PATHOGENICITY OF *MELOIDOGYNE EXIGUA* ON COFFEE (*COFFEA ARABICA* L.) IN POTS

M. Di Vito,[1] R. Crozzoli,[2] and N. Vovlas[1]

Istituto di Nematologia Agraria, Consiglio Nazionale delle Ricerche, Via G. Amendola 165/A 70126 Bari, Italy,[1] and Laboratorio de Nematología Agrícola, Instituto de Zoología Agrícola, Universidad Central de Venezuela, Apto, 4579 Maracay-2101-A, Venezuela.[2]

ABSTRACT

Di Vito, M., R. Crozzoli, and N. Vovlas. 2000. Pathogenicity of *Meloiodogyne exigua* on coffee (*Coffea arabica* L.) in pots. Nematropica 30:55-61.

The relationship between a geometric series of fourteen initial population densities (*Pi*) of *Meloidogyne exigua* between 0 and 512 eggs and juveniles/cm^3 soil and growth of coffee (*Coffea arabica*) was investigated in one-liter clay pots. The Seinhorst model, $y = m + (1 - m)z^{P-T}$, was fitted to average plant height, internode length, and fresh top weight. Tolerance limits (T) to the nematode for height, weight and lengths of internodes of coffee plants were 5.9, 1.2 and 6.2 eggs and juveniles/cm^3 soil, respectively. The minimum relative yields (m) were 0.7 and 0.4 at $Pi \geq 256$ eggs and juveniles/cm^3 soil for height and internode length of plants, respectively, and 0.5 at $Pi \geq 128$ eggs and juveniles/cm^3 soil for plant top weight. Maximum nematode reproduction was 422-fold at lowest initial population densities (*Pi*). The histopatology of coffee roots infected by *M. exigua* reveals swollen root tips and axes. Nematode egg masses were visible on root surfaces only in small root-galls whereas commonly they were embedded in the root tissues. Multiple infection sites were common, resulting in galls containing several females. Formation of specialized cells (giant cells) in the stele, disruption of the vascular system, and hyperplasia of the vascular parenchyma was the most common anatomical alterations observed in infected coffee roots.

Key words: Coffea arabica, coffee, histopatology, *Meloidogyne exigua*, pathogenicity, root-knot nematode, tolerance limit.

RESUMEN

Di Vito, M., R. Crozzoli y N. Vovlas. 2000. Patogenicidad de *Meloidogyne exigua* en café (*Coffea arabica* L.) en maceteros. Nematrópica 30:55-61.

Se estudió la relación entre una serie geométrica de catorce densidades poblacionales iniciales (*Pi*) de *Meloidogyne exigua* las cuales oscilaron entre 0 y 512 huevos y juveniles/cm^3 de suelo y el crecimento de café (*Coffea arabica*) en maceteros de arcilla de un litro de capacidad. Valores de altura, peso fresco de follaje y longitud de los entrenudos del tallo fueron introducidos en el modelo de Seinhorst $y = m + (1 - m)\ z^{P-T}$. Los límites de tolerancia (T) de altura, peso de las plantas y longitud de los entrenudos del tallo al nematodo fueron 5.9, 1.2 y 6.2 huevos y juveniles/cm^3 de suelo, respectivamente. Los rendimentos mínimos relativos (m) fueron 0.7 y 0.4 a $Pi \geq 256$ huevos y juveniles/cm^3 de suelo para altura de las plantas y longitud de los entrenudos, respectivamente y 0.5 a $Pi \geq 128$ huevos y juveniles/cm^3 de suelo para el peso aéreo de la planta. La reproducción máxima del nematodo fue de 422 veces, alcanzada con la menor densidad poblacional inicial (*Pi*). Se estudió la histopatología del nematodo en las raíces de café infectadas. Dichas raíces mostraban engrosamientos tanto en el ápice como a lo largo de su eje. Las masas de huevos de los nematodos fueron visibles en la superficie solamente en las agallas mas pegueñas pero comúnmente se encontraron dentro del tejido vascular. Múltiples sitios de infección fueron comunes resultando en agallas que contenían más de una hembra. La formación de células especializadas (células gigantes) en el cilindro central, disrupción del sistema vascular e hiperplasia del parénquima vascular son las alteraciones anatómicas más comunes observadas en raíces infectadas.

Palabras claves: Café, *Coffea arabica*, histopatología, límite de tolerancia, *Meloidogyne exigua*, nematodo agallador, patogenicidad.

