Host–parasite relationships of *Meloidogyne incognita* on spinach

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Host–parasite relationships in root-knot disease of spinach caused by *Meloidogyne incognita* race 1 were studied under glasshouse conditions. Nematode-induced mature galls were large and usually contained one or more females and egg masses with eggs. Feeding sites were characterized by the development of giant cells containing granular cytoplasm and many hypertrophied nuclei. The cytoplasm in these giant cells was aggregated alongside the thickened cell walls. Stelar tissues within galls appeared disorganized. The relationship between initial nematode population density ($P_i$) in a series from 0–128 eggs and second-stage juveniles per cm$^3$ soil and growth of spinach cv. Symphony F$_1$ seedlings was tested under glasshouse conditions. A Seinhorst model $\left[y = m + \left(1 - m\right)zP - T\right]$ was fitted to fresh top- and total plant-weight data for inoculated and control plants. Tolerance limits ($T$) of spinach cv. Symphony F$_1$ to *M. incognita* race 1 for fresh top and total plant weights were 0·25 and 0·5 eggs and second-stage juveniles per cm$^3$ soil, respectively. The minimum relative values for fresh top and total plant weights were zero in both cases at $P_i \geq 32$ eggs and second-stage juveniles per cm$^3$ soil. Root galling was least at low initial population densities and greatest at 16 eggs and second-stage juveniles per cm$^3$ soil. Maximum nematode reproduction rate was 33·1-fold at the lowest $P_i$.

**Keywords:** histopathology, nematode reproduction, pathogenicity, root-knot nematode, *Spinacia oleracea*, threshold level

**Introduction**

Spinach (*Spinacia oleracea*) is an economically important leafy vegetable crop in many countries. Of 32 000 ha of spinach grown annually throughout the European Union, 70% are in Mediterranean countries (FAO, 2002). Fungal and viral diseases are generally recognized as the most economically important constraints in spinach production (Correll et al., 1994). However, several plant-parasitic nematodes have been reported to damage the crop. Nematode species associated with spinach include cyst (*Heterodera schachtii*), root-lesion (*Pratylenchus penetrans*) and root-knot nematodes (*Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*) (Olthof & Potter, 1973; Olthof et al., 1974; Potter & Olthof, 1974; Castillo & Jiménez-Díaz, 2003; Manchini et al., 2003). Among these, *Meloidogyne* spp. are the most common and damaging nematodes of spinach in several countries (Potter & Olthof, 1993).

Although attacks by *Meloidogyne* spp. are probably an important constraint for spinach cultivation, little information exists regarding the host–parasite relationships between these nematodes and spinach. Plant growth impairment caused by *Meloidogyne* spp. to vegetable crops is influenced by nematode species and/or physiological race, as well as the initial nematode population density in soil at sowing (Sasanelli, 1994). Thus identification of *Meloidogyne* populations, their pathogenic race characterization, and the evaluation of population densities infesting soil are essential for designing effective control measures in the context of sustainability and integrated pest management. This is especially important with root-knot nematodes as host-plant resistance, which could be used to reduce the initial nematode population density at threshold level, is scarce among vegetable crops (Sasser & Carter, 1985). It is well established that the extent of crop growth impairment by the nematode is influenced by nematode population density at planting, and that a minimum population density is required before measurable yield loss occurs (the tolerance limit) (Seinhorst, 1965, 1979).

Reproductive fitness and virulence are major components of pathogenicity (Shaner et al., 1992) and thus important for the assessment and understanding of disease reactions of plants to pathogens. When a compatible host–parasite interaction has been established by a nematode, infection
of the plant can be followed by a sequence of disruptions in physiological processes that leads to pathogenesis (Melakeberhan & Webster, 1993). Pathogenesis studies demonstrated that threshold densities of *M. hapla* for growth of spinach were 6–18 eggs and second-stage juveniles per cm³ soil in microplot experiments (Potter & Olthof, 1974). Recently, severe feeder-root infections of spinach and heavy soil infestations (44–378 eggs and second-stage juveniles per cm³ soil) by *M. incognita* were found in commercial fields of spinach in Andalusia, southern Spain (Castillo & Jiménez-Díaz, 2003). Infected plants showed yellowing and decline, with heavily damaged root systems and an abundance of galls that suggested a highly specialized nematode–plant interaction. The objectives of this study were to determine: (i) the histopathology of nematode-feeding sites in spinach roots infected by *M. incognita*; and (ii) the relationship between the initial population density of the nematode and growth of spinach seedlings under glasshouse conditions.

