

## RESEARCH NOTE - NOTA INVESTIGATIVA

### EFFECT OF *ROTYLENCHULUS RENIFORMIS* ON THE GROWTH OF PAPAYA IN POTS

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#### ABSTRACT

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The relationship between a geometric series of ten initial densities ( $P_i$ ) of *Rotylenchulus reniformis* between 0 and 64 eggs, juveniles and young females/cm<sup>3</sup> soil and growth of papaya (*Carica papaya*) "Paraguanera type" was investigated in one-liter clay pots. The Seinhorst model,  $y = m + (1-m)z^{p-t}$ , was fitted to plant average fresh and dry top weight. Tolerance limits ( $T$ ) to the nematode for fresh and dry top weight of papaya plants were 0.25 and 0.18 eggs, juveniles and young females/cm<sup>3</sup> soil, respectively. The minimum relative yields ( $m$ ) were 0.67 and 0.65 at  $P_i \geq 16$  eggs, juveniles and young females/cm<sup>3</sup> soil for fresh and dry top weight of plants, respectively. The population of *R. reniformis*, determined 120 days after inoculation, fitted the model  $P_f = axy(1-q^p)(-\log q)^{-1} + (1-x)P_i + sx(1-y)P_i$ . Maximum nematode reproduction was 36-fold for the lowest initial population density ( $P_i$ ), and the equilibrium density was 14 eggs, juveniles and young females/cm<sup>3</sup> soil. The nematodes penetrated roots and stimulated the production of a number of specialized cells to form a distinct stellar syncytium.

*Key words:* *Carica papaya*, papaya, pathogenicity, reniform nematode, *Rotylenchulus reniformis*, tolerance limit.

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#### RESUMEN

Crozzoli, R., G. Perichi, N. Vovlas y N. Greco. 2004. Efecto de *Rotylenchulus reniformis* en el crecimiento de papaya en macetas. *Nematropica* 35:53-58.

Se estudió la relación entre una serie geométrica de diez densidades poblacionales iniciales ( $P_i$ ) de *Rotylenchulus reniformis* entre 0 y 64 huevos, juveniles y hembras inmaduras/cm<sup>3</sup> de suelo y el crecimiento de plantas de papaya (*Carica papaya*) "tipo Paraguanera" en macetas de arcilla de un litro. Los valores de peso aéreo fresco (PAF) y seco (PAS) de las plantas se ajustaron a la ecuación de Seinhorst,  $y = m + (1-m)z^{p-t}$ . Los límites de tolerancia ( $T$ ) para PAF y PAS fueron de 0,25 y 0,18 juveniles y hembras inmaduras/cm<sup>3</sup> de suelo, respectivamente, mientras que el rendimiento mínimo ( $m$ ) relativo fue de 0,67 y 0,65 para  $P_i \geq 16$  nematodos/cm<sup>3</sup> de suelo, respectivamente. Los valores de población final de *R. reniformis*, determinados 120 días después de la inoculación, se ajustaron a la ecuación  $P_f = axy(1-q^p)(-\log q)^{-1} + (1-x)P_i + sx(1-y)P_i$ . La reproducción máxima del nematodo fue de 36 veces, alcanzada con la menor población inicial ( $P_i$ ), y la densidad de equilibrio fue de 14 huevos, juveniles y hembras inmaduras/cm<sup>3</sup> de suelo. El nematodo penetró en las raíces y estimuló la producción de numerosas células especializadas que formaron un sincitio en la región estelar.

*Palabras clave:* *Carica papaya*, límite de tolerancia, nematodo reniforme, papaya, patogenicidad, *Rotylenchulus reniformis*.

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Papaya (*Carica papaya* L.) is native to tropical America and is widely cultivated in Central and South America for the production of fresh fruit (Bustillo *et al.*, 2000;

Rosales and Suárez, 2001). Of the several nematodes reported to be associated with papaya, only two genera, *Meloidogyne* and *Rotylenchulus*, are of economic importance

and are distributed worldwide in papaya plantations (Cohn and Duncan, 1990). *Meloidogyne* spp. are generally more damaging than *Rotylenchulus reniformis* Linford & Oliveira (Pinochet and Ventura, 1980; Figueroa, 1989; Pinochet and Guzman, 1987; Bridge *et al.*, 1996). Severe damage to papaya by reniform nematode has been reported in Puerto Rico (Ayala *et al.*, 1971) and Trinidad (Singh and Farrell, 1972).