INTRODUCTION

Coffee (*Coffea arabica* L.) is an important crop in tropical and subtropical countries and is cultivated on 11 167 000 ha yielding 6 456 000 MT. In Venezuela, 150 000 ha each year are devoted to this crop with a production of 50 600 MT of green coffee (FAO, 1998). The world yield loss due to nematode infestations was estimated to be 15% annually (Mendes *et al.*, 1977). Among nematode pests, several species of root-knot nematodes (*Meloidogyne* spp.) are pathogens of coffee. *Meloidogyne exigua* Goeldi, is a common nematode in Central and South America, where it causes severe loss of coffee yield (Campos *et al.*, 1990; Flores and Yépez, 1969). Although several authors have reported yield suppression in coffee infested with nematodes (Campos *et al.*, 1990), few have determined threshold levels for damage by this nematode. Therefore, a study was conducted in pots in a greenhouse to determine the effect of population densities of a Venezuelan population of *M. exigua* on the growth of coffee and on the dynamics of nematode populations on this plant species. The anatomical alterations induced by *M. exigua* in coffee roots were also investigated.

MATERIALS AND METHODS

A Venezuelan population of *M. exigua* from coffee was reared in Bari, Italy on tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers in a greenhouse at 26 ± 3°C. When large egg masses appeared on the tomato roots, the roots were gently washed free of adhering soil, finely chopped, and numbers of eggs and juveniles in ten 5 g samples were estimated by shaking in jars in 100 ml of 1% aqueous solution of sodium hypochlorite for 4 min (Hussey and Barker, 1973). The remaining roots were then thoroughly mixed with 3 kg of steam sterilized sandy soil and used as inoculum. Eighty-four clay pots were each filled with 1 000 cm^3 of steam sterilized sandy soil (sand 88%, silt 5%, clay 7% and organic matter 2.5%). Appropriate amounts of the inoculum were mixed into the soil of each pot to give population densities of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 or 512 eggs and juveniles/cm^3. We used chopped infested tomato roots because they were found to be more efficient than dispersed eggs (Di Vito *et al.*, 1986).

A single three-month old seedling of a Sao Tomè local coffee variety was transplanted in each pot. The pots were arranged in a randomized complete block design, having six replications, on benches in a glasshouse at 26 ± 3°C. Four months after transplanting, fresh top weight, height and number of stem internodes of plants were recorded. The length of internodes was calculated by dividing the height of each plant by the number of internodes of the same plant. The root systems were gently washed and weighed. Nematodes were extracted by macerating roots in a blender for 90 seconds in a 1% sodium hypochlorite solution, followed by centrifugation according to Coolen's method (Coolen, 1979). Eggs and second stage juveniles of the nematode present in the soil were also extracted by Coolen's modified method (Coolen, 1979; Di Vito *et al.*, 1985). The sum of the nematodes extracted from roots and soil of the same pot was considered as the final population density (*Pf*).

The Seinhorst model, $y = m + (1 - m)z^{Pi-T}$ (Seinhorst, 1965; 1979) was fitted to the data for top fresh weight, plant height, and length of stem internodes. In this model, *Pi* is the initial population density, *y* is the ratio between the yield at *Pi* and that at *Pi* ≤ *T*, *m* is the minimum relative yield (*y* at very large *Pi*), *z* is a constant < 1 with z^T = 1.05, and *T* is the tolerance limit (*Pi* below which no yield is lost).

In a separate study, galled coffee roots were collected from infected plants after 60 days of exposure to the nematode, washed free of adhering soil and debris and individual galls and uninfected pieces of roots selected. Galled and healthy material was fixed in chrome-acetic acid and glacial acid solution, dehydrated with a tert-butyl alcohol series (from 50 to 100% concentration) and embedded in 58°C (melting point) histoplast. Sections 10-12 μm were cut and stained with safranin and fast green according to Johansen's method (Johansen, 1940). Selected sections containing the nematode feeding sites were compared with tissue from uninfected roots.

RESULTS AND DISCUSSION

The Venezuelan population of *M. exigua* affected the growth of coffee plants negatively (Fig. 1a). Symptoms of nematode attack were evident at an initial population density (*Pi*) of 8 eggs and juveniles/cm^3 soil and consisted of a marked reduction of growth of the plant top. The tolerance limits (*T*) of coffee plant fresh top weight, height and stem internode length to *M. exigua* were 1.2, 5.9 and 6.2 eggs and juveniles/cm^3 soil, respectively. The minimum relative yields (*m*) were 0.7 and 0.4 at *Pi* ≥ 256 eggs and juveniles/cm^3 soil for plant height and stem internode length, respectively, and 0.5 at *Pi* ≥ 128 eggs and juveniles/cm^3 for plant fresh top weight (Figs. 2, 3 and 4).

