NOTAS DE INVESTIGACION—RESEARCH NOTES

# GROWTH RESPONSE OF *IN VITRO* PRODUCED BANANA PLANTLETS TO *MELOIDOGYNE JAVANICA* IN POTS

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RESUMEN

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La relación entre densidades iniciales de inóculo (Pi) de *Meloidogyne javanica* en el crecimiento de plántulas de banana producidas *in vitro* se estudió en maceteros bajo condiciones de invernadero (25 ± 3 °C). Las plántulas de banana (Musa AAA Cavendish subgrupo: Dwarf Cavendish) fueron expuestas por 60 días a densidades de nematodos en progresión geométrica (de 0.062 a 512 huevos y J2/cm$^2$ de suelo). La respuesta en el crecimiento de las plantas de banana inoculadas con *M. javanica* Pi fue ajustada al modelo de daño de Seinhorst, la cual mostró límites de tolerancia de las plantas a los nematodos de 0.146 y 0.32 huevos y J2/cm$^2$ de suelo por el peso fresco y la altura, respectivamente. La máxima reproducción de los nematodos se estimó en 19 veces el Pi y ocurrió con el nivel poblacional inicial más bajo. Cortes transversales y longitudinales de raíces infectadas de *M. javanica* mostraron de 3–5 células gigantes multinucleadas acomodadas alrededor de la parte anterior del nematodo y adyacentes al xilema. Interrupciones de elementos del xilema por las células gigantes y reacciones hipertróficas e hiperplásticas del parénquima vascular y tejidos corticales fueron observadas conformemente en los cortes de raíces agalladas.

*Palabras clave:* banana, daño de nematodos, histopatología, *Meloidogyne javanica, Musa* spp., nematodo agallador, relación hospedante-parásito.

Of the various plant-parasitic nematode species found in association with stunted bananas (*Musa* spp.), the most widespread and detrimental are *Radopholus similis* (Cobb) Thorne, certain *Pratylenchus* spp., and *Helicotylenchus multicinctus* (Cobb) Golden (7,10,11,19). It also is common to find *Meloidogyne* spp. in the tropics; however, these nematodes are considered to be minor pests of full-grown bananas (1,7,19). In subtropical areas and in plastic greenhouses such as in Lebanon and Greece, where bananas are grown at suboptimal temperature, the presence of root-knot nematodes may be important and damage of these pests to banana plantlets can be serious (15,16,20). To obtain a better understanding of the impact of root-knot nematodes under such conditions, we examined the effects of *Meloidogyne javanica* (Treub) Chitwood on young micropropagated banana plantlets in a glasshouse in southern Italy.

A population of *M. javanica* host race 1 (3,17) from southern Italy was increased on tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers. After determining the numbers of eggs and J2 in random samples of chopped roots following NaOCl extraction (8), root pieces (0.5 to 1.0 cm long) were thoroughly mixed with 3 L of steam-sterilized sand to serve as inoculum. Root fragments infected with nematodes only were used to minimize nematode mortality during the inoculation procedures (5). Appropriate amounts of inoculum were mixed with 1.9 L of a steam-sterilized sandy soil (7% clay, 3.9% silt, 89.1% sand, and 2.3% organic matter; pH 7.5) per pot to achieve a geometric series of 14 population densities (Pi) from 0.062 to 512 eggs and J2/cm³ soil.

Plantlets of dessert banana (*Musa* AAA Cavendish subgroup: Dwarf Cavendish), derived from parent plants from Crete were propagated *in vitro* using meristematic domes cultured in basic growth media (18). After being rooted and acclimated for 4–5 weeks in a growth chamber (25 °C, 16-hr photoperiod), plantlets were individually transplanted into sterile soil in greenhouse pots. Ten days afterward, they were transplanted again to the freshly inoculated pots. Six replicated pots were included for each inoculum level and arranged randomly on a glasshouse bench. Control pots without nematodes were also added to give 15 treatments. Ambient temperature was controlled at 25 ± 3 °C.

