RESEARCH NOTES—NOTAS DE INVESTIGACION

PARASITISM AND REPRODUCTION OF *MELOIDOGYNE INCOGNITA* AND *ROTYLENCHULUS RENIFORMIS* ON CANTALOUPE IN TWO SOILS

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RESUMEN

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Densidades iniciales de nematodos de 5 *Meloidogyne incognita* de segundos estadios juveniles/cm³ de suelo y 53 *Rotylenchulus reniformis* juveniles y hembras pre-infectivas/cm³ de suelo, solas y en combinación, suprimieron el crecimiento e indujeron clorosis de la hoja de melón cv. Perlita, de manera similar, cuando las plantas fueron mantenidas bajo condiciones de invernadero en Delmita arena fina limosa e Hidalgo limo arenoso. Aunque 'Perlita' fue un buen hospedero para los dos nematodos, la densidad inicial que se uso de *R. reniformis*, aumentó a una gran densidad final más que la de *M. incognita* en los dos suelos, ya sea en uno o en las dos infestaciones. Estudios histológicos de secciones de raíz infectadas simultáneamente con *M. incognita* y *R. reniformis* demostraron que cada nematodo estableció un lugar de alimentación en diferentes tejidos del cilíndro central. El sincito de *R. reniformis* fue localizado en la periféria del cilíndro central, mientras las células gigantes de *M. incognita* se hallaron en el centro del cilíndro central.

Palabras claves: Cucumis melo, histopatología, Meloidogyne incognita, melón, nematodo nodulador, nematodo reniforme, patogenecidad, *Rotylenchulus reniformis*, textura de suelo.

In the lower Rio Grande Valley of Texas, U.S.A., production of canteloupe (*Cucumis melo* L.) is reduced in fields infested with *Meloidogyne incognita* (Kofoid & White) Chitwood and *Rotylenchulus reniformis* Linford & Oliveira (2,7). Concomitant infections of cantaloupe by *M. incognita* and *R. reniformis* are common in this area. The pathogenicity of *M. incognita* and *R. reniformis* to cantaloupe is well established (1,2,7) and yield increase has been obtained in *M. incognita* and *R. reniformis* infested fields treated with nematicides (2). However, little information

exists on the influence of two different soil types occurring in the Rio Grande Valley on infection of cantaloupe by these two nematode species. Unpublished (Heald) field observations indicate that in the spring (Feb – March), when cantaloupe is planted in the Rio Grand Valley, *R. reniformis* population densities are about tenfold greater than *M. incognita* and average about 50 preinfective juveniles and females/cm^3 of soil in contrast to five *M. incognita* second-stage juveniles (J2)/cm^3 of soil. The objectives of this study were to determine under greenhouse conditions: i) the effect of *R. reniformis* and *M. incognita* on growth of cantaloupe in two different soil types; ii) the histopathology of cantaloupe roots infected with mixed populations.

Twenty-four 15-cm-d white plastic pots were each filled with 1 500 cm^3 of sterilized Delmita loamy fine sand (87% sand, 6% clay, 7% silt, pH 7.0) and 24 with Hidalgo sandy loam (79% sand, 13% clay, 8% silt, pH 7.8). Three cantaloupe seeds (cv. Perlita) were planted in each pot. *Meloidogyne incognita* and *R. reniformis* were reared in the greenhouse on tomato (*Lycopersicon esculentum* Mill.) cv. Homestead and cantaloupe cv. Perlita, respectively. To obtain inocula, *M. incognita* J2 and *R. reniformis* juveniles and preinfective females were extracted from greenhouse cultures by the Baermann funnel method. Three days after planting, soils were infested alone or in combination with five *M. incognita* J2 and 53 *R. reniformis* preinfective stages/cm^3 of soil. Nematodes suspended in 100 ml of water were injected with a syringe into the soil at 15 locations, 5 cm deep, evenly distributed over the soil surface. Seven days after planting, seedlings were thinned to one plant per pot. Treatments were arranged in a randomized complete block design and replicated six times with noninfected plants as controls. Plants were maintained at 28±4 C on a greenhouse bench and received normal maintenance. Thirty-one days after soil infestation, the percentage of chlorotic leaves per plant was recorded. The experiment was terminated 49 days after