The highest final population density (*Pf*) of the nematode was 249 eggs and juveniles/cm^3 soil and occurred at *Pi* = 16 eggs and juveniles/cm^3 soil (Table 1). The maximum reproduction rate (*Pf/Pi*) of *M. exigua* on coffee was 422-fold at the lowest initial population density (*Pi*) and decreased with increasing initial population densities (Table 1).

Galls commonly occurred individually (at root tips or along the axes) or in clusters involving the entire root circumference, thus resulting in root diameters 2-8 times larger than that for uninfected roots (Fig. 1). Randomly selected individual galls showed that more than 75% of galls contained egg masses. Galls usually contained feeding sites of more than one female nematode.

Root sections stained with safranin and fast green revealed both hypertrophy and hyperplasia, disorganized and disrupted xylem elements and vascular tissues. Nematode feeding sites were comprised of giant cells (3-8 per specimen) usually surrounding the heads of females, although undersized giant cells were associated with pre-adult males. Active multinucleated giant cells contained granular cytoplasm and numerous hypertrophied nuclei and nucleoli (Fig. 1b-f). Dense giant cell cytoplasm lined deeply stained thick cell walls.

The anatomical changes induced by *M. exigua* in coffee seedling roots in our experiment are similar to those that most root-knot nematodes induce in their hosts (Vovlas and Di Vito, 1991), and particularly to those illustrated by Mendes *et al.* (1977) on coffee roots, although they studied material which was exposed to nematode infection for a longer period, with the likelihood that more nematode generations had developed.

The experiment confirms the destructive effect of *M. exigua* on coffee. In fact the tolerance limits of this crop to the nematode are very low and similar to those obtained by other authors (Ferreira Dias Rodrigues and Crozzoli, 1995). Therefore,