In Venezuela, *Meloidogyne incognita* (Kofoid & White) Chitwood and *R. reniformis* are the two most common plant parasitic nematodes associated with papaya cultivation (Petit, 1990; Bustillo *et al.*, 2000; Rosales and Suárez, 2001). The damage threshold for *M. incognita* on papaya has been determined (Bustillo *et al.*, 2000), but information on *R. reniformis* remains lacking. Therefore, an investigation was conducted in pots in a screen house to determine *i*) the effect of increasing population densities of a Venezuelan population of *R. reniformis* on the growth of papaya "Paraguaná type", *ii*) the dynamics of the nematode population and *iii*) the anatomical alterations induced by *R. reniformis* on this plant species.

#### Screen House Experiment

The original *R. reniformis* population used in this experiment was isolated from pigeon pea (*Cajanus cajan* L.) at Barquisimeto (Estado Lara, Venezuela) and reared on cowpea (*Vigna unguiculata* (L.) Walp.) cv Bayo. When egg masses developed, the cowpea roots were gently washed in running tap water, and then macerated in a blender for 120 s. The eggs and motile life stages were counted and used as inoculum in an aqueous suspension. Twenty-day-old seedlings of "Paraguaná type" papaya were transplanted singly into 17-cm-diameter clay pots containing 1000 cm<sup>3</sup> of steam-pasteurized soil (sand 60.8%, silt 34%, clay 5.2%, organic matter 6.8%, pH 7.2). One week later, appropriate amounts of the

nematode suspension were poured uniformly into five holes made around the papaya roots to obtain population densities of 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 or 64 eggs, juveniles and immature females/cm<sup>3</sup> soil. There were six replicates for each nematode population density. All pots were arranged on benches in a screen house in Maracay (Estado Aragua, Venezuela), according to a randomized block design. During the course of the experiment, 200 ml of a fertilizer (15-15-15, NPK) solution (2.5 g/l) were applied to pots soon after inoculation and every 15 days. The pots were irrigated as required.

Plants were harvested 120 days after inoculation. The roots in each pot were washed and cut into pieces approximately 0.5-cm-long. Roots from a non infested pot and from a pot infested with the highest nematode density were selected for histological observation. To determine the increase of the nematode populations, nematodes from the roots of each plant were extracted by macerating in a blender for 90 seconds and then counted. Nematodes from the soil were extracted with the Oostenbrink elutriator (s'Jacob and Van Bezooijen, 1971). Final population density (*Pf*) from each pot was considered as the sum of the nematodes extracted from roots and soil.

Data of dry and fresh plant weights were fitted to the equation  $y = m + (1 - m)z^{Pi/T}$  (Eq. 1), proposed by Seinhorst (1965; 1986b). In this equation, *y* is the relative yield (expressed as a proportion of the nematode-free growth), *m* is the minimum relative yield (the value of *y* at the highest *Pi*), *Pi* is the nematode population density at planting, *T* is the tolerance limit (*Pi* at which yield reduction first becomes apparent), and *z* a constant <1 with  $z^T = 1.05$ .

*Rotylenchulus reniformis* populations determined at harvest were fitted to the equation  $Pf = axy(1 - q^{Pi})(-\log q)^{-1} + (1 -$

$x)P_i+sx(1-y)P_i$  (Eq. 2) proposed by Seinhorst (1970, 1986a). In this model,  $P_i$  is the soil population density of the nematode at the time of inoculation,  $P_f$  is the nematode population density from soil and roots determined 120 days after inoculation,  $a$  is the maximum reproduction rate of the nematode,  $y$  is the proportion of food supply available to the nematode at a given  $P_i$  (generally equal to  $y$  of the previous equation),  $x$  is the proportion of the nematode population that could potentially infect the roots (for this nematode, immature females,  $\max = 1$ ), and  $s$  is the proportion of the nematode population (mostly immature females) remaining in the soil in the absence of the host plant. In this experiment, it was assumed that all eggs hatched even in soil with few or no roots.

*Rotylenchulus reniformis* tolerance limits ( $T$ ) for fresh and dry top weights were 0.25 and 0.18 eggs, juveniles and immature females/cm<sup>3</sup> soil, respectively. Minimum growth reduction ( $m$ ) were 67 and 65% for fresh and dry top weight, respectively, and occurred at  $P_i \geq 16$  eggs, juveniles and immature females/cm<sup>3</sup> soil (Fig. 1).

