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EFFECT OF ROOT-KNOT NEMATODES *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA* ON THE GROWTH OF COFFEE (*COFFEA ARABICA* L.) IN POTS

by
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Summary. The relationship between initial population densities (*Pi*) of two Italian root-knot nematode species (*Meloidogyne incognita* race 1 and *M. javanica*) and growth of coffee (*Coffea arabica*) seedlings was studied in a glasshouse experiment. Initial population density of both nematode species consisted of (0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 or 256) eggs and second stage juveniles/cm^3 of soil. The response of the coffee plants to the initial population density was fitted to the model $y = m + (1\text{-}m)z^{P\text{-}T}$. The tolerance limit (*T*) of coffee seedlings to *M. incognita* race 1 was 2.09 eggs and juveniles/cm^3 soil and to *M. javanica* 1.9 and 1.34 eggs and juveniles/cm^3 soil for total and fresh top weight, respectively. A minimum relative yield (*m*) of coffee was 0.4 at Pi $\geq$ 128 eggs/cm^3 soil of *M. incognita* and 0.4 and 0.5 for total and fresh top weight, respectively at the same levels of inoculum for *M. javanica*. Cross sections of *M. incognita* infected roots showed several moderately sized giant cells arranged around the nematode head, compared with the well developed large multinucleate giant cells induced by *M. exigua*.

Nematodes known to attack coffee roots include root-knot *Meloidogyne* species which may be very destructive to coffee plantations depending on the region and species involved. Yield losses due to *Meloidogyne* spp. attacks have been estimated at 15% in Brazil (Gonzaga and Lordello, 1986), while in pot experiments conducted in Puerto Rico by Negron and Acosta, (1987) significant reductions of roots and shoots (32 and 34%) of coffee seedlings were dimostrated with inoculations of 21.3 eggs/cm^3 of soil of *M. incognita*. *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw. damage coffee trees in Puerto Rico, Guatemala, Cuba, Jamaica, Brazil, Ivory Coast and S. Tomé and Principe but the economic conseguences of infestation have not yet been assessed (Vovlas and Lamberti, 1985; Gonzaga and Lordello, 1986). We describe the relationship between initial population densities of *M. incognita* and *M. javanica* and the growth of young coffee (*Coffea arabica* L.) plants and illustrate the anatomical alterations induced by *M. incognita* in coffee roots compared with those induced by *M. exigua* Goeldi in coffee seedlings collected in Brazil.

Materials and methods

The Italian populations of *Meloidogyne incognita* race 1 (Taylor and Sasser, 1978) and *M. javanica* were reared separately on tomato (*Lycopersicon esculentum* Mill.) cv. Roma VF in a glasshouse at 25 ± 3°C. When large egg-masses were formed, the roots were finely chopped and numbers of nematodes (infective stages) were estimated by processing ten samples with 1% aqueous solution of sodium hypochlorite (Hussey and Baker, 1973); then the roots were thoroughly mixed with 5 kg of sterilized sandy soil and used as inoculum. Ninety six clay pots were each filled with 0.5 l of steam sterilized sandy soil (sand 88.1%, silt 3.9%, clay 7.0% and organic matter 2.5%). Appropriate amounts of the inoculum were mixed into the soil in each pot to give population densities of 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 or 256 eggs and juveniles/cm^3 soil. The use of infected roots was selected because it was considered to be more efficient than dispersed eggs (Di Vito *et al.*, 1986). Four pots of each inoculum level and nematode population were used. A single three- month old seedling of *Coffea arabica* «Sao Tomè local variety» was transplanted in each pot. The pots were arranged in a randomized block design on a glasshouse bench at 25 ± 3°C.

Three months after transplanting, the coffee plants were uprooted and the root systems gently washed. Fresh weights of tops and roots were recorded. Nematodes in the roots were extracted by Coolen's method (Coolen, 1979) and counted. Data of total and top plant fresh weight were analyzed by Seinhorst's equation (Seinhorst, 1965) and root fresh weight and nematodes extracted from the roots were statistically analyzed and LSD's calculated.

Histological observations were made on roots infested with *M. incognita* and *M. exigua*. Root segments were fixed in a formaldehyde-acetic acid-alcohol solution, dehydrated in TBA (tertiary butyl alcohol) series and embedded in paraffin. Root sections 10-12 µm thick were stained with safranin and fast-green, mounted in Dammar balsam and examined with the aid of a compound microscope (Johansen, 1940).