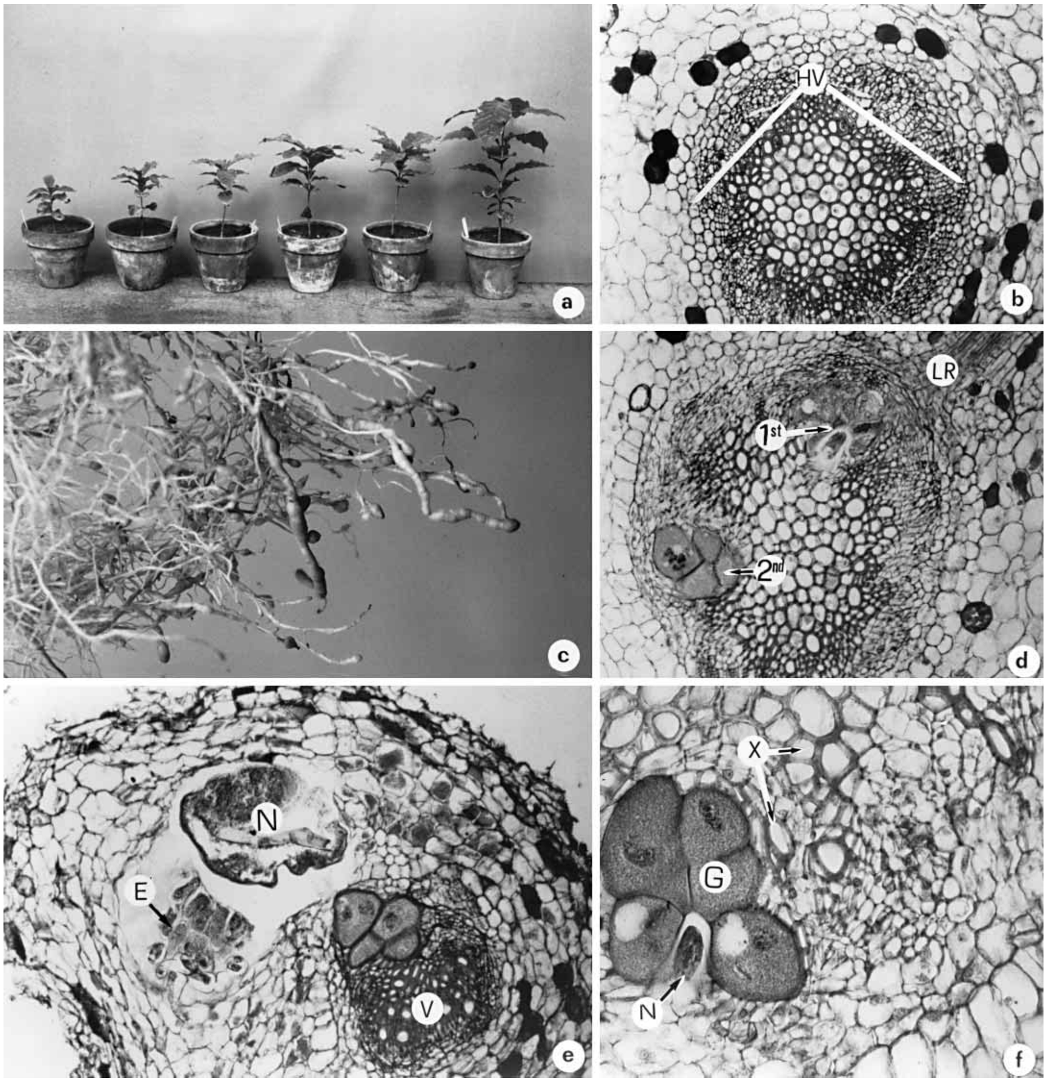

Fig. 1. Coffee plants infected with *Meloidogyne exigua*: a) effect of increasing population densities of the nematode on the growth of coffee plants; b) transverse section of uninfected coffee root, for comparison, showing healthy vascular system (HV); c) infected roots with galls; d, e, f) cross sections of infected coffee roots showing nematode females (N) with eggs (E) and well developed large multinucleated giant cells (G); note two feeding sides (1st and 2nd) induced by visible specimens, abnormal and compressed xylem elements (X) caused by giant cell expansion, and undersized giant cells induced by the nematode near the lateral root (LR).

potential sites for establishment of coffee orchards should be checked for nematode infestation, and the use of plants free of infection by the nematode is a prerequisite to ensure good crop productivity.

The decline of *M. exigua* populations at high *Pi* on local coffee variety Sao Tomè likely resulted from reduced food supply for the nematodes because of poor plant growth. However the reproduction of the

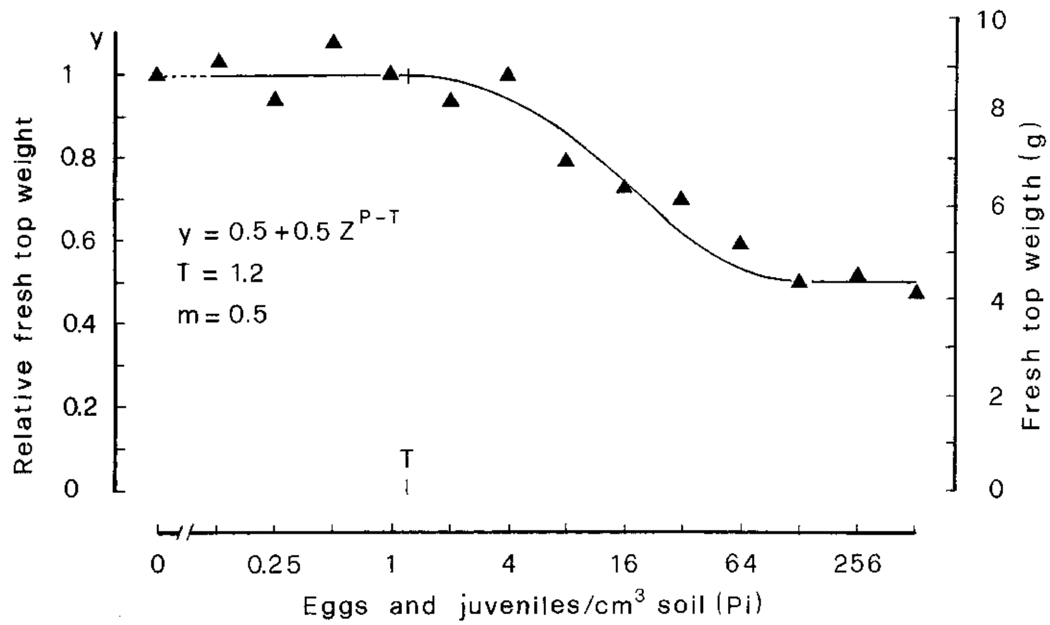

Fig. 2. Relationship between initial population density (*Pi*) of a Venezuelan population of *Meloidogyne exigua* and relative fresh top weight (*y*) of coffee plants grown in clay pots maintained in a glasshouse at 26 ± 3°C.

