Effect of the root-knot nematode *Meloidogyne incognita* on parsley

Yndira Aguirre*, Renato Crozzoli* and Nicola Greco**

***Universidad Central de Venezuela, Facultad de Agronomía, Instituto de Zoología Agrícola, Laboratorio de Nematología Agrícola, Apdo. 4579, Maracay 2101-A, Venezuela,**
****C.N.R., Istituto per la Protezione delle Piante, Sezione di Bari, 70126, Bari, Italy.**

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Summary. A screen house experiment was conducted in 1000 cm^3 plastic pots to evaluate the effect of increasing population densities of *M. incognita* race 1 on yield of parsley cv. Double Curled. Initial nematode densities were: 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 eggs + second stage juveniles (J2) cm^{-3} soil. Fitting the Seinhorst model, $y = m+(1-m)z^{Pi-T}$, to average plant top and total fresh weight and top dry weight, gave tolerance limits (T) of 0.17, 0.02 and 0.025 eggs + J2 cm^{-3} soil. The minimum relative yields (m) were 0.5 at $Pi \geq 16$ eggs + J2 cm^{-3} soil, 0.55 at $Pi \geq 2$ eggs + J2 cm^{-3} soil and 0.34 at $Pi \geq 4$ eggs + J2 cm^{-3} soil for top fresh weight, total fresh weight and top dry weight. The maximum nematode reproduction rate was 37-fold at $Pi = 0.25$ eggs + J2 cm^{-3} soil and an equilibrium density was estimated at 5 eggs + J2 cm^{-3} soil. Anatomical modifications observed in galled roots were similar to those induced by this root-knot nematode species in other hosts.
Key words: *Meloidogyne incognita*, parsley, pathogenicity, *Petroselinum sativum*, root-knot nematode, Venezuela.

Among nematode pests, several species of root-knot nematodes (*Meloidogyne* spp.) are pathogens to parsley. Of these, *M. incognita* is the most important, although *M. arenaria* and *M. hapla* have been reported to also damage parsley (Netsher & Sikora, 1990). *Meloidogyne incognita* is very common in Venezuela (Greco *et al.*, 2000) and results in a severe loss of parsley yield (Crozzoli, unpublished) although precise information on the pathogenicity of this nematode to parsley is lacking. Therefore, a pot-based study was conducted in a screenhouse to *a*) assess the effect of different population densities of a Venezuelan population of *M. incognita* on the yield of parsley and *b*) observe the dynamics of the nematode populations on parsley. Anatomical alterations induced by *M. incognita* in parsley roots were also investigated.

MATERIALS AND METHODS

Meloidogyne incognita race 1, originally isolated from tomato (*Lycopersicon esculentum* Mill.), was reared on tomato cv. Rutgers in a screen house at 26 °C in Maracay, Venezuela. When large egg masses appeared, tomato roots were gently washed in running water, cut into 0.5 cm long pieces and mixed. To estimate the number of eggs and second-stage juveniles available, five sub-samples, each of 10 g, of infested root samples were processed by the sodium hypochlorite method (Hussey & Barker, 1973). Appropriate amounts of the infested roots were thoroughly mixed with steam-sterilized soil (sand 60.8%, silt 34%, clay 5.2%, organic matter 6.8%, pH 7.2) to provide population densities of 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 eggs + J2 cm^{-3} soil and used to fill six replicate 1000 cm^3 plastic pots. Also, six non-infested pots served as control. One seed of parsley (*Petroselinum sativum* L.) cv. Double Curled was sown in each pot. All pots were arranged on benches 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^{-1}) were applied to pots soon after seed germination and thereafter every 15 days. Pots were irrigated as required.

To derive plant growth curves, the number of leaves per plant was recorded at two-week intervals. At harvest (75 days after sowing), fresh top weight, fresh total weight and dry top weight were measured and average values per plant were calculated.

To determine the increase of nematode populations in each pot, roots were washed free of adhering soil, dried, weighed and cut into approximately 0.5 cm long pieces. Nematodes were extracted by macerating total plant roots in a blender for 120 s and counted. Nematodes in the soil were extracted using an Oostenbrink elutriator (s'Jacob & Van Bezooijen, 1971). The final population density (*Pf*) of each pot was considered as the sum of the nematodes extracted from both roots and soil.