Results and discussion

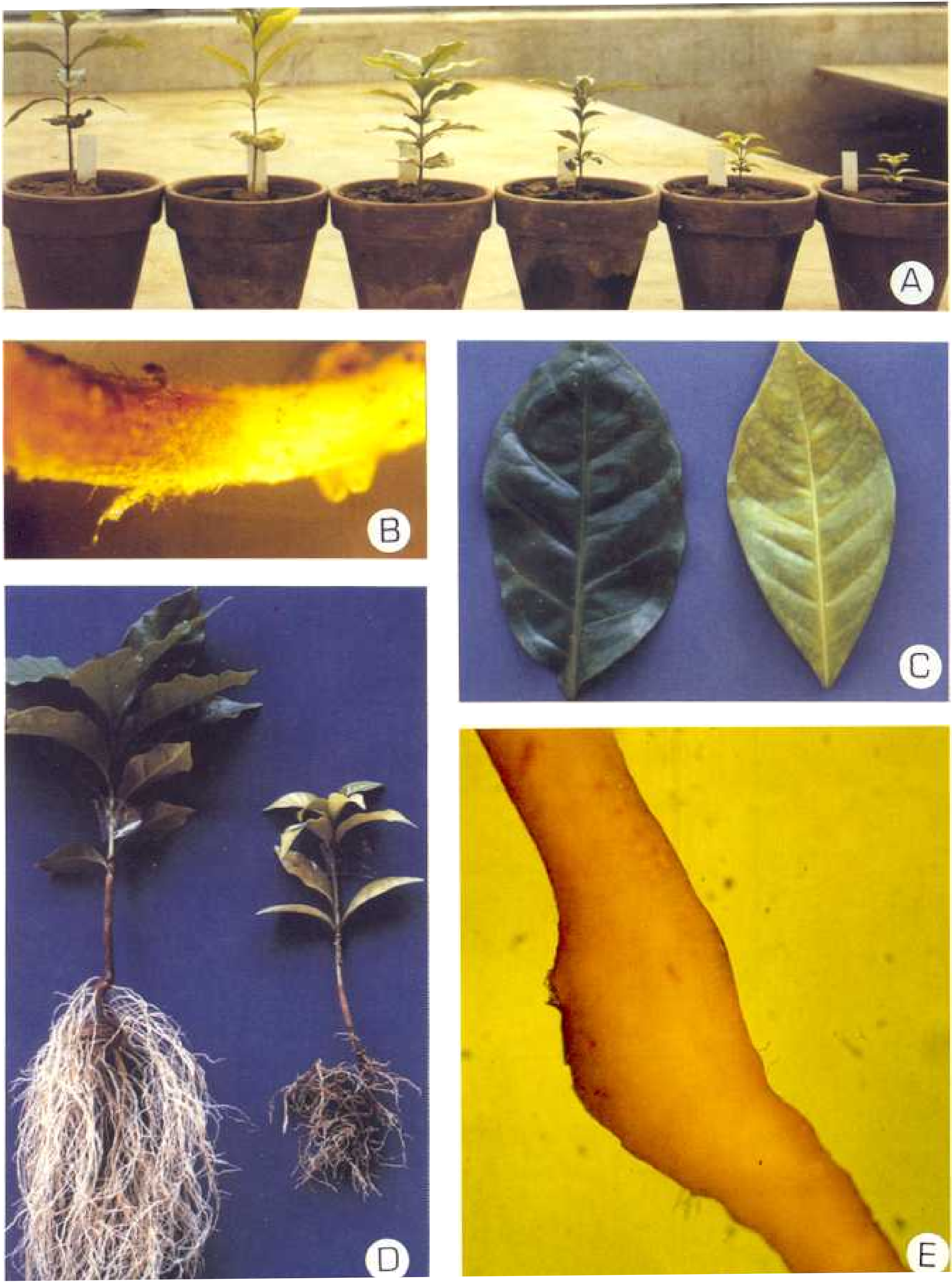
The Italian populations of *M. incognita* and *M. javanica* negatively affected the growth of coffee plants (Figs. 1, 2). Symptoms were evident at population densities Pi ≥ 16 eggs/cm^3 soil where there was marked reduction of growth and chlorosis of the top of plants. The fresh weight of coffee plants at the initial population density (*Pi*) of *M. incognita* and *M. javanica* was fitted to the equation $y = m + (1\text{-}m)z^{P\text{-}T}$ proposed by Seinhorst (1965), where $y =$ the ratio between the yield (fresh top weight) at *Pi* and that at $P \leq T$, $m =$ the minimum relative top weight (y at very large Pi), $z =$ a constant <1 with $z^{-T} = 1.05$, $T =$ the tolerance limit (*Pi* at which no yield is lost), and $P =$ initial population density of nematode.