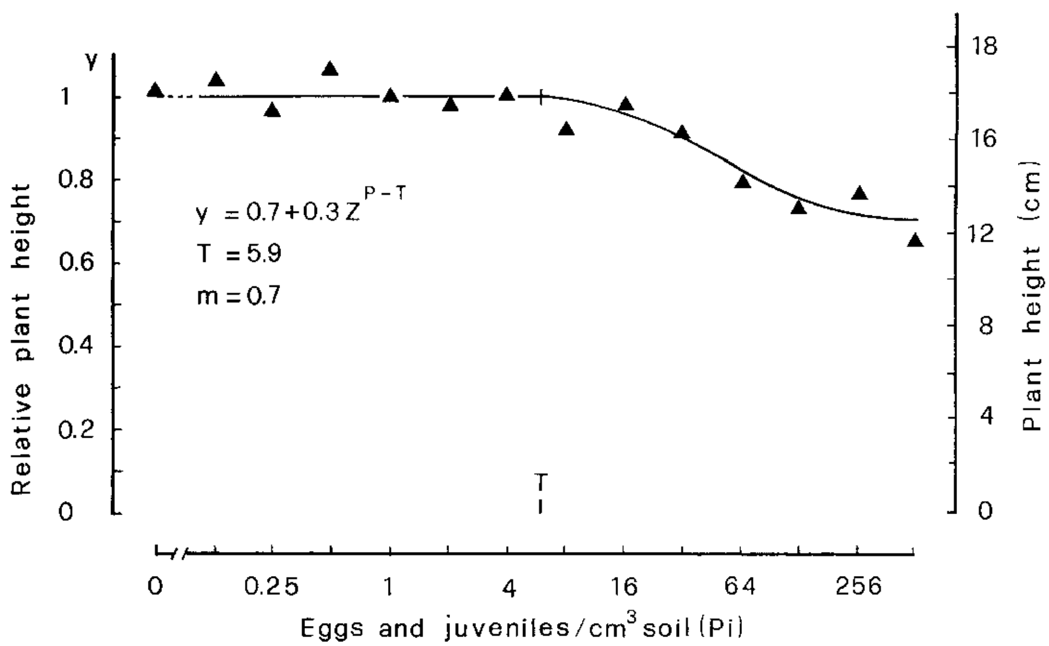

Fig. 3. Relationship between initial population density (*Pi*) of a Venezuelan population of *Meloidogyne exigua* and relative height (*y*) of coffee plants grown in clay pots maintained in a glasshouse at 26 ± 3°C.

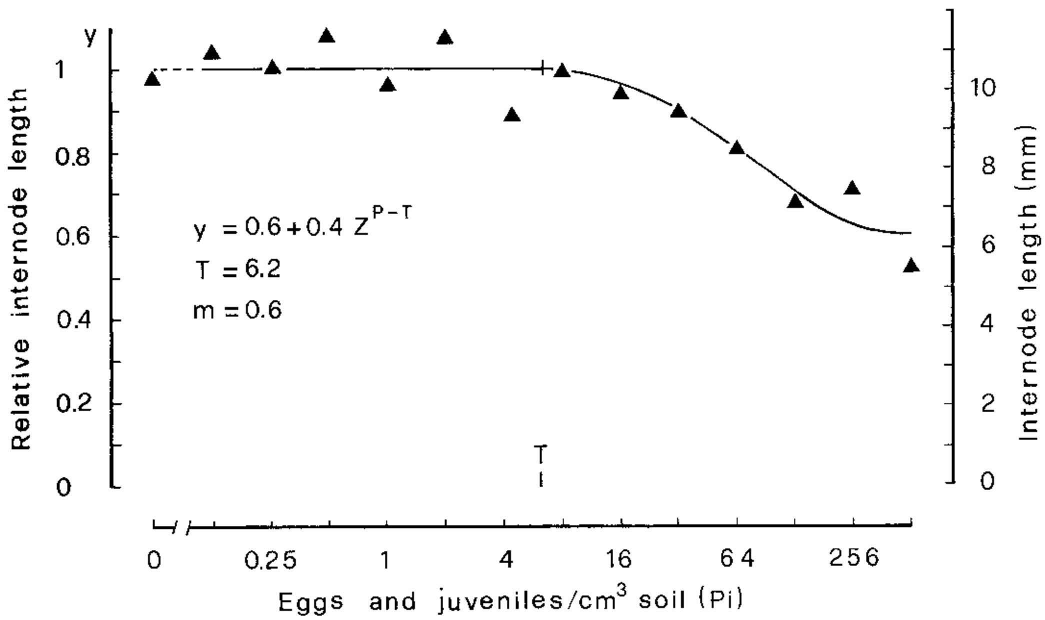

Fig. 4. Relationship between initial population density (*Pi*) of a Venezuelan population of *Meloidogyne exigua* and relative length of internodes (*y*) of coffee plants grown in clay pots maintained in a glasshouse at 26 ± 3°C.

