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PATHOGENIC POTENTIAL OF THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, ON RICE IN VENEZUELA

by

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Summary. An experiment was carried out in a glasshouse to investigate the effect of increasing densities of three Venezuelan populations of *Meloidogyne incognita* on growth of rice. Initial nematode densities were 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 eggs and juveniles/cm³ soil for the population from Zulia and 0, 2, 8, 32, 64 and 128 eggs and juveniles/cm³ soil for the populations from Barinas and Lara. Tiller height was recorded at five different plant stages and fresh plant weight and nematode populations in roots and soil per pot were determined at harvest. The population from Lara State was the most aggressive; all plants in pots inoculated with 128 nematodes/cm³ soil were yellowing at emergence and died 40 days later. Tolerance limits, derived according to Seinhorst, for shoot weight and tiller height of rice were 8 and 6.6, 6.6 and 4, 2.4 and 3.5 eggs and juveniles/cm³ soil for the Zulia, Barinas and Lara populations, respectively. A minimum relative yield of 0 could have been achieved at 512-420 and 156-226 eggs and juveniles/cm³ soil, for plant weight and shoot height, for the Zulia and Lara populations, respectively. Maximum reproduction rates of the nematode were 50, 19.5, and 57-fold and the equilibrium densities were 119, 256 and 62 eggs and juveniles/cm³ soil, respectively, for the populations from Zulia, Barinas and Lara.

In Venezuela 173,000 ha per year are cropped with rice mainly in the States of Cojedes, Guarico and Portuguesa (Anonymous, 1997). Two crops per year may be practiced, one under rainfed conditions, from May to October, and another under irrigation, from November to April.

Among the several nematodes reported in association with rice in many countries *Meloidogyne incognita* has been reported to damage this crop in Costa Rica and Cuba (Bridge *et al.*, 1990). This species is very common in Venezuela; therefore an experiment was undertaken in 1998 to relate different densities of three Venezuelan populations of *M. incognita* with the growth of rice.

Materials and methods

Populations of *Meloidogyne incognita* (Kofoid *et* White) Chitw. were collected from sweet potato [*Ipomea batatas* (L.) Lam.] at Barinas (Barinas State), tomato (*Lycopersicon esculentum* Mill.) at Anzoátegui (Lara State) and guava (*Psidium guajava* L.) at Mara municipality (Zulia State), the last identified as race 1 according to Taylor and Sasser (1978) by Crozzoli and Casasas (1998). All populations were reared on tomato cv. Rutgers in a glasshouse maintained at 25±3 °C. Three months later, when a large number of egg masses had formed, the plants were uprooted, and the roots gently washed in running water, cut in 0.5 cm long pieces and mixed. To es-

timate the amount of eggs and second stage juveniles available, five 10 g root samples infested with each population were processed by the sodium hypochlorite method (Hussey and Barker, 1973). Appropriate amounts of infested roots were then thoroughly mixed with steam sterilised sandy soil (89% sand) to provide population densities of 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 eggs/cm³ soil for the Zulia population and 2, 8, 32, 64 and 128 eggs/cm³ soil for populations from Barinas and Lara States; the infested soil was then used to fill 3 dm³ clay pots. There were six pots for each nematode population and density. Six pots were not infested and served as control. Pots were sown with eight seeds of the rice (*Oryza sativa* L.) cultivar Llano 5, usually cropped locally, on 6 June and thinned to four plants per pots one week after seed germination. Six additional pots for each nematode population were also infested with 128 eggs/cm³ soil, but were left uncropped to determine the decline of the nematode population in the absence of a host. All pots were arranged on benches in a glasshouse at 25±3 °C according to a randomised block design.

During the course of the experiment a fertiliser solution was applied to pots soon after seed germination and then every twenty days. The pots were watered as required.

The growth of plants was monitored by measuring separately the height of all tiller per plant on 24 June, 6 and 20 July, 10 August and 1 September. At the same time observations were made on the presence of symptoms of nematode attack (yellowing and death of the plants). At harvest (September 1) the fresh weight of each plant was measured and averages per plant and per pot were calculated.

To determine the increase of nematode population, the roots in each pot were washed free of the adhering soil, dried, weighed and cut in approximately 0.5 cm long pieces. A 20 g root sample was then processed by the sodium hypochlorite method (Hussey and Barker, 1973) and numbers of eggs counted. Further, the soil

in each pot was mixed and eggs and juveniles of the nematode extracted from 500 cm³ by Coolen (1979) method as modified by Di Vito *et al.* (1985).