Plant height data and leaf number per plant were used to construct growth curves, and averages of fresh top weight, fresh total weight and dry top weight were fitted to the equation $y = m + (1-m)z^{Pi-T}$, as proposed by Seinhorst (1965; 1986b) by using transparent overlays of standard curves. In this equation, *y* is the relative yield (the rate between the average growth parameter, at *Pi* and that at $Pi \leq T$), *m* the minimum relative yield (the value of *y* at the largest *Pi*), *Pi* the nematode population density at sowing, *T* is the tolerance limit (the magnitude of *Pi* above which yield reduction begins to occur), and *z* a constant < 1 with $z^{-T} = 1.05$.

The population of *M. incognita* determined at harvest was fitted to the model $Pf = axy(1-q^{Pi})(-^{e}log q)^{-1}+(1-x)Pi+sx(1-y)Pi$ as proposed by Seinhorst (1970, 1986a). In this model *Pi* is the soil population density of the nematode at the time of sowing, *Pf* is the nematode population density determined at harvest, *a* is the maximum reproduction rate of the nematode, *y* is the proportion of the 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 *M. incognita* juveniles + hatchable eggs, max = 1), and *s* is the rate of the nematode egg population that is not stimulated to hatch in the absence of the host plant.

In a separate experiment, galled parsley roots were collected from infected plants after 45 days exposure to *M. incognita*, washed free of adhering soil and debris and individual galls selected. Root galls were fixed in chrome-acetic acid and glacial acid solution, dehydrated with a ter-butyl alcohol series (from 50 to 100% concentration) and then embedded in 60 °C (melting point) histoplast. Sections of 10-12 μm were cut and stained with safranin and fast green according to the method of Johansen (1940). Selected sections containing the nematode feeding sites were observed under a compound microscope.

RESULTS AND DISCUSSION

Leaf production was reduced from 15 and 30 days after sowing/inoculation onwards in pots inoculated with 4-256 eggs + J2 cm^{-3} soil and 0.125-2 eggs + J2 cm^{-3}, respectively. At harvest the number of leaves, compared to that of control pots, was 70%, 85% and 93% at *Pi* = 256, 4-128 and 0.125-2 eggs + J2 cm^{-3} soil (Fig. 1).

The tolerance limits (*T*) of parsley fresh total weight, fresh top weight, and dry top weight to *M. incognita* were 0.05, 0.17 and 0.025 eggs + J2 cm^{-3} soil. The minimum relative yields (*m*) were 0.55, 0.5 and 0.34 at $Pi \geq 2$, 16 and 4 eggs + J2 cm^{-3} soil, for fresh total weight, fresh top weight, and dry top weight (Fig. 2).

The experiment confirmed the destructive effect of *M. incognita* to parsley. Tolerance limits of parsley to the nematode were very low and similar to those of other crop plants obtained in Venezuela and elsewhere. Crozzoli *et al.* (1997) reported a tolerance limit in different cultivars of *Phaseolus vulgaris* L. to *M. incognita* of 0.02-0.03 eggs + J2 cm^{-3} soil and minimum yield (*m*) of 0.37-0.53 at $Pi \geq 2$ eggs + J2 cm^{-3} soil and of *Vigna unguiculata* (L.) Walp. to the same nematode of 0.03 eggs + J2 cm^{-3} soil and minimum yield of 0.28 at $Pi \geq 2$ eggs + J2 cm^{-3} soil. Di Vito *et al.* (1986) reported a tolerance limit in *Solanum melongena* L. to *M. incognita* of 0.054 eggs + J2 cm^{-3} soil but a lower minimum yield (*m* = 0.05).

The population of *M. incognita* determined at harvest also fitted the model $Pf = axy(1-q^{Pi})(-^{e}log q)^{-1}+(1-x)Pi+sx(1-y)Pi$. The maximum reproduction rate of the nematode was 37-fold at *Pi* = 0.25 eggs + J2 cm^{-3} soil, the equilibrium density was 5 eggs + J2 cm^{-3} soil and the maximum nematode density that parsley could have supported, assuming that no damage had occurred, was 19 eggs + J2 cm^{-3}.