nematode in our experiment was higher than that reported in a similar experiment in Venezuela (Ferreira Dias Rodrigues and Crozzoli, 1995). The discrepancy may be due to differences in coffee varieties and climatic conditions in our greenhouse

Table 1. Effect of initial population densities (*Pi*) of *Meloidogyne exigua* on final population densities (*Pf*) and reproduction rate (*Pf/Pi*) of the nematode maintained on coffee plants grown in clay pots.

Eggs and juveniles/cm^3		Reproduction rate
Initial population densities (*Pi*)	Final population densities (*Pf*)	(*Pf/Pi*)
0.125	52.8	422.4
0.25	84.0	336.0
0.5	99.5	199.0
1	161.5	161.5
2	130.2	65.1
4	110.3	27.6
8	178.5	22.3
16	249.0	15.6
32	197.3	6.2
64	79.5	1.2
128	128.5	1.0
256	101.6	0.4
512	109.7	0.2

experiment compared to those in the previous report.

ACKNOWLEDGEMENTS

We thank Mrs. G. Zaccheo and F. Catalano for technical assistance, and Mr. V. Radicci for preparing the drawings.

LITERATURE CITED

CAMPOS, V. P., P. SIVAPALAM, and N. C. GNANAPRAGASAM. 1990. Nematode parasites of coffee, cocoa and tea. Pp. 387-429 *in* M. Luc, R. A. Sikora, and J. Bridge, eds. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford, U.K.

COOLEN, W. A. 1979. Methods for the extraction of *Meloidogyne* spp. and other nematodes from roots and soil. Pp. 317-330 *in* F. Lamberti and C.E. Taylor, eds. Root-knot Nematodes (*Meloidogyne* species) Systematics, Biology and Control. Academic Press, New York, NY, U.S.A.

DI VITO, M., N. GRECO, and A. CARELLA. 1985. Population densities of *Meloidogyne incognita* and yield of *Capsicum annuum*. Journal of Nematology 17:45-49.

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

FAO. 1998. FAO production yearbook, Vol. 51. Food and Agriculture Organization of the United Nations, Rome, Italy.

FERREIRA DIAS RODRIGUES, I., and R. CROZZOLI. 1995. Efectos del nematodo agallador *Meloidogyne exigua* sobre el crecimiento de plantas de café en vivero. Nematologia Mediterranea 23:325-328.

FLORES, J. M., and T. YÉPEZ. 1969. *Meloidogyne* in coffee in Venezuela. Pp. 251-256 *in* J. E. Peachey, ed. Nematodes of Tropical Crops. C.A.B, St. Albans, U.K.

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

JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Company, New York, NY, U.S.A.

MENDES, B. V., S. FERRAZ, and C. SHIMOYA. 1977. Observationes histopatologicas de raizes de caffeeiro parasitadas por *Meloidogyne exigua* Goeldi, 1887. Nematologia Brasileira 2:208-229.

SASSER, J. N., and D. W. FRECKMAN. 1987. A world perspective on nematology: the role of the Society. Pp. 7-14 *in* J. A. Veech and D. W. Dickson, eds. Vistas on Nematology. Society of Nematologists, Hyattsville, MD, U.S.A.

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

SEINHORST, J. W. 1979. Nematodes and growth of plants: formulation of the nematode-plant system. Pp. 231-256 *in* F. Lamberti and C. E. Taylor, eds. Root-knot Nematodes (*Meloidogyne* species) Systematics, Biology and Control. Academic Press, New York, NY, U.S.A.

VOVLAS, N., and M. DI VITO. 1991. Effect of root-knot nematodes *Meloidogyne incognita* and *M. javanica* on the growth of coffee (*Coffea arabica* L.) in pots. Nematologia Mediterranea 19:253-258.

Received: *12.X.1999*
Recibido:

Accepted for publication: *27.XII.1999*
Aceptado para publicación:

BLANK PAGE USED IN PAGE COUNT