Nematode reproduction was observed at all initial population levels and was higher at the lowest  $P_i$  than at the highest  $P_i$ . The highest final population density ( $P_f$ ) of the nematode was 16.9 eggs, juveniles and immature females and eggs/cm<sup>3</sup> soil, and occurred at  $P_i = 2$  eggs (Fig. 2). The maximum rate of nematode reproduction was 36-fold and occurred at the lowest initial population density ( $P_i = 0.25$  eggs, juveniles and immature females/cm<sup>3</sup> soil) (Fig. 2).

The Oostenbrink elutriator used to extract nematodes from the soil does not extract eggs, so the  $P_f$  and maximum reproductive rate of *R. reniformis* are expected to be higher than those shown in Fig. 2. Moreover, most of the  $P_f$ , especially in pots with  $P_i \leq 1$ , derives from new generations of the

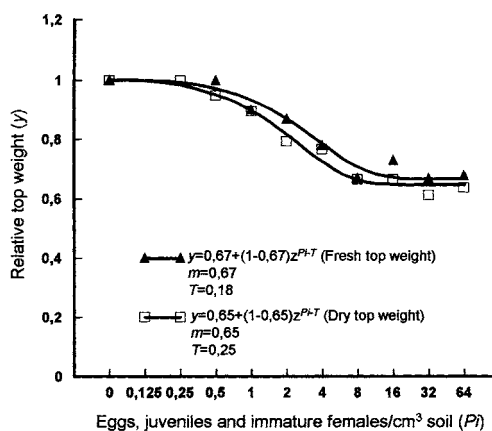


Fig. 1. Relationships between initial population densities ( $P_i$ ) of the Venezuelan population of *Rotylenchulus reniformis* and relative top fresh and dry weights ( $y$ ) of papaya "Paraguana" type in pots in screen house.

nematode and is represented by the first term of Eq. 2. Eggs of *R. reniformis* hatch and juveniles become immature females (infective stage) even in the absence of the host plant. Inserra *et al.* (1994) showed that up to 30% juveniles and immature females survived in pots with non host plants after 15 months. As our experiment lasted only four months, it can be assumed that at least

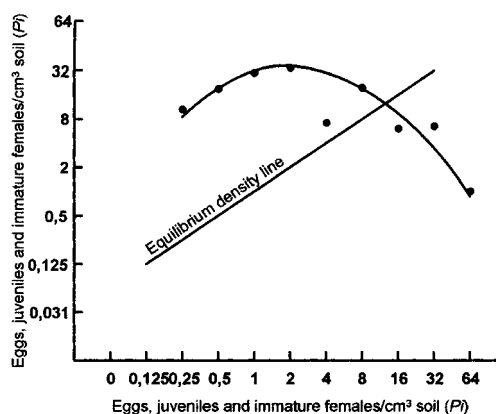


Fig. 2. Relationships between *Rotylenchulus reniformis* population densities at inoculation ( $P_i$ ) and 120 days after inoculation ( $P_f$ ) on papaya "Paraguana" type in pots in screen house.

50% of the *Pi* survived in absence of the host. Therefore, in the third term of Eq. 2,  $s$  could have been as high as 0.5, and the total *Pi* surviving at harvest could have been at least  $0.5(1-y)Pi$ .

#### Histological Observations

Non infected and nematode-infected papaya roots selected for histological observations were fixed in chrome-acetic