Galls contained more than one female nematode and egg masses were located outside galls (Fig. 4A). Root sections stained with safranin and fast green revealed both hypertrophy and hyperplasia, disorganized and disrupted xylem elements and vascular tissues. Nematode feeding sites comprised 5-8 giant cells per specimen, surrounding the heads of females. Active multinucleated giant cells contained granular cytoplasm and numerous hypertrophied nuclei and nucleoli (Fig. 4 B & C). Dense giant cell cytoplasm lined the highly stained thick cell walls. The anatomical changes induced

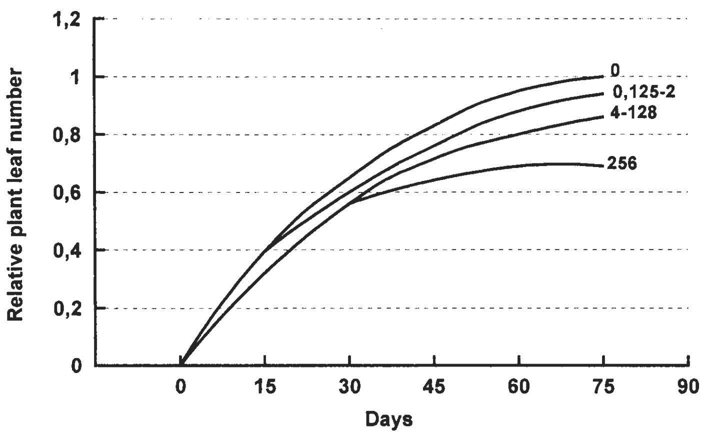

Fig. 1. Growth curves of parsley cv. Double Curled in soil infested with increasing population densities of *Meloidogyne incognita.* Relative plant leaf number is the ratio between average plant leaf number at harvest of plants in pots not affected by the nematode and averages observed at different days observations in pots infested with increasing population densities of the nematode. Figures next to curves are population densities cm^{-3} soil at sowing (*Pi*).

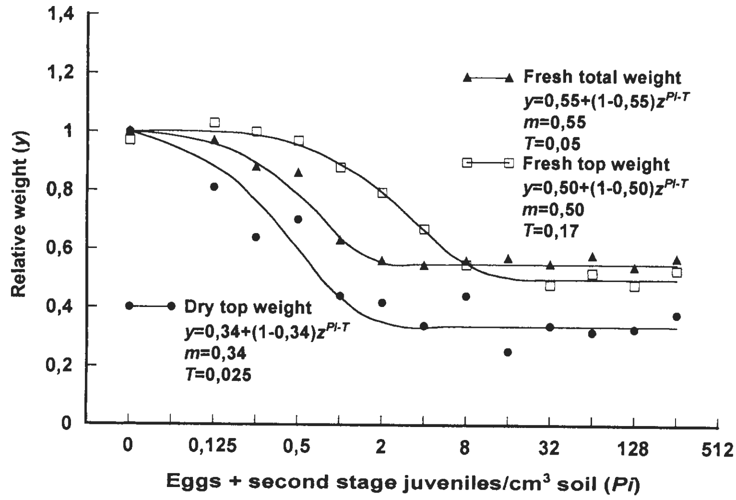

Fig. 2. Curves relating initial population densities of *Meloidogyne incognita* (*Pi*) and relative total fresh weight, fresh top weight and dry top weight of parsley cv. Double Curled, 75 days after sowing.

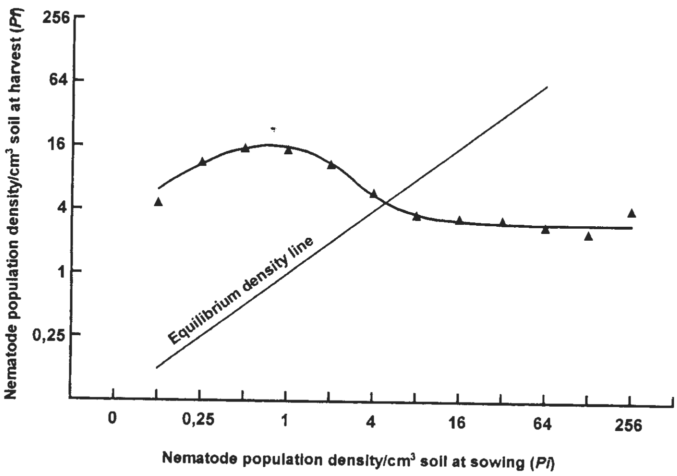

Fig. 3. Relation between population densities of *Meloidogyne incognita* at sowing (*Pi*) and those observed at harvest (*Pf*), on parsley cv. Double Curled, in pots in screenhouse.