After 60 days, plant heights and top and root weights were recorded. Eggs

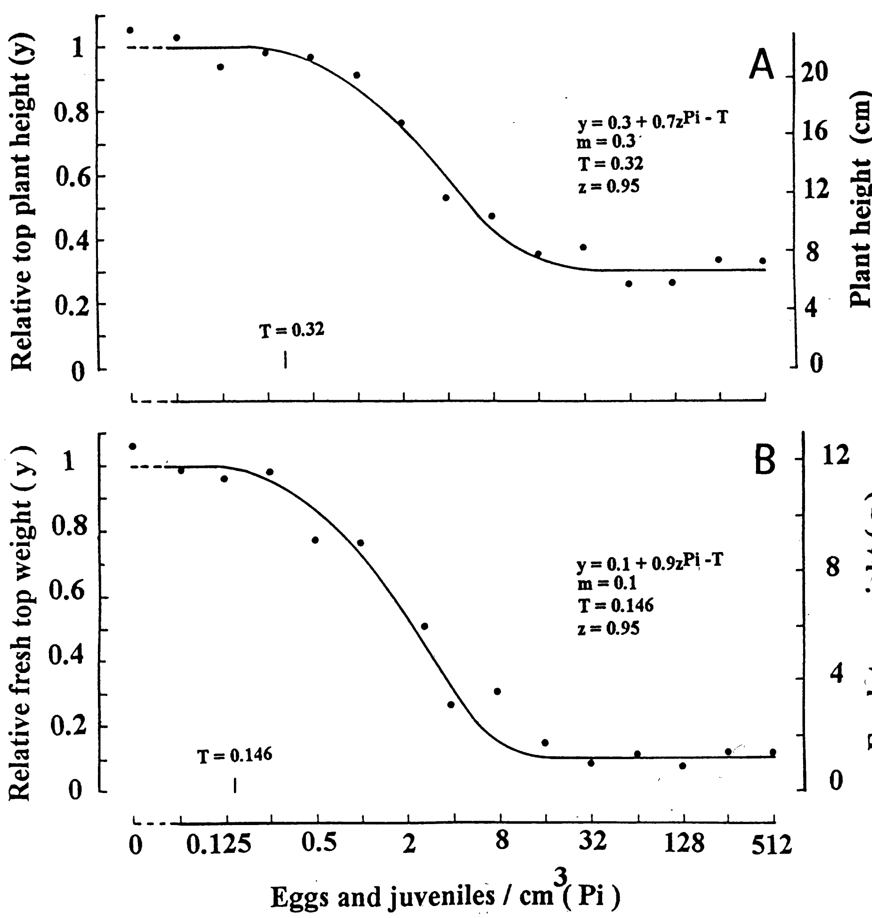



Fig. 1. Growth response of banana (Cavendish Dwarf) plantlets from tissue culture to *Meloidogyne javanica* 60 days after inoculation with a geometric series of increasing nematode densities. A) Relationship between initial nematode density (Pi) and relative height (y) of plantlets. B) Relationship between initial nematode density (Pi) and relative weight (y) of plantlet tops. Both relationships are expressed according to the Seinhorst's plant damage model.