Data on the tiller height were used to construct growth curves of rice (Figs. 1-3), while data on fresh weight and height at harvest were used to derive curves to relate nematode population densities with the yield of rice (Figs 4-6) according to Seinhorst's model (Seinhorst, 1965; 1986b). Also, a relation between populations of the nematode at sowing (*Pi*) and at harvest (*Pf*) was derived according to Seinhorst (1970, 1986a) (Fig. 7).

Results

At emergence most of the seedlings in the pots inoculated with 128 eggs/cm³ soil of the Lara population of *M. incognita* were yellowing and all plants had died by 20 July. At the same population density no plants had died in the pots inoculated with the population from Barinas and dead plants were observed in four pots inoculated with the population from Zulia.

To built growth curves, average total tiller height was calculated for each population and density, at different dates; then the ratios between each of these average values and those observed at harvest (1 September) in the pots inoculated with ineffective population densities were calculated and presented graphically (Figs 1-3).

In the pots inoculated with the nematode population from Barinas, no growth reduction was observed up to a density of 32 eggs/cm³ soil (Fig. 1). Growth of rice at 64 and 128 eggs/cm³ soil was clearly delayed since June 24 and at harvest it was 81% and 68%, respectively, of that of unaffected pots. From Fig. 1 it appears that at these nematode densities the plant could have reached the same size as those in less infested pots if they had been allowed to grow for a longer period.

Until 8 August rice growth was not affected by the Zulia population up to 64 eggs/cm³ soil (Fig. 2). Thereafter, the growth remained unaffected in pots inoculated with 0-16 eggs of the nematode/cm³ soil but it was suppressed in those inoculated with 32 or 64 eggs/cm³ soil in which the average total tiller height per plant at harvest was 80% and 72%, respectively, of that of the previous nematode densities. At 128 eggs/cm³ soil plant growth was always less than that at all other nematode initial densities and reached a maximum of 28% at the end of the experiment. It appeared, however, that plants in pots infested with ≥ 32 eggs/cm³ soil would not reach the normal plant size even if allowed to grow longer.

With the Lara population the growth of plants was not affected until 10 August by den-

sities up to 32 nematode eggs/cm³ soil (Fig. 3). In pots with 32 eggs/cm³ soil plant growth was only 79% of that in less infested pots. Growth reduction was very obvious soon after germination, at larger nematode densities. At harvest the growth of rice in pots with 64 eggs/cm³ soil was only 0.5 of that of lower densities. At the largest nematode density, there was some plant growth (max 2%) but only until July 7.

Averages of growth parameters (weight and height of the shoots) fitted the equation $y = m + (1-m)z^{P-T}$, proposed by Seinhorst (1965, 1986b). In this equation y is the relative yield (the rate between the average, of the growth parameter considered, at P and that at $P \leq T$), m the minimum relative yield (the value of y at the largest P), P the nematode population density at sowing, T is the tolerance limit (the magnitude of