The tolerance limit (*T*) of total and top fresh weight of coffee to *M. incognita* race 1 was 2.09 eggs and juveniles /cm^3 soil, and the minimum relative yield was 0.4 at $Pi \geq$ 128 eggs juveniles/cm^3 soil (Fig. 3). The tolerance limits (*T*) of coffee to *M. javanica* were 1.9 and 1.34 eggs/cm^3 soil for total and fresh top plant weight, and the minimum yields to the same nematode were 0.4 and 0.5 at $Pi \geq$ 128 eggs/cm^3 soil for total and fresh top plant weight of coffee, respectively (Fig. 3). The root systems of coffee plants were greatly reduced by *M. incognita* and *M. javanica* infestation. In fact the initial population densities of both nematode species can negatively affect the growth of roots (Tab. I) and at higher inoculum level of these pathogens the root weight was about 50% lower than that found at low levels of inoculum.

The reproduction of *M. incognita* and *M. javanica* on coffee was inconsistent or nil and only juvenile stages were found in the roots. However the number of nematode specimens recovered from the roots of coffee was lower

Fig. 1 - Effect of different population densities of *Meloidogyne incognita* on the growth of coffee seedlings. Stunted and yellow plants correspond to the higher inoculum densities.

Fig. 2(*Front page*) - Coffee plants infected with *Meloidogyne incognita*: A), effect of increasing population densities (left to right) on the growth of coffee plant; B), detail of a necrotic area of an infected coffee plant; C), green and yellowed leaves respectively from healhtly and nematode-infected plants; D) Non infected (left) and stunted infected plant (right); E), detail of a single gall without egg-masses.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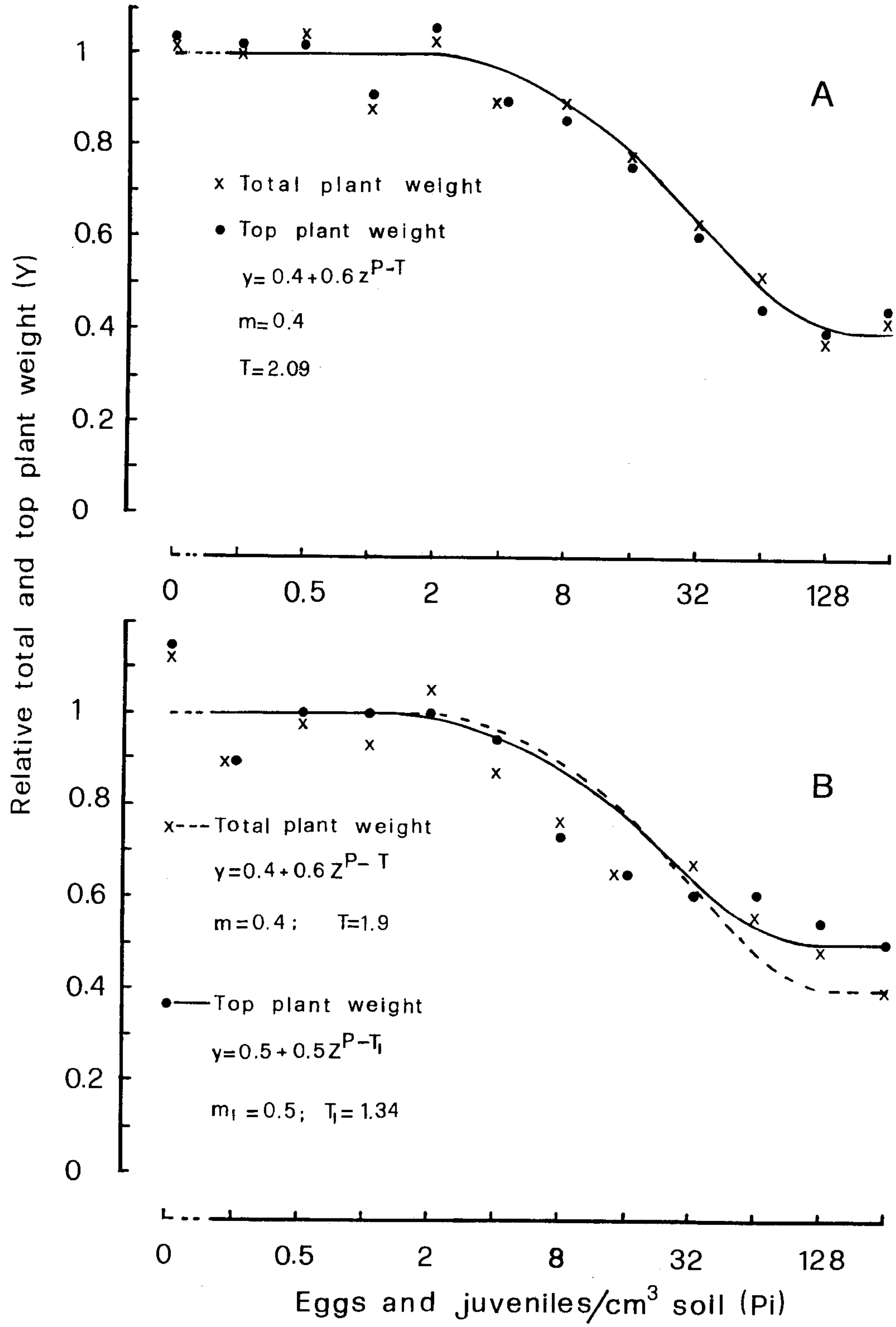