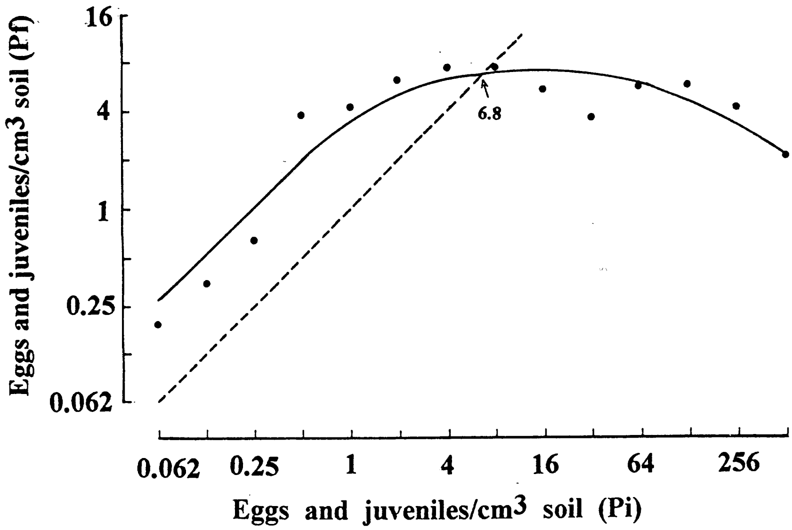



Fig. 2. Relationship between initial (Pi) and final (Pf) population densities of *Meloidogyne javanica* on Cavendish Dwarf bananas grown in pots for 60 days. Data fit to Seinhorst's nematode population model, Pf = ay ($^e$log $q^{-1}$) (1 – $q^{Pi}$) + s (1 – x) Pi. Dashed line is the maintenance line where Pi = Pf.

and J2 were extracted from macerated roots with the NaOCl method (8), and from soil with Coolen's modified method (2,4). Final population density (Pf) was expressed as total numbers of eggs and J2 in soil and roots, divided by the 1 900-$cm^3$ soil volume to give nematodes per 1.0 $cm^3$. Thin sections of selected root pieces were prepared for histological examination according to Johansen (9). The relationships of plantlet height and plantlet top weight to the initial nematode level (Pi) were evaluated using the plant damage model of Seinhorst (12,14), y = m + (1 – m) $z^{Pi - T}$, where y = the ratio between the relative yield (plant's top fresh weight or height) at Pi and that at P ≤ T, m = the minimum relative fresh top weight and height (y at very large Pi), z = a constant < 1 with $z^{-T}$ = 1.05, T = the tolerance limit (Pi at which no yield is lost), and Pi = the initial nematode population density. Seinhorst's nematode population model (13), Pf = ay ($^e$log $q^{-1}$) (1 – $q^{Pi}$) + s (1 – x) Pi, in which Pf and Pi are as above, a = the maximum multiplication rate (for Pi tending to 0), y = the ratio between root weight at a given Pi and that in absence of the nematode, s = the proportion of the eggs that do not hatch in absence of host roots, and x = the proportion of eggs that hatch in the presence of the host roots, was also used to interpret data on nematode reproduction.

Twenty-five days after inoculations, stunted growth was evident at initial densities (Pi) ≥ 16 eggs and J2/$cm^3$ soil. The plant damage model that best fit plantlet heights at 60 days indicated a tolerance limit of 0.32 eggs and J2/$cm^3$ soil and a maximum growth suppression (at Pi ≥ 16) of 70%, compared to growth of the control (Fig. 1 A). The tolerance limit for plantlet top weight was 0.146 eggs and J2/$cm^3$ soil, and at Pi ≥ 64 mean plant top weight was only 10% that of the control (Fig. 1 B).