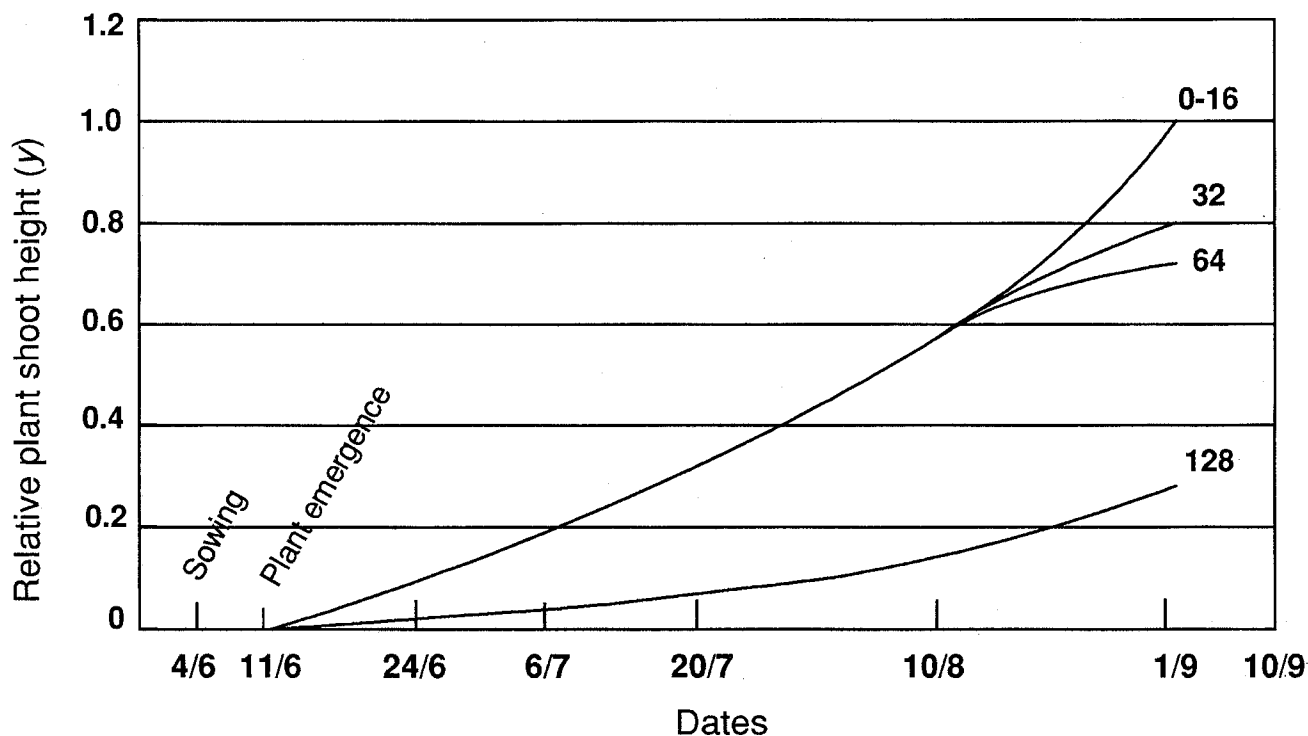


Fig. 1 - Growth curves of rice cv. Llano 5 in soil infested with increasing population densities of *Meloidogyne incognita* from Zulia. Relative plant shoot height is the ratio between average tiller length at harvest of plants in pots not affected by the nematode and averages observed at different dates in pots infested with increasing population densities of the nematode. Figures next to curves are population densities/cm³ soil at sowing (P).

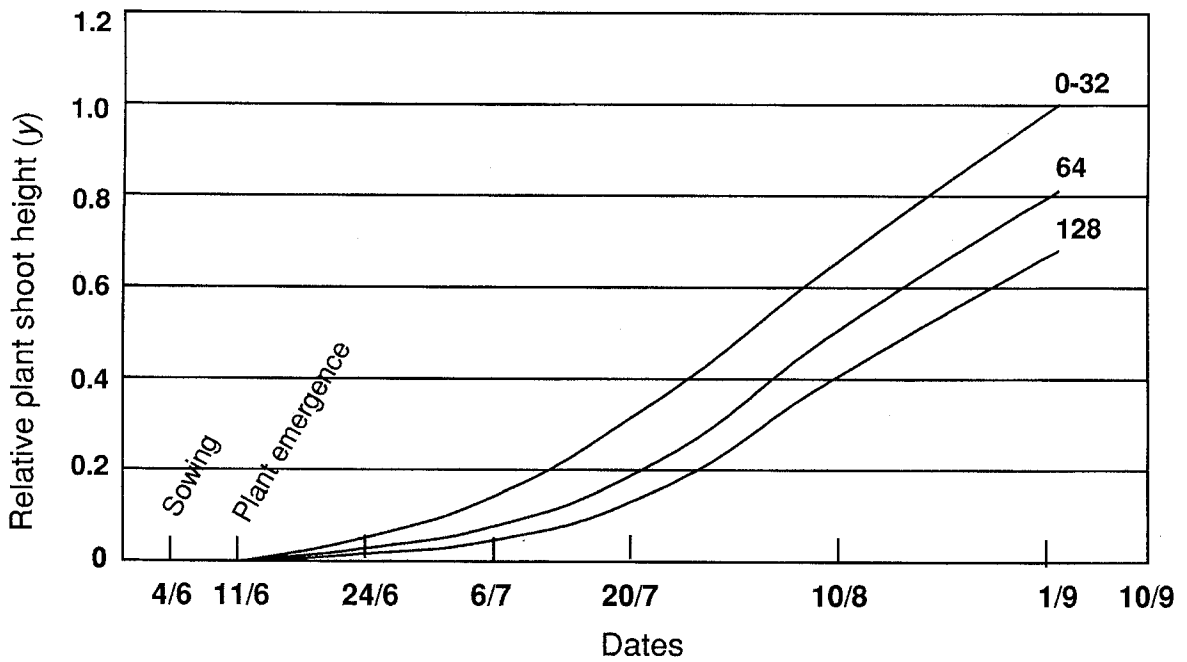


Fig. 2 - Growth curves of rice cv. Llano 5 in soil infested with increasing population densities of *M. incognita* from Barinas. Relative plant shoot height is the ratio between average tiller length at harvest of plants in pots not affected by the nematode and averages observed at different dates in pots infested with increasing population densities of the nematode. Figures next to curves are population densities/cm³ soil at sowing (*P*_i).