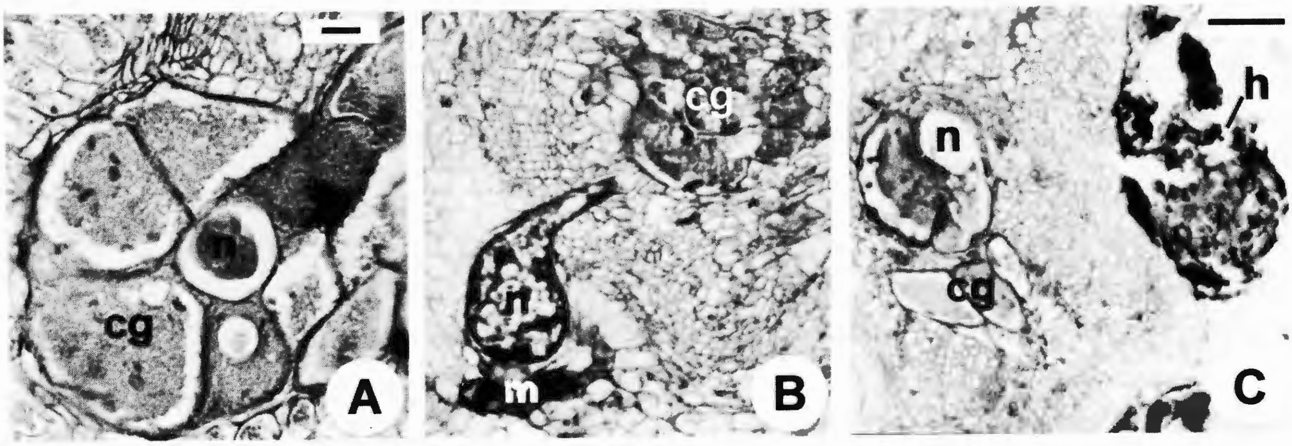

Fig. 4 A, B, C. Cross sections of infected parsley roots showing nematode female (n) and well developed large multinucleated giant cells (cg) and incipient gelatinous mass (m) and egg mass outside the root (h). Bar in A = 100 µm; Bar in C = 100 µm; Bar in B the same of C.

by *M. incognita* in parsley roots in our experiment were similar to those previously reported for hosts of many root-knot nematode species (Crozzoli *et al.*, 1994; Bustillo *et al.*, 2000).

The results of our experiment indicate that where *M. incognita* infests parsley, it may cause severe damage to the crop even at relatively low *Pi*. Therefore, potential sites for establishment of this crop should be checked for the presence of this nematode.

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Aguirre Y., Crozzoli R., Greco N. Воздействие *Meloidogyne incognita* на петрушку.
Резюме. Для выяснения влияния численности популяции *M. incognita* на урожай петрушки были проведены эксперименты в пластиковых контейнерах в защищенном грунте. Первоначальные уровни заражения составляли 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 и 256 яиц и личинок на см^3 почвы. Оценки общего и сухого веса надземной части растения в соответствии с моделью Сейнхорста $y=m+(1-m)z^{Pi-T}$ давали значения порога толерантности (T) 0.17, 0.02 и 0.025 яиц и личинок на см^3 почвы. Минимальный относительный выход урожая (m) составил 0.5 при $Pi \geq 16$ яиц и личинок на cm^3 почвы, 0.55 при $Pi \geq 2$ яиц и личинок на cm^3 почвы и 0.34 при $Pi \geq 4$ яиц и личинок на cm^3 почвы по весу свежесрезанных надземных частей растений, общему свежесрезанному весу и сухому весу надземных частей растений. Максимальная степень размножения нематод была 37-кратной при $Pi = 0.25$ яиц и личинок на cm^3 почвы, а равновесная плотность была оценена на уровне 5 яиц и личинок на cm^3 почвы. Анатомические особенности образующихся галлов были схожи с таковыми на других растениях-хозяевах.