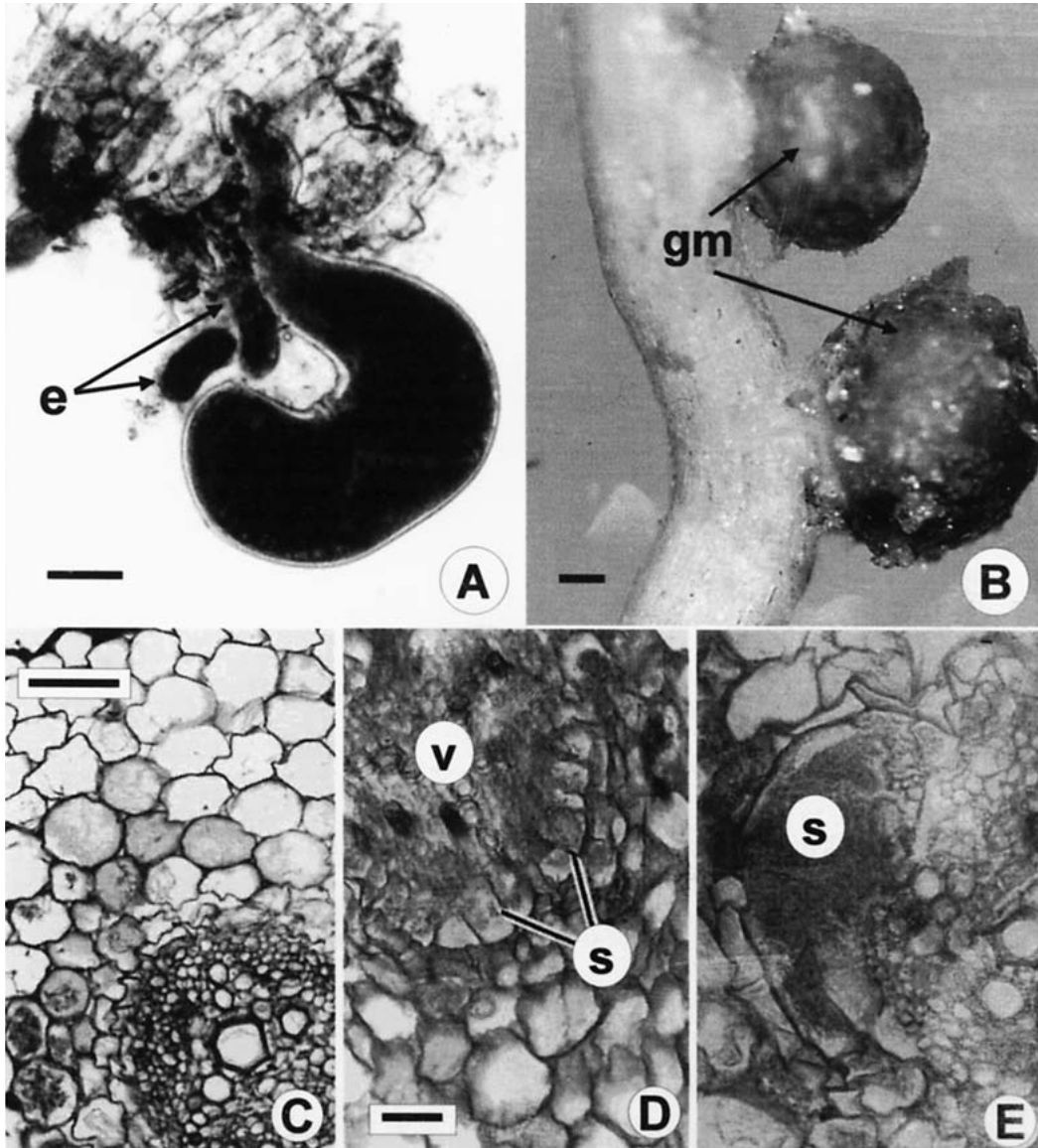


Fig. 3. Papaya roots infected with *Rotylenchulus reniformis*. A. Root portion with a partially inserted *R. reniformis* (R) female with two eggs (e); B. Soil particles adhering to the gelatinous matrix (gm); C. Cross section of an uninfected papaya root; D. Cross sections of infected roots with stelar syncytia (s), (v = vascular system); E. Feeding sites showing syncytia (s). Bars in A and B = 50  $\mu$ m; C = 40  $\mu$ m; D and E = 20  $\mu$ m.

acid and glacial acid solution, dehydrated with a ter-butyl alcohol series (from 50 to 100% v/v) 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). Sections containing the nematode feeding sites were selected, observed and photographed.

Light microscopy observations (Fig. 3) of cross sections of infected roots revealed that nematodes penetrated roots and established permanent feeding sites, as reported in the literature (Razak and Evans, 1976). The nematode penetrated roots perpendicularly to the axis, and after crossing all cortical cell layers, stimulated the production of a number of specialized cells to form a distinct stelar syncytium. The size of the feeding cell always appeared larger than that of the other syncytial cells which, usually, extended for 6-10 cell layers from the feeding point in all directions. For each female, 150-180 mononuclear nurse cells were involved in the formation of a single syncytium.