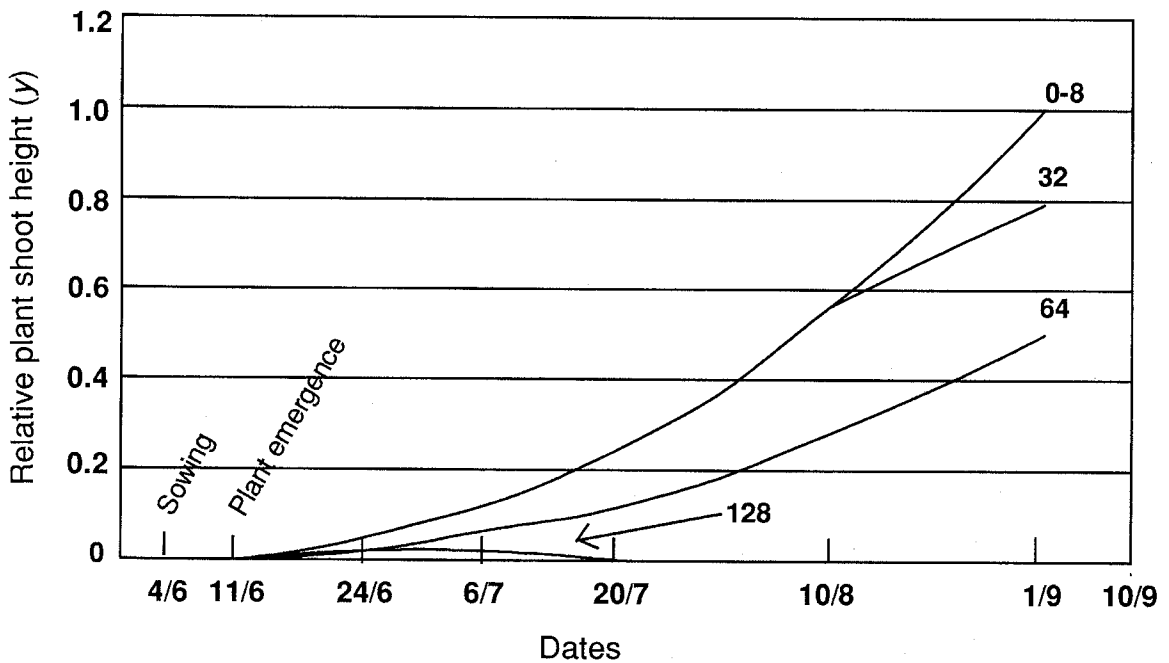


Fig. 3 - Growth curves of rice cv. Llano 5 in soil infested with increasing population densities of *M. incognita* from Lara. Relative plant shoot height is the ratio between average tiller length at harvest of plants in pots not affected by the nematode and averages observed at different dates in pots infested with increasing population densities of the nematode. Figures next to curves are population densities/cm³ soil at sowing (*P*_i).

P above which yield reduction begins to occur), and z a constant < 1 with $z^{-T} = 1.05$. Fitting average growth parameters per pots or per plants were similar and therefore only data of average per plant are presented.

Data of growth parameter of the Zulia population fitted the above equation reasonably well, with the exception of data of shoot weight of plants grown in pots infested with 8 nematodes/cm³ soil which is not reported (Fig. 4). Tolerance limits derived with this fit were 8 and 6.6 eggs and juveniles of the nematode/cm³ soil for shoot weight and height, respectively. The estimated minimum yields (m) were 0 at P_i of 512 and 420 nematodes/cm³ soil for shoot weight and shoot height, respectively. Data of the other populations fitted much better to the Seinhorst equation (Figs. 5, 6). With the Barinas population the derived tolerance limits of rice to the nematode were 6.6 eggs and juve-

niles/cm³ soil for shoot weight and 4 eggs and juveniles/cm³ soil for shoot height (Fig. 5). The estimated minimum yields were 0.4 and 0.6, respectively, and were estimated to occur at 420 and 256 eggs and juveniles/cm³ soil. In pots infested with the Lara population the tolerance limits of rice to *M. incognita* were 2.4 eggs and juveniles/cm³ soil for shoot weight and 3.5 eggs and juveniles/cm³ soil for shoot height (Fig. 6). With this population a minimum yield of 0 was estimated to occur at 156 and 226 eggs and juveniles/cm³ soil for weight and height of the shoots, respectively.

