee roots, but 9 to 58% of the initial inoculum penetrated tomato roots. Very few J2 were observed in the Caturra and Catuai roots over an 8-day period, and it did not appear that low numbers of nematodes in roots was due to nematode egress. No development or reproduction of *M. javanica* was observed 40 or 90 days post-inoculation, whereas tomato supported development and reproduction. Since other *M. javanica* isolates have been reported to parasitize coffee, these results are in accord with the suggestion that geographic isolates of *M. javanica* vary in host range.

Key words: Caturra, Catuai, *Coffea arabica*, *Meloidogyne javanica*, nematode egress, nematode development, nematode reproduction, nematode resistance.

RESUMEN

Araya, M. y E. P. Caswell-Chen. 1995. *Coffea arabica* cvs. Caturra and Catuai no hospedantes para un aislamiento de California de *Meloidogyne javanica*. Nematropica 25:165-171.

La penetración radical, la salida, desarrollo y reproducción de un aislamiento californiano de *Meloidogyne javanica* sobre *Coffea arabica* cvs. Caturra y Catuai fueron evaluados. El tomate, *Lycopersicon esculentum*, se incluyó como un control susceptible. Todos los experimentos se condujeron bajo condiciones de cámaras bioclimáticas a 25°C ó invernaderos con malla a 22-28°C. Las plantas se inocularon con juveniles (J2) de *M. javanica* a 10, 40 o 90 dias después de la inoculación. Se tomaron datos de crecimiento así como del número de nemátodos teñidos que se encontraron cuando extraídos por cámara nebulizadora o el embudo de Baermann, las variedades Caturra y Catuai no fueron hospedantes del aislamiento Californiano de *M. javanica*. La penetración de raíces en ambas variedades fue baja a los 10 días después de inoculadas. Menos del 2% de J2 penetraron raíces de café y entre el 9 y 58% se observaron en las raíces de café por 8 días y tampoco los bajos números observados se debieron a la salida de los nemátodos. No se observaron indicaciones de reproducción y desarrollo del nematodo. Aislamientos de *M. javanica* se han reportado parasitando al café, estos resultados sustentan la sugerencia que los aislamientos de *M. javanica* difieren en gama de hospedantes.

Palabras clave: caturra, catuai, *Coffea arabica*, *Meloidogyne javanica*, salida de nemátodos, desarrollo, resproducción y resistencia.

INTRODUCTION

Approximately 18 *Meloidogyne* species have been reported as parasites or associates of coffee. It appears that a *Meloidogyne* sp. reported to damage coffee in one country is not necessarily harmful to the same *Coffea* sp. in another country. The variable damage caused by *Meloidogyne* species in different geographic regions may result from variability in *Meloidogyne* spp., *Coffea* species or environments among countries. Coffee has been reported to be relatively resistant to *M. javanica* (Treub, 1885; Chitwood, 1949) (22). However, *M. javanica* has been reported associated with coffee in El Salvador (1,2), Colombia (15), Brazil (7,16), Tanzania (7), Zaire (7), India (3,7), Cuba (21), Puerto Rico (21), Guatemala (21), Jamaica (21), and the Ivory Coast (21). Yield losses due to *M. javanica* have not been defined. However, Vovlas and Di Vito (21) estimated a damage threshold of 1.9 and 3.4 eggs and juveniles/cm^3 of soil, respectively, for total plant weight and plant top weight of *C. arabica* cv. Sao Tome seedlings when infected by an Italian *M. javanica* population.

Quantitative data on the penetration, development, reproduction and early damage caused by *M. javanica* infecting *C. arabica* is unavailable, and the research presented here was initiated to define these parameters using a California isolate of *M. javanica* and Costa Rican *C. arabica* cv. Caturra and Catuai, with tomato (*Lycopersicon esculentum* Mill.) included as a susceptible control.

MATERIALS AND METHODS

General experimental procedures: Root penetration by *M. javanica* was assessed in experiments 1, 2, and 3, while development and reproduction of *M. javanica* within roots was assessed in experiments 4 and 5. All experiments were conducted using *M. javanica* (UCD strain VW4) second-stage juvenile (J2) inoculum obtained from hydroponic tomato cultures (14). The species identity of this isolate was confirmed by morphology (11), North Carolina Host Range Differential Test (11), isozyme pattern (9), mitochondrial DNA (17) and preliminary RAPD analyses (8). *Coffea arabica* cvs. Caturra and Catuai were used, and tomato (*Lycopersicon esculentum* Mill) cv. UC 204 C was the susceptible control unless otherwise indicated. Steam-sterilized washed mortar sand or white silica sand (No. 60 silica sand, Corona Industrial Sand Co., Corona CA) were used as soil to assure nematode movement, soil aeration, and ease of recovering roots.