The results of this experiment confirm the ability of reniform nematode to damage papaya. Maximum yield reduction observed in our experiment (35%) was greater than that caused in a similar experiment by the southern root-knot nematode, *M. incognita* (22.5%), on the same papaya type (Bustillo *et al.*, 2000). In Venezuela (Crozzoli, personal communication), soil population densities of *R. reniformis* in plantation of papaya Paraguanera range from 1.2 to 30 individuals/cm<sup>3</sup>. The lowest population density is above the tolerance limit of papaya to the nematode established in the present work. Therefore, yield loss would occur in every papaya orchard in Venezuela where the nematode is present, and could be as large as the maximum (33-35%) observed in our experiment. However, damage levels mea-

sured in pots differ substantially from those under field conditions. Many factors other than nematodes may affect the plant response observed under field conditions (Barker *et al.*, 1985). The results of this pot experiment should be confirmed by field studies. The wide host range of the reniform nematode complicates its management on papaya because it is very difficult to find lands free of either weeds or the nematode in many tropical areas.

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#### LITERATURE CITED

- AYALA, A., N. ACOSTA, and J. A. ADSUAR. 1971. A preliminary report on the response of *Carica papaya* to foliar applications of two systemic nematicides. *Nematropica* 1:10.
- BARKER, K. R., D. P. SCHMITT, and J. L. IMBRIANI. 1985. Nematode population dynamics with emphasis on determining damage potential to crops. Pp. 135-148. in K. R. Barker, C. C. Carter and J. N. Sasser, eds. *An Advanced Treatise on Meloidogyne*. Volume II. Methodology. International *Meloidogyne* Project. North Carolina State University Graphics, Raleigh, U.S.A.
- BRIDGE, J., D. HUNT, and P. HUNT. 1996. Plant parasitic nematodes of crops in Belize. *Nematropica* 26:111-119.
- BUSTILLO, Y., R. CROZZOLI, N. GRECO, and F. LAMBERTI. 2000. Efecto del nematodo agallador *Meloidogyne incognita* sobre el crecimiento de la lechosa (*Carica papaya*) en vivero. *Nematologia Mediterranea* 28:163-170.
- COHN, E., and L. DUNCAN. 1990. Nematode parasites of subtropical and tropical fruit trees. Pp. 347-361 in M. Luc, R. A. Sikora and J. Bridge, eds. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, U.K.
- FIGUEROA, W. 1989. Nematode survey on commercial papaya farms. *Journal of Agriculture of the University of Puerto Rico* 73:165-166.
- INSERRA, R. N., R. A. DUNN, and N. VOVLAS. 1994. Host response of ornamental palms to *Rotylen-*

- chulus reniformis*. Journal of Nematology (supplement) 26:737-743.
- S'JACOB, J. J., and J. VAN BEZOOIJEN. 1971. A Manual for Practical Work in Nematology. Agricultural University, Wageningen, The Netherlands.
- JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Company, New York, USA.
- PETIT, P. 1990. Reconocimiento de nematodos fitoparásitos asociados a frutales de importancia económica en Venezuela. Fitopatología Venezolana 3:2-5.
- PINOCHET, J., and R. GUZMÁN. 1987. Nematodos asociados a cultivos agrícolas en El Salvador; su importancia y manejo. Turrialba 37:137-145.
- PINOCHET, J., and O. VENTURA. 1980. Nematodes associated with agricultural crops in Honduras. Turrialba 30:43-47.
- RAZAK, A. R., and A. A. EVANS. 1976. An intracellular tube associated with feeding by *Rotylenchulus reniformis* on cowpea root. Nematologica 22:182-189.
- ROSALES, L. C., and Z. SUÁREZ. 2001. Nematodos fitoparásitos asociados al lechoso y distribución geográfica en Venezuela. Fitopatología Venezolana 14:21-23.
- SEINHORST, J. W. 1965. The relationship between nematode density and damage to plants. Nematologica 11:137-154.
- SEINHORST, J. W. 1970. Dynamics of populations of plant parasitic nematodes. Annual Review of Phytopathology 8:131-156.
- SEINHORST, J. W. 1986a. The development of individuals and populations of cyst nematodes Pp. 101-117 in F. Lamberti and C. E. Taylor, eds. Cyst nematodes. Plenum Press, New York and London.
- SEINHORST, J. W. 1986b. Effect of nematode attack on the growth and yield of crop plants. Pp. 191-209 in F. Lamberti and C. E. Taylor, eds. Cyst nematodes. Plenum Press, New York and London.
- SINGH, N., and K. FARRELL. 1972. Occurrence of *Rotylenchulus reniformis* in Trinidad, West Indies. Plant Disease Reporter 56:551.

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