The population of *M. incognita* determined at harvest also fitted the model

$$Pf = axy(1 - q^{Pi})(-e \log q)^{-1} + (1 - x)Pi + sx(1 - y)Pi$$

proposed by Seinhorst (1970, 1986a). In this model P_i is the soil population density of the

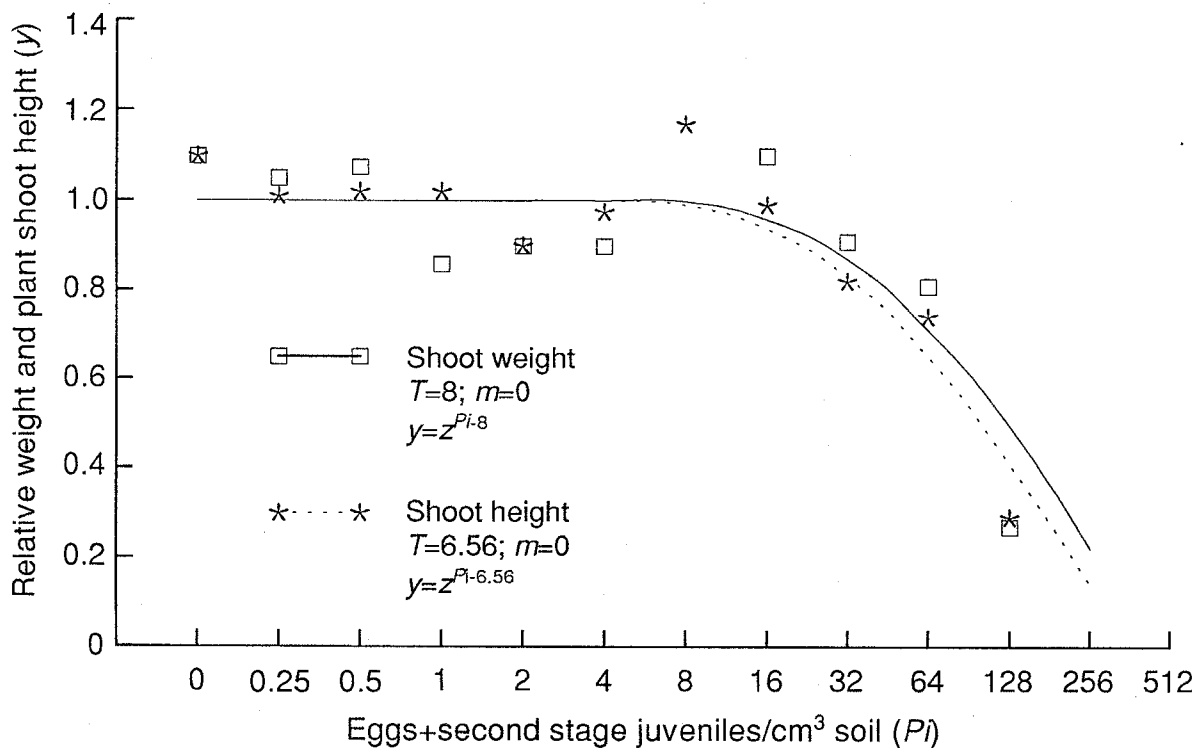


Fig. 4 - Curves according to Seinhorst (1965, 1986b) relating population densities at sowing (P_i) of a population of *M. incognita* from Zulia and relative height and fresh weight of plants of rice cv. Llano 5, in pots.