The population model that best fit the

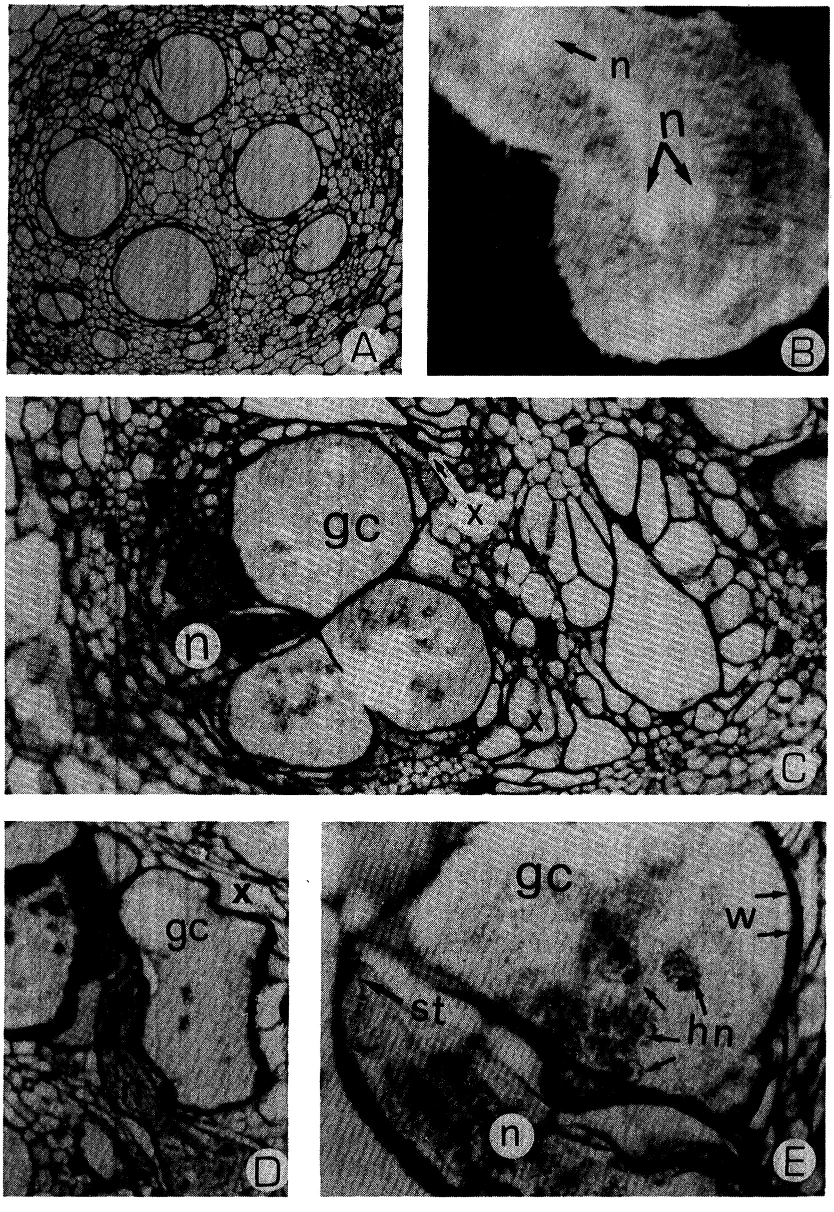
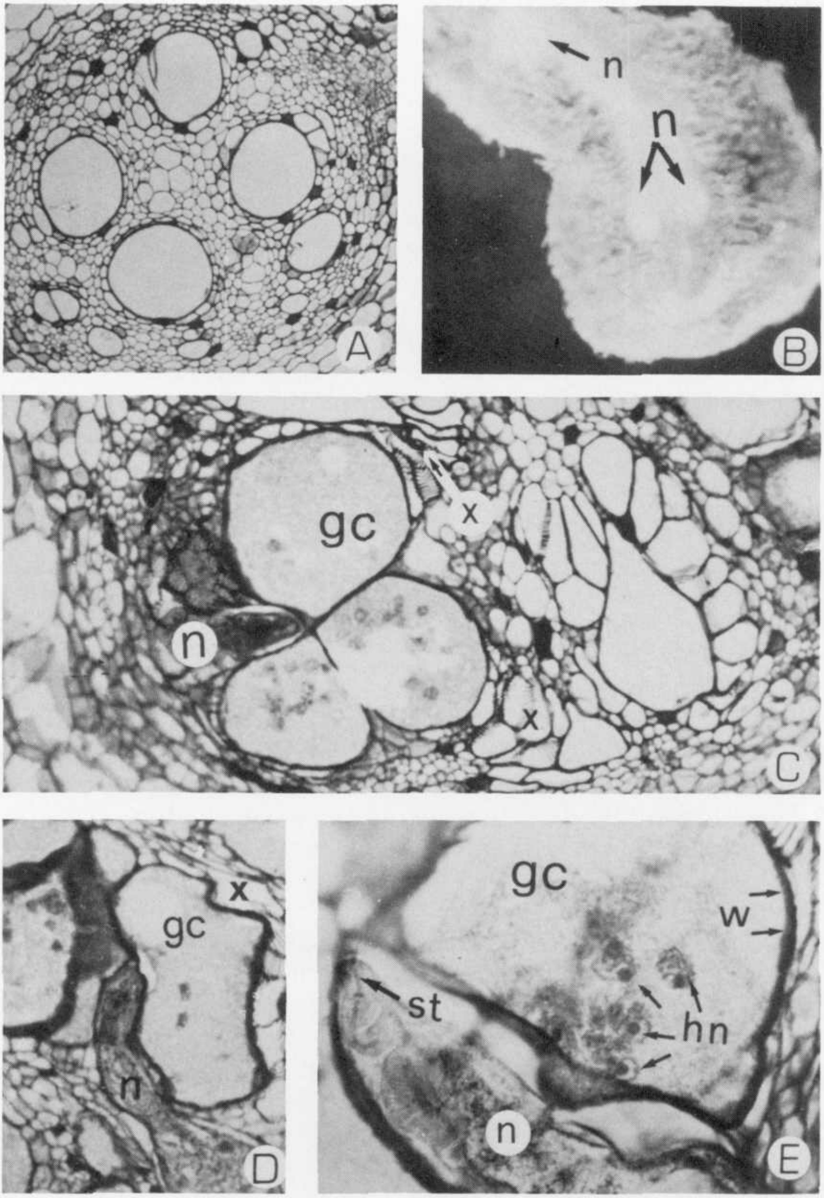