Coffea arabica seeds (Costa Rican Coffee Institute) were planted 0.5-1.0 cm deep and allowed to grow for 45 days in an open box containing autoclaved white silica sand. The box was kept in an incubator at 30° C with 12 hrs light per day. Tomato seed were germinated on moist Whatman's No. 4 filter paper in a petri dish for 4 days at 30° C. Plants were inoculated by adding an aqueous suspension of *M. javanica* J2, obtained directly from hydroponic cultures, to the sand surface. Seed and seedlings were irrigated daily with distilled water. Unless indicated otherwise, all experiments were conducted in an incubator at 25° C with 12 hrs light per day. A control treatment without nematodes was included for each coffee cultivar. Unless stated otherwise, the tap root of Caturra and Catuai was not trimmed when seedlings were transplanted.

Roots were collected, rinsed and blotted dry with paper towels. They were weighed and root lengths were measured. All roots from each plant were cleared and stained with acid-fuchsin (6,12), pressed between glass plates (7.5 × 5.0 cm^2), and the number of nematodes within roots

counted at ×40 magnification. Data collected in all experiments, as appropriate, included: plant data (shoot weight and length, number of leaves, fresh root weight and length), Pf/Pi, J2 per root system, J2 per gram of root, and J2 per cm of root. The number of J2 per cm of root and per root system were regressed with nematode Pi using PC SAS (19).

Experiment 1: Plants were transplanted individually into vials (Baxter Diagnostic 20 dram snap-cap, Hayward, California) containing 90 g of autoclaved, white silica sand. The bottoms of the vials were perforated for drainage (3.0-mm hole), and a 2.5-cm-diam mesh patch was placed in the bottom of each vial to prevent sand loss. The tap root of Caturra and Catuai seedlings was cut 1.5 cm from the base of the stem before transplanting. All vials were placed in open plastic boxes in a completely randomized design with 6 nematode densities per plant and 7 replicates. The boxes were placed in an incubator. Ten days after the seedlings were transplanted, the vials were inoculated with 0, 3, 6, 9, 12 or 15 J2/g sand. Ten days after inoculation, roots were rinsed free of sand, weighed, cleared and stained (6,12) for counting nematodes.

Experiment 2: Caturra, Catuai and tomato seedlings were transplanted individually into 464-ml cups containing 550 g of sterilized, washed mortar sand. Cups were arranged in a completely randomized design as in experiment 1. Ten days after the seedlings were transplanted, cups were inoculated with 0, 3, 6, 9, 12, or 15 J2/g sand. The tomato control consisted of 7 replicates inoculated with 6 J2/g sand. Ten days after inoculation, roots were rinsed, and stained nematodes in the roots counted.

Experiment 3: This eight-day experiment was conducted to determine whether J2 of *M. javanica* entered coffee roots but then egressed. Caturra, Catuai, and tomato seedlings were transplanted individually into 464-ml cups containing 550 g of sterilized washed mortar sand. Cups were arranged in a completely randomized design with 3 treatments and 40 replicates. Fifty-five days after Caturra and Catuai, and 15 days after tomato seedlings were transplanted, each cup was inoculated with 3 J2/g sand. Following inoculation, the number of J2 within roots was determined daily for 8 days. Each day 5 replicate plants were sampled destructively. Roots were rinsed and stained to determine the number of nematodes within roots.

Experiment 4: Development and reproduction of *M. javanica* within roots of Caturra, Catuai, and tomato were assessed 40 days after inoculation. Caturra, Catuai, and tomato seedlings were transplanted into 464-ml cups containing 550 g of sterilized, washed mortar sand. Cups were arranged in a completely randomized design with 6 nematode densities per plant and 7 replicates. Forty-five days after transplanting Caturra and Catuai and 10 days after transplanting tomato, the cups were inoculated with 0, 6, 12, 18, 24 or 30 J2/g sand. As a control for inoculum viability, seven replicates of tomato were inoculated with 6 J2/g sand. Every third day plants were fertilized with nutrient solution (14). Forty days after inoculation, plants were uprooted, and the foliage from each coffee plant was weighed and the number of leaves recorded. Roots were weighed and root lengths measured. All roots were cleared and stained, subsequently digested with cellulase and pectinase (4), and the released nematode growth stages were counted.

Experiment 5: This 90-day experiment was conducted to assess nematode reproduction and development on Caturra, Catuai, and tomato. Caturra, Catuai and tomato seedlings were transplanted into

530-ml cups containing 600 g of sterile washed mortar sand. All cups were kept in the lath house (ca. 22-28° C). Plants were irrigated with filtered water as necessary and were fertilized twice weekly with Hoagland's solution. Cups were arranged in a completely randomized design with 6 nematode densities per plant and 7 replicates. Twelve days after transplanting, cups were inoculated with densities of 0, 0.5, 1, 2, 3, and 4 J2/g sand. Fifty days after inoculation, the tomato shoots were cut about 10 cm from the base of the stem to prevent flowering and shading.