Fig. 3 - Relationship between initial population densities (*Pi*) of *Meloidogyne incognita* (A), or *M. javanica* (B) and relative plant weight (y) of coffee plants, 60 days after transplanting.

with *M. javanica* infection than with *M. incognita*, 45.7 vs. 69.4 per g of roots, respectively at $Pi = 256$ eggs/cm^3 soil (Tab. I). Coffee roots infected by *M. exigua* were deformed by spheroidal galls (3-10 times the root diameter) located along the root axis, with large egg masses protruding from the root surface. Histological examination of these galls indicated that *M. exigua* induces the formation of well developed giant cells located in the stele and surrounding the anterior portion of the nematode (Fig. 4B). The histological modifications observed in coffee roots are similar in their patterns to those reported for other root-knot nematodes by Taylor and Sasser (1978).

In contrast to the extensive galls induced by *M. exigua*, moderately developed galls were observed in *M. incognita* infected roots (Fig. 2E). Infested plants had poorly developed and deformed roots, with small elongated galls (3-4 times the root diameter) without egg masses present along the root axis. Extensive lesions (necrotic areas on the root surface) were also frequently observed on infected roots (Fig. 2B). Sectioned galls showed undersized giant cells in contrast to the well developed large multinucleate giant cells observed in *M. exigua* — infected coffee roots.

Negron and Acosta (1987) reported significative egg-production and well developed giant cells of *M. incognita* infected coffee seedlings in Puerto Rico. Probably they worked with *M. incognita* race 3, a common nematode in several coffee growing areas (Taylor *et al.*, 1982). Although no reproduction of *M. incognita* and *M. javanica* occurred in coffee seedlings, our findings indicate that coffee can be severely damaged by both nematodes particularly in soil enriched by new inoculum reared on weed plant hosts common in coffee plantations. Also in the favorable conditions, such as relatively high temperatures, of the coffee-growing countries the root-knot nematodes can reproduce quickly and reach damaging population levels relatively early. Therefore potential sites for the establishment of nurseries or coffee plantations should be evaluated not only on the basis of their agronomical and edaphic qualities, but also on their nematological history. Nematode damage in our experiment was severe because of the young seedlings used. The value of the minimum yield (*m*) will undoubtedly increase with plant age (Seinhorst, 1979).

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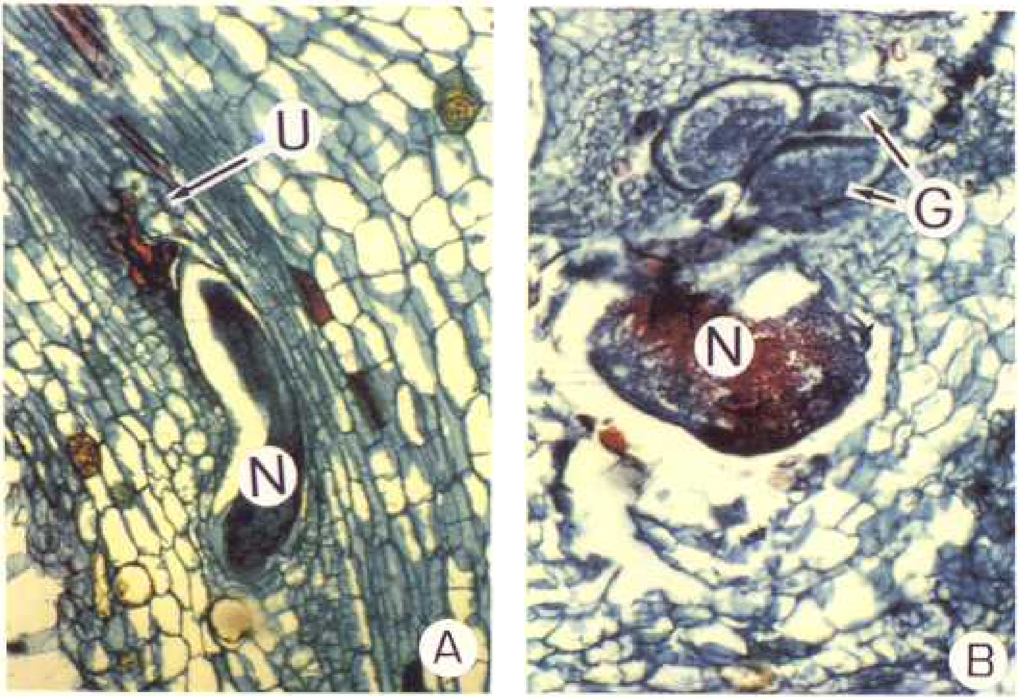