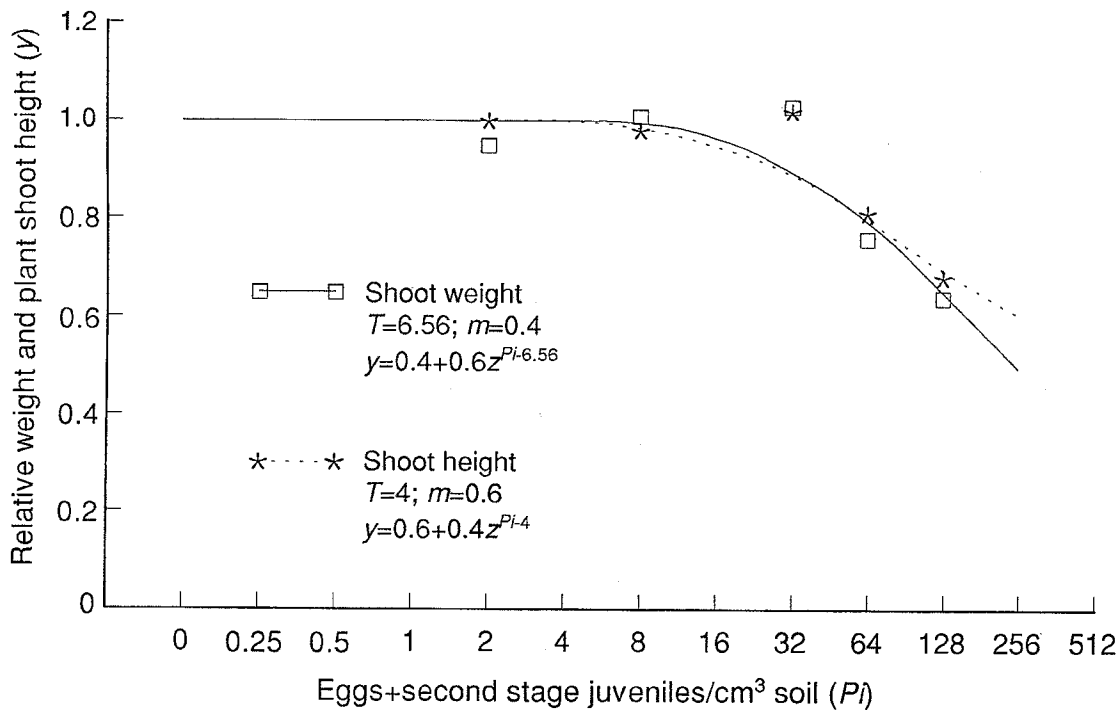


Fig. 5 - Curves according to Seinhorst (1965, 1986b) relating population densities at sowing (Pi) of a population of *M. incognita* from Barinas and relative height and fresh weight of plants of rice cv. Llano 5, in pots.

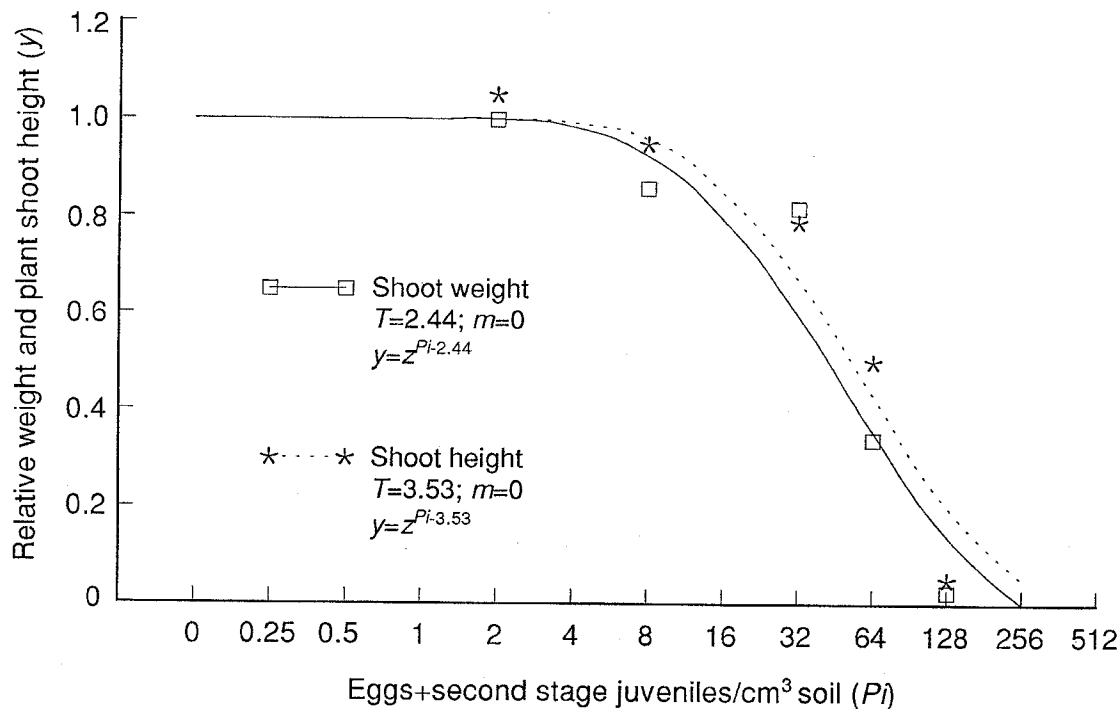


Fig. 6 - Curves according to Seinhorst (1965, 1986b) relating population densities at sowing (Pi) of a population of *M. incognita* from Lara and relative height and fresh weight of plants of rice cv. Llano 5, in pots.