Ninety days after inoculation coffee shoots were weighed, number of leaves counted, shoot lengths measured, and root weights and lengths recorded. These measurements were not collected for tomato because plants shoots had been trimmed during the experiment. A root-gall index was estimated using a scale from 0 to 8 where 0 indicated gall-free roots (0% galling) and 8 indicated ca. 100% galling (10). From each pot, J2 were extracted from root subsamples in a mist chamber for 5 days, and from 250 g sand in a Baermann funnel for 5 days (5). The root subsamples consisted of all roots or, if there was too much root mass, 15 g of roots. The sum of total soil J2 and total root J2 were considered the final Pf per cup. An estimated reproductive index (Pf/Pi) was calculated for roots and soil by dividing the final Pf per cup by the initial J2 inoculum per cup (Pi).

RESULTS

Experiment 1 and 2: In Experiment 1, the penetration of Caturra, Catuai and tomato roots increased with increasing Pi (Fig. 1). Linear regression of mean number of nematodes per root system on Pi was significant for Caturra ($P \leq 0.05$, $r^2 = 0.65$), Catuai ($P \leq 0.05$, $r^2 = 0.72$), and tomato ($P \leq 0.05$, $r^2 = 0.71$). A significant linear regression of mean number of J2 per cm of root on Pi was obtained for tomato ($F = 20.1$, $P \leq 0.01$, $r^2 = 0.83$), with the greatest infection density (112.5 J2/cm of root) observed at a Pi = 12 J2/g sand. In Experiments 1 and 2, penetration was 9 to 58% of the initial inoculum in tomato roots, but was never greater than 2% in Caturra and Catuai. At comparable Pi, the number of J2 in Caturra and Catuai roots was approximately one-tenth of the penetration observed in tomato (Fig. 2). The root length of Caturra and Catuai was not proportional to *M. javanica* Pi.

Experiment 3: More J2 penetrated tomato than Caturra and Catuai (Fig. 3). Penetration of tomato plateaued 3 days after inoculation. The number of J2 in the root system of Caturra ranged from 0 to 5.8 during the 8 days of the experiment. Nematode penetration of Catuai roots was low, ranging from 0 to 1.6 J2 per root system, and no clear evidence of egress of large numbers of J2 was detected.

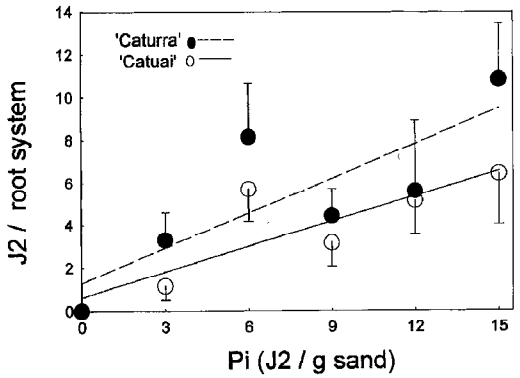

Fig. 1. Number of *Meloidogyne javanica* juveniles (J2) in roots of *Coffea arabica* (cvs. Caturra and Catuai) roots 10 days after inoculation with different initial densities (Pi) of *Meloidogyne javanica*. Linear regression for *Coffea arabica* cv. caturra: $y = 1.28 + 0.54 \times Pi$; $F = 7.69$, $P \leq 0.05$, $r^2 = 0.66$. Linear regression for Catuai: $y = 0.6 + 0.4 \times Pi$; $F = 10.3$, $P \leq 0.01$, $r^2 = 0.72$. (Data are means of 7 replicates + SE).

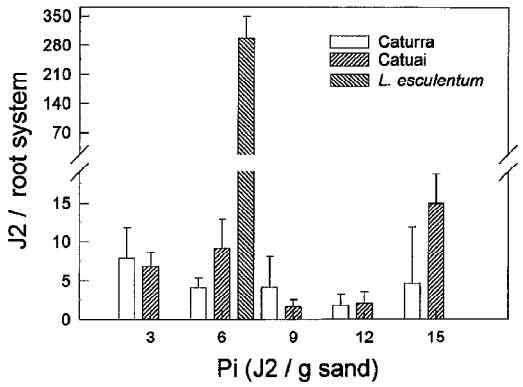

Fig. 2. The total number of juveniles (J2) 10 days post-inoculation in roots of *Coffea arabica* cvs. Caturra and Catuai, and tomato inoculated with different initial densities of *Meloidogyne javanica*. (Data are means of 7 replicates ± SE).