Fig. 4 - Anatomical changes induced by *Meloidogyne incognita* (A) and *M. exigua* in coffee roots (B). Note the undersized giant cells (U) induced by *M. incognita* in our experiment, in contrast to the well developed large multinucleate giant cells (G) induced by *M. exigua* on coffee roots collected in Brazil. (N = Nematode).

TABLE I - *Effect of initial population densities of* Meloidogyne incognita *race 1 and* M. javanica *on the number of nematode specimens in the coffee roots and on the weight of coffee roots.*

Eggs and juveniles/ cm³ soil (*Pi*)	Root weight of coffee (g)		N. of specimens/g of coffee roots	
	M. incognita	*M. javanica*	*M. incognita*	*M. javanica*
0	2.2		—	—
0.25	3.0		0.5	0
0.5	3.5		0	0
1	2.6		0.6	5.8
2	3.0		0	1.3
4	2.7		3.2	3.4
8	2.8		4.1	12.8
16	2.4		7.1	5.9
32	1.9		18.5	8.6
64	1.5		39.1	15.9
128	1.0		40.6	28.7
256	1.1		69.4	45.7
LSD $P \leq 0.05$	1.5 NS		23.8	21.1
$P \leq 0.01$	2.1 NS		32.0	28.3

Literature cited

COOLEN W.A., 1979 - Methods for the extraction of *Meloidogyne* spp. and other nematodes from roots and soil, pp. 317-330, *in*: «Root-knot Nematodes (*Meloidogyne* species) Systematics, Biology and Control» (F. Lamberti and C.E. Taylor eds.), Academic Press, London.

DI VITO M., GRECO N. and CARELLA A., 1986 - Effect of *Meloidogyne incognita* and importance of the inoculum on the yield of eggplant. *J. Nematol. 18*: 487-490.

GONZAGA L. AND LORDELLO E., 1986 - «Plant parasitic Nematodes of Bananas, Citrus, Cotton, Grapes and Tobacco», Union Carbide. Agr. Products Company Inc., pp. 71.

HUSSEY R.S. and BAKER K.R., 1973 - A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Reptr. 57*: 1025-1028.

JOHANSEN D.A., 1940 - Plant Microtechnique. Mc Graw Hill, New York, pp. 523.

NEGRON I.A. and ACOSTA N., 1987 - Studies on host- parasite relationships of *Meloidogyne incognita* and *Coffea arabica* cv. Borbon. *Nematropica, 17*: 71-79.

SEINHORST J.W., 1965 - The relationship between nematode density and damage of plants. *Nematologica, 11*: 137-154.

SEINHORST J.W., 1979 - Nematodes and growth of plants: formulation of the nematode-plant system, pp. 231-256 *in*: Root-knot Nematodes (*Meloidogyne* Species), Systematics, Biology and Control. (F. Lamberti and C.E. Taylor eds). Academic Press, London.

TAYLOR A.L. and SASSER J.N., 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Dept. Plant Pathol. North Carolina State University Graphics, Raleigh, N.C. (USA), pp. 111.

TAYLOR A.L., SASSER J.N. and NELSON L.A., 1982 - Relationship of climate and soil characteristics to geographical distribution of *Meloidogyne* species in agricoltural soils. Dept. Plant Pathol. North Carolina State University Graphics, Raleigh, N.C. (USA), pp. 65.

VOVLAS N. and LAMBERTI F., 1985 - Some Tylenchids from coffee plantations of S. Tomé. Proc. ONTA 17th ann. Meeting, July 28-Aug. 2, 1985, S. Augustine, Trinidad, p. 39.

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