**Materials and methods**

The stock nematode culture used in the experiments was derived from a single egg mass of *M. incognita* that originated from infected feeder roots of spinach cv. Polka from Encinarejo (Córdoba) in southern Spain (Castillo & Jiménez-Díaz, 2003). The stock culture was established by placing an egg mass of the nematode beneath the root system of a cv. Rutgers tomato (*Lycopersicon esculentum*) seedling. Inoculum for experiments was obtained by extracting eggs and second-stage juveniles (J2) from 2-month-old tomato plant cultures using 1% sodium hypochlorite solution (Hussey & Barker, 1973).

**Differential hosts test**

The response of the *M. incognita* population to differential hosts was evaluated in a glasshouse at 25 ± 3°C (Taylor & Sasser, 1978). The differential host set included cotton (*Gossypium hirsutum* cv. Delta Pine 16), peanut (*Arachis hypogaea* cv. Florunner), pepper (*Capsicum annuum* cv. Early California Wonder), tobacco (*Nicotiana tabacum* cv. NC 95), watermelon (*Citrullus vulgaris* cv. Charleston Grey) and tomato cv. Rutgers. Twenty-day-old seedlings of the differential host plants were transplanted (one per pot) into 700 mL clay pots filled with autoclaved sandy soil. Two days later, individual seedlings were inoculated by adding 10 mL of a suspension containing 10,000 eggs and J2s to the population of *M. incognita*. Control plants received the same amount of water. Plants were maintained in a glasshouse adjusted to 25 ± 3°C. There were four plants for each host. Fifty days after inoculation, plants were uprooted and their roots gently washed free of adhering soil and stained with safranin and fast-green, mounted permanently in 40% xylene solution of a polymethacrylic ester (Synocril 9122X, Gray Valley Products, NJ, USA), examined microscopically and photographed (Johansen, 1940).

**Histopathology**

Galled roots from naturally *M. incognita*-infected spinach plants sampled at Encinarejo (Córdoba) and from plants artificially infected in the inoculum density-plant growth experiment (see below) were selected for histopathological studies. Roots were gently washed free of adhering soil and debris, and individual galls were selected together with healthy roots. These root tissues were fixed in formaldehyde chromoacetic solution for 48 h, dehydrated in a tertiary butyl alcohol series (40–70–85–90–100%) and embedded in 58°C-melting-point paraffin. Embedded tissues were sectioned with a rotary microtome. Sections 10–12 μm thick were stained with Phloxine B (Gurr, Sigma-Aldrich, Poole, UK), examined microscopically, and photographed (Johansen, 1940).

**Inoculum density–plant growth impairment relationship**

Inoculum of *M. incognita* was produced on tomato cv. Rutgers plants inoculated as described above and maintained in a glasshouse adjusted to 25 ± 3°C. Two months after inoculation, at the time that egg masses were well formed in tomato roots, the inoculated plants were uprooted and their roots gently washed free of adhering soil and finely chopped. To estimate the numbers of eggs and J2s in the chopped tissue, six 5 g aliquots of infected chopped roots were suspended in 1% aqueous solution of sodium hypochlorite in 100 mL jars for 4 min (Hussey & Barker, 1973). For inoculation, chopped infected roots were thoroughly mixed with 2 kg steam-sterilized sandy soil and the mixture was used as inoculum to provide a range of inoculum densities (Di Vito et al., 1986). Appropriate amounts of this inoculum were mixed with a potting mixture of steam-sterilized sandy soil (87.5% sand, 5% silt, 5% clay, 2.5% organic matter) to give a range of increasing population densities of 0, 0-0625, 0-125, 0-25, 0-5, 1, 2, 4, 8, 16, 32, 64 and 128 eggs and J2s per cm³ soil, and 700 mL clay pots were filled with the infested soil mixtures. A single pregerminated seed of spinach cv. Symphony F₁ (widely used in the Mediterranean region) was sown in each pot. There were six pots for each inoculum level, arranged in a randomized complete block design in a glasshouse at 25 ± 3°C. Plants in pots were watered as needed and fertilized with 100 mL of a 0-1%, 20-5-32 + micronutrients hydrosol fertilizer solution (Haifa Chemicals Ltd, Haifa, Israel) every week. Data on the appearance of symptoms of nematode attack (stunting and yellowing) were recorded during the experiment. Fifty days after sowing, fresh top and total plant weights were measured. Plants were uprooted, and the roots washed free of adhering soil and weighed. Root infection by the nematode was assessed by estimating RGS on a 0–5 scale: 0 = no galls; 1 = 1–5 galls; 2 = 6–20 small galls; 3 = >20 galls homogeneously distributed in the root system; 4 = reduced and deformed root system with some

large galls; $5 = \text{completely deformed root system with few but large galls}$ (Di Vito et al., 1979). Eggs and J2s in the egg masses in roots were extracted by the sodium hypochlorite method (Hussey & Barker, 1973) and counted. Nematodes in soil were extracted by a modification of Coolen’s method (Coolen, 1979; Di Vito et al., 1985). The final nematode population density ($P_f$) was calculated as the total of that from roots and soil.