Fig. 3. Anatomical changes induced by *Meloidogyne javanica* on banana roots. A) Vascular elements of non-infected root for comparison. B) Deformed root apex. Note the concomitant invasion of three nematode females (n). C) Adult female (n) feeding from enlarged giant cells (gc) that are adjacent to disrupted cells (x) in a cross section of the gall. D) Well-developed giant cells (gc) surrounding the anterior nematode body portion (n) and adjacent to xylem elements in a longitudinal section. E) Magnified feeding site of a nematode female (n), showing apparently granular cytoplasm and hypertrophied nuclei (hn); st = nematode stylet; w = wall ingrowths.

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relationship among plant weights, initial inoculum levels (Pi), and final counts of eggs and J2 (Pf) indicated a maximum reproductive factor of 19 and an equilibrium density of 6.8 eggs and J2/cm³ soil (Fig. 2).

Anatomical modifications observed in galled banana roots were generally similar to those induced by root-knot nematodes in other hosts (17), with 3–8 characteristically polynucleate giant cells within the central cylinder surrounding the anterior end of each female. Giant cells contained granular appearing cytoplasm; cell wall thickenings and ingrowths were observed in several sections (Fig. 3).

Our results confirm that at suboptimal temperatures, young banana plantlets previously rooted *in vitro* can be severely damaged by *M. javanica*, even at low nematode densities occurring commonly in Crete (0.5–1.0 nematode/cm³ soil). However, as reported for other fruit crops [such as commercial fig (*Ficus carica* L.) trees infected by *Heterodera fici* Kirjanova (6)], root-knot nematode damage on banana should be expected to be more severe in small plantlets than in full-grown plants. It is well known that the maximum growth suppression caused by nematodes decreases with plant age (13). *Meloidogyne javanica* is abundant and widespread in many subtropical banana growing areas and its effects on banana growth in those areas deserve additional study. Of particular interest would be the effects of temperature and cultivar on susceptibility, and the tolerance of bananas grown from suckers or bullheads when compared with material originated from tissue culture.

In Crete, banana plantlets are usually planted directly in the ground favoring early nematode infection of young banana plants, which are severely damaged by these pests as shown by the results of these tests. Alternative cultural practices such as the transplanting of the *in vitro* propagated plantlets into uninfested soil and in clean containers should be considered as a preventive measure before the final transplant of larger banana plants, which are more tolerant to nematodes, in nematode contaminated ground. Additional sanitation and agronomic practices to prevent nematode buildup in the sites destined to banana plantations should be adopted. Chemical control of root-knot nematodes in these banana plantations is not always economically feasible and is also environmentally risky.

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