Experiment 4: No nematode development or reproduction was observed in either Caturra or Catuai seedlings, while all 5 developmental stages were recovered from tomato, with an average of 18 000 eggs and 170 adult females observed per tomato root system. Caturra and Catuai plant growth parameters were not affected by exposure to *M. javanica* J2. Root weight varied from 0.23 to 0.28 in Caturra, and from 0.21 to 0.24 g/root system in Catuai. Root length in Caturra ranged from 10.5 to 11.1, and 9.8 to 10.4 cm per root system in Catuai. Number of leaves per plant of Caturra varied from 2.29 to 3.42 and from 2.5 to 3.1 for Catuai.

Experiment 5: Tomato had a root-gall index of 8.0. In tomato, the Pf/Pi and the number of J2 per g of root decreased with increasing Pi, and the greatest number of J2 per g of root was observed when plants were inoculated with the lowest Pi (0.5 J2/g sand). No galls were found on roots of Caturra and Catuai, and no J2 were observed in soil or roots. Plant growth parameters of Caturra and Catuai were not affected by *M. javanica* at any of the Pi densities examined.

DISCUSSION

Our experiments reveal that *C. arabica* cvs. Caturra and Catuai were not readily penetrated by a California isolate of *M. javanica*. Those few juveniles that did enter the roots did not develop and reproduce. It is possible that the low penetration was because coffee was not attractive to this nematode isolate. However, the experimental conditions, sand as the soil substrate and the small soil volumes, should have enhanced J2 ability to find roots while minimizing their energy expenditure.

By using the North Carolina Differential Host Test, the International *Meloidogyne* Project found that "Variation in pathogenicity to the differential hosts among populations of the major species of *Meloidogyne* is relatively uncommon" (11). However, others have noted that host-range, in the broad sense, varies among geographic isolates of *Meloidogyne* spp. (18), and our results lead us to suspect that that suggestion is probably accurate. Understanding host-range variation in

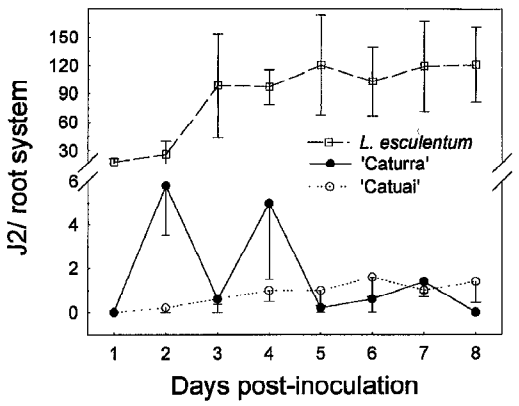

Fig. 3. Number of nematodes in roots of *Coffea arabica* cvs. Caturra, Catuai and tomato inoculated with 1650 (J2) *Meloidogyne javanica* in 550 g sand and kept at 25° C. Roots were collected and stained each day until 8 days post-inoculation. (Data are means of 5 replicates ± SE).

Meloidogyne spp. associated with coffee is important for designing management strategies based on cover cropping or rotations.

Our results generally agree with Whitehead (22) that coffee is very resistant to *M. javanica*; however, our findings on the host status of *C. arabica* to *M. javanica* are not in accord with other reports that C. arabica is parasitized by *M. javanica* (1,2,3,7,21). It is possible that different *C. arabica* cultivars are differentially susceptible to *M. javanica*. More likely, there is considerable variation among different geographic isolates of *M. javanica* with respect to their abilities to parasitize various plant species (18), including coffee. As has been noted elsewhere, *Meloidogyne incognita* displays differential pathogenicity to coffee in different geographic regions and in different soil types (20). It is also possible that detection of *M. javanica* in coffee plantations result from *M. javanica* parasitizing weeds, as reported in Cuban coffee plantations (13). Finally, it may be that the *Meloidogyne* species have been incorrectly identified in some studies, perhaps due to a mixture of species and the difficulties associated with their identification (18).

The hypothesis that low numbers of J2 in roots was due to J2 egress from roots was not supported, as numbers were low and stayed low. It is possible that nematodes egressed from Caturra roots during the first five days, although if they did, they only did so at very low numbers. If large numbers of nematodes were penetrating and then leaving the roots, the number of J2 observed in the roots should have varied widely over our short sampling interval, and this was not observed. In addition, coffee seedling age did not seem to affect nematode penetration in these experiments as plants inoculated at 55-days-old (Exp. 1 and 2) or 100-days-old (Exp. 3) had similar, low J2 penetration. The low penetration and limited development observed in these experiments was not a result of using a hypovirulent *M. javanica* isolate since VM4 was pathogenic and showed typical parasitism on tomato in all experiments. The experimental temperatures were within an acceptable range for *M. javanica* growth and reproduction (20). Thus, the coffee examined in these studies was weakly suitable for *M. javanica* penetration and showed no nematode reproduction.

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