nematode at the time of sowing rice, Pf is the nematode population density determined at harvest, a is the maximum reproduction rate of the nematode, y is the proportion of food supply available to the nematode at a given Pi (generally equal to y of the previous equation), x is the proportion of the nematode population that could potentially infest the roots (for this nematode juveniles+hatchable eggs, $\max=1$), s is the rate of the nematode egg population that is not stimulated to hatch in the absence of the host plant. In this experiment it was assumed that all eggs hatched even in soil with poor or no roots. Moreover, the nematode population observed at harvest, in the plots infested with 128 nematodes/cm³ soil and left fallow was negligible ($\max 0.6$ eggs/cm³ soil). Then in this experiment x was =1 and s was =0 from which it came that the second and third addenda of the equation, which represent the proportion of Pf derived from Pi that could not reproduce, was also =0. Therefore, the population of the nematode observed at harvest (Pf) derived only from new generations of the nematode, whose magnitude depended on the reproduction rates of the nematode and was proportional to the amount of food supply (y). According to curves in Fig. 7, the maximum reproduction rates of *M. incognita* were 50-fold for the population from Zulia, 19.5 for the population from Barinas, and 57-fold for the population from Lara State. The equilibrium densities of the nematodes were 119, 256, and 62 eggs and juveniles/cm³ soil, respectively. Moreover, the maximum nematode densities that rice could have supported, assuming that no damage had occurred, were 423, 393 and 300 eggs and juveniles/cm³ soil for the Barinas, Zulia and Lara populations respectively.

Discussion and conclusion

Usually under field or microplot conditions tolerance limits of crop plants to *Meloidogyne* spp. are rather low: about one egg or much less

per cm³ soil (Sasanelli, 1994). In our experiments the least tolerance limit of rice to *M. incognita* was 2.4 eggs and second stage juveniles/cm³ soil for the Lara population but as high as 8 eggs and juveniles/cm³ soil for the Zulia population. Moreover, the minimum yield was estimated =0 for the Lara and Zulia populations, but it occurred at 156-226 eggs and juveniles/cm³ soil for the former population and 420-512 eggs and juveniles/cm³ soil for the latter. With the population from Barinas the tolerance limit was intermediate (4-6.6 eggs and juveniles/cm³ soil), while the minimum yield was estimated to be not less than 0.4-0.6 even at 256 eggs and juveniles/cm³ soil. Thus it is clear that populations of different geographical origin of this species of root-knot nematode may greatly differ in their virulence to rice. Whether this is the result of differences in races is not known since only the race of the Zulia population has been determined (Crozzoli and Casassa, 1998).

Di Vito *et al.* (1996) reported a tolerance limit of Asian rice to *M. javanica* of 0.26-1 egg and juvenile/cm³ soil and minimum yields of 0 at 16-64 eggs and juveniles/cm³ soil. In this experiment the tolerance limit was not less than 2.4 eggs/cm³ soil and a minimum of 0 occurred at a Pi of not less than 156 eggs and juveniles/cm³ soil. This indicates that *M. incognita* is less pathogenic than *M. javanica* to rice. However, in Nigeria Babatola (1984) observed a varying degree of susceptibility to *M. incognita* among rice cultivars; this author also observed a grain yield decrease of over 60% in soil infested with eight eggs and juveniles/cm³ soil, a level that in this experiment caused a yield loss of no more than 8% with the Lara population, which was the most aggressive one.

The results of our experiment indicate that where Venezuelan populations of *M. incognita* infest rice they are not likely to cause severe damage to upland rice under field conditions as large population densities are uncommon. Among the other *Meloidogyne* spp, reported to