Statistical analysis

The relationship between the initial nematode population density ($P_i$) and plant growth (indicated by the fresh top and total plant weights) was determined by fitting the data to the Seinhorst model: $y = m + (1 - m)z^{-y/T}$ when $P \geq T$, and $y = 1$ when $P < T$ (Seinhorst, 1965, 1979). In this model $y$ is the relative value of the plant growth parameter; $m$ is the minimum value of $y$ (at a very large initial nematode population density); $P_i = \text{initial nematode population density}$; $T = \text{tolerance limit (initial population at which plant growth is not impaired)}$; and $z$ is a constant $<1$ reflecting nematode damage, with $z^{-y} = 1 - 0.05$ (Seinhorst, 1965, 1979). The Seinhorst equation was fitted using the SEINFIT program (Viane et al., 1997). The coefficient of determination ($R^2$) and the residual sum of squares were used to indicate the goodness-of-fit of data to the model. The experiment was performed twice. Similarity among experiments was tested by preliminary ANOVAs using experimental runs as factors, so that experiment $\times$ treatment interaction could be determined. This interaction was not significant and allowed data to be combined for ANOVA and fitting to the Seinhorst model.

Data on RGS, final nematode population ($P_f$) and reproduction rate ($R_f$ = final population/initial population) in the inoculum density–plant growth impairment experiment were normalized before analysis by transforming them into $\log_{10}(X + 1)$ (Gomez & Gomez, 1984). ANOVAs were carried out using STATISTIX 8-0 (NH Analytical Software, Roseville, MN, USA). Means of RGS, $P_i$ and $R_i$ values were compared using Fisher’s protected least significant difference test (LSD) at $P = 0.05$.

Results

Differential hosts test

Inoculation of the M. incognita population onto differential hosts indicated that it was unable to parasitize cotton, peanut and tobacco, but was able to reproduce on tomato, watermelon and pepper. Therefore this population was identified as race 1 of M. incognita.

Histopathology

Root galls induced by M. incognita race 1 on spinach varied in size and location (Fig. 1a). Generally, large, spherical, regular galls were present on root tips, and these were also present along root axes (Fig. 1a). Galls occurred either singly or in clusters which could encircle the entire root. In the latter case the root diameter was two to six times greater than that of uninfected roots (Fig. 1a). The majority of individual galls selected at random for inspection contained an egg mass and, in many cases, up to six mature globose females were found associated with the largest galls. Occasionally an egg mass was found inside the cortical root tissues, but the majority of egg masses were observed at the root surface irrespective of the age of the plant root system.

Comparative histological observation of healthy (Fig. 2a) and M. incognita-infected spinach roots (Fig. 2b–c) showed cellular alterations in tissues of the cortex, endodermis, pericycle and stele induced by the nematode. In the permanent feeding sites, nematode-induced formation of large multinucleate giant cells adjacent to the stele tissues was observed in all infected spinach roots. This formation led to distortion and crushing of tissues (Fig. 2b–c). Giant cells showed dense cytoplasm and variable numbers of hypertrophied nuclei and nucleoli (Fig. 2b–c). Hyperplasia of tissues adjacent to giant cells contributed to the formation of root galls (Fig. 1a).

Inoculum density–plant growth impairment relationship

The initial population densities of the nematode included in the study negatively affected the growth of spinach plants (Fig. 1b). The relationships between initial nematode population density and fresh top and total plant weights were appropriately described by the Seinhorst equation (Fig. 3a,b). Symptoms of attack (stunting and yellowing) by M. incognita race 1 and reduction of plant top growth were evident 15 days after inoculation with an initial population density of only 16 eggs and J2s per cm$^3$ soil. Twenty-five days after inoculation, all plants inoculated with more than 32 eggs and J2s per cm$^3$ soil were dead. The spinach tolerance limits ($T$) to M. incognita race 1 were 0.25 and 0.5 egg and J2s per cm$^3$ soil for fresh top and total plant weights, respectively (Fig. 3a,b). The minimum relative value ($m$) for fresh top and total plant weights was 0 at $P_i = 32$ eggs and J2s per cm$^3$ soil in both cases (Fig. 3a,b). The maximum nematode reproduction rate ($R_f$) was 33.1 at the lowest initial population density ($P_f = 0.0625$ eggs and J2s per cm$^2$ soil). In general, reproduction rate decreased as the initial nematode population increased (Table 1) and the highest final population density ($P_f$) was found in plants inoculated with an initial population density of eight eggs and J2s per cm$^3$ soil (Table 1). Similarly, the severity of root galling of spinach was lowest at low initial population densities, and highest at 16 eggs and J2s per cm$^2$ soil (Table 1), with all plants that received higher numbers dying.

Discussion

Knowledge of the relationship between initial nematode population densities in soil and plant growth is essential for prediction of crop losses caused by plant-parasitic nematodes and for the choice of possible management strategies. With the reduced availability of nematicides
and consequent higher costs, growers will probably increase their reliance on soil sampling to assess the risk of nematode damage and to decide on cropping sequences and possible control tactics.

The histological studies revealed a typical susceptible reaction of spinach cv. Symphony F1 to infection by *Meloidogyne incognita* race 1. The extensive giant cells and modifications of tissues induced by nematodes sequester nutrients from the host plant and limit water and nutrient translocation from infected roots to above-ground plant tissues (Hussey & Williamson, 1997). The development and parasitic habit of *M. incognita* race 1 observed in spinach in the present study were similar to those found by other researchers (Potter & Olthof, 1974; Manachini *et al.*, 2003), so there are many reasons to believe that *M. incognita* has the potential to damage spinach severely.

The severity of root galling of spinach roots and the severe impairment of plant growth with increase in initial inoculum densities in soil confirms the great damage potential and reproductive capacity of *M. incognita* race 1 on spinach. The tolerance limit of this plant to the nematode was as low as 0·25 and 0·5 eggs and J2s per cm$^3$ soil for fresh top and total plant weights, respectively. An initial population density of this parasite exceeding 32 eggs and J2s per cm$^3$ soil was lethal for spinach plants. The Seinhorst damage function adequately described the pathogenic relationship of *M. incognita* race 1 on spinach (Viane *et al.*, 1997). The findings of the present study on the pathogenicity of this nematode on spinach agree with those of other researchers in India (Pankaj *et al.*, 2001) and Canada (Potter & Olthof, 1974), who found that *M. incognita* and *M. hapla* significantly reduced the growth of this crop. However, the present results on threshold levels are not in agreement with the results of Potter & Olthof (1974), who found that in microplots spinach infected with *M. hapla* at initial population densities of 18

![Figure 1](a) Galled roots of spinach artificially infected with *Meloidogyne incognita* race 1. (b) Effect of increasing densities (from zero on left to 32 eggs and J2s per cm$^3$ soil on the right) of *M. incognita* race 1 on the growth of spinach (cv. Symphony F1), showing marked reduction in top growth.
eggs and J2s g\(^{-1}\) soil were not seriously reduced in growth (maximum reduction 13%). These authors established that, with this initial inoculum density of the nematode in soil, plants might survive and produce a saleable crop. However, the results of the present study indicate that a similar nematode inoculum density of *M. incognita* race 1 would have an almost lethal effect on plant growth. Differences in the susceptibility of spinach cultivars, the virulence of nematode species, and soil type and climatic conditions may be responsible for this difference.

Figure 2  (a) Transverse section of a healthy spinach root (cv. Symphony F1). (b,c) Transverse sections of naturally (b) and artificially (c) infected roots showing giant cells induced by *Meloidogyne incognita* race 1. Abbreviations: gc = giant cells; hn = hypertrophied nuclei; n = nematode female; st = stylet. Scale bars = 50 µm.
A reduction in nematode reproduction rate with increasing initial nematode inoculum density has been reported for infections of several crops by *M. incognita* (Di Vito et al., 1985, 1986). However, reproduction rates of *M. incognita* race 1 on spinach were much lower than those reported for tomato cv. Roma (472.8-fold $P_i$) infected by a population of *M. incognita* race 1 from Italy (Di Vito & Ekanayake, 1983), or those reported for a Spanish population of *M. arenaria* race 2 (434.8-fold $P_i$) infecting white mulberry (Castillo et al., 2001). Thus the present data indicate that this population of *M. incognita* race 1 is highly pathogenic to spinach. The findings could be a consequence of severe damage to root tissues of spinach, as well as nematode competition for nutrients and/or root tissue availability (feeding sites), as a result of which a smaller proportion of the inoculum would develop successfully.

Management procedures need to be implemented in order to guarantee production of spinach, because plant growth reduction can start at soil population densities as low as 0.25 eggs and J2s per cm$^3$ soil and can reach 50% in soil infested with four eggs and J2s per cm$^3$ soil, while plant growth is negligible at $P_i = 32$ eggs and J2s per cm$^3$ soil. Strategies that aim to prevent these nematodes from infesting spinach-growing fields should be used, along with measures that aim to reduce nematode damage (e.g. use of nematicides, soil solarization and organic amendments).

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References