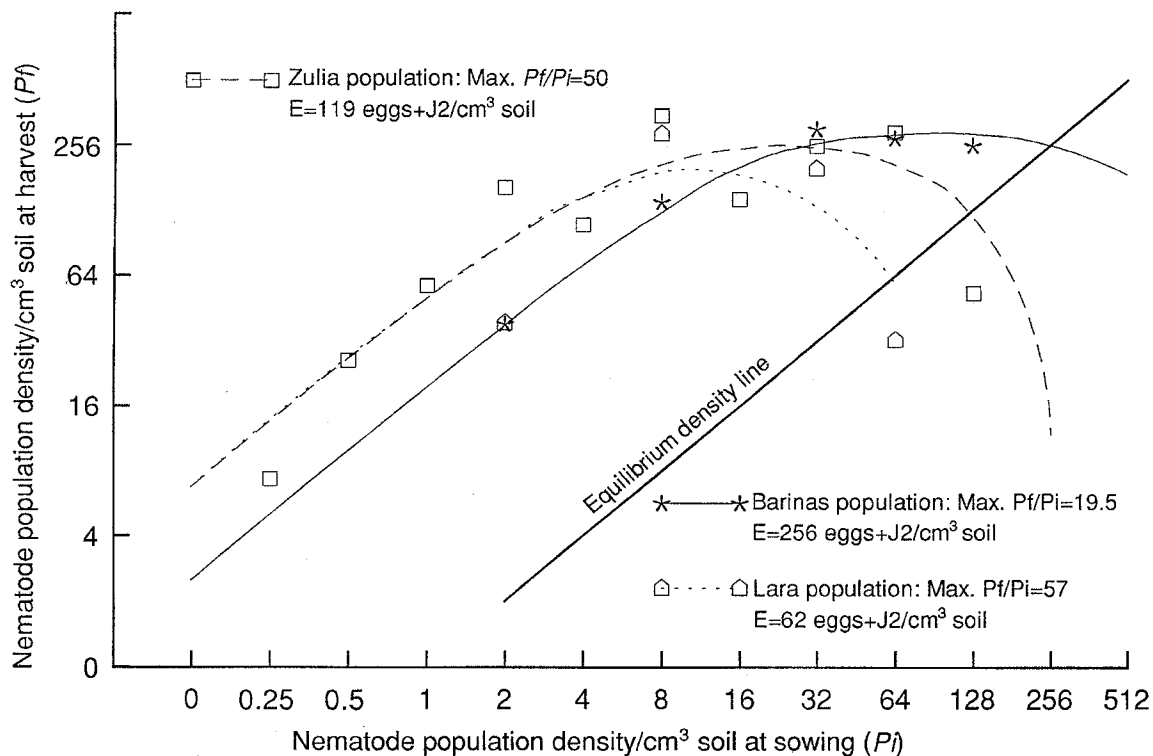


Fig. 7 - Relationship between population densities of three Venezuelan populations of *M. incognita* inoculated at sowing (P_i) and those observed at harvest (P_f), on rice cv. Llano 5 grown in pots in a glasshouse.

be severe pests of rice (Bridge *et al.*, 1990), only *M. arenaria* (Loof, 1964) and *M. javanica* (Yépez and Meredith, 1970) are known to occur in Venezuela, but they are not widespread.

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Literature cited

ANONYMOUS, 1997. Anuario Estadístico Agropecuario 1997. República de Venezuela, Ministerio de Agricultura y Cría, Dirección General Sectorial de Planificación y Políticas. Dirección de Estadística, Caracas, 319 pp.

BABATOLA J. O., 1984. Rice nematode problems in Nigeria: their occurrence, distribution and pathogenesis. *Tropical Pest Management*, 30: 256-265.

BRIDGE J., LUC M. and PLOWRIGHT R. A., 1990. Nematode parasites of rice, pp. 69-108. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (Luc M., Sikora R. A. and Bridge J. eds). CAB International, Wallingford, UK.

COOLEN W. A., 1979. Methods for the extraction of *Meloidogyne* spp. and other nematodes from roots and soil, pp. 317-329. In: *Root-knot Nematodes* (*Meloidogyne* spp.) *Systematics, Biology and Control* (Lamberti F. and Taylor C.E. eds). Academic Press, London. U.K.

CROZZOLI R. and CASASSA A. M., 1998. Especies y razas de *Meloidogyne* en cultivo del guayabo en Venezuela. *Revista de la Facultad de Agronomía*, 15: 107-108.

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

DI VITO M., VOVLAS N., LAMBERTI F., ZACCHEO G. and CATALANO F., 1996. Pathogenicity of *Meloidogyne javanica* on Asian and African rice. *Nematol. mediterr.*, 24: 95-99.

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

LOOF P. A. A., 1964. Free-living and plant-parasitic nematodes from Venezuela. *Nematologica*, 10: 201-300.

SASANELLI N., 1994. Tables of nemato-pathogenicity. *Nematol. mediterr.*, 22: 153-157.

- 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: Cyst Nematodes* (Lamberti F. and Taylor C.E. eds). 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: Cyst Nematodes* (Lamberti F. and Taylor C.E. eds). Plenum Press, New York and London.
- TAYLOR A. and SASSER J. N., 1978. *Biology, Identification and Control of Root-Knot Nematodes* (*Meloidogyne* spp.). North Carolina State University Graphics, Raleigh, NC, USA, pp. 111.
- YÉPEZ G. and MEREDITH J., 1970. Nematodos fitoparásitos en cultivos de Venezuela. *Rev. Fac. Agron.*, 5(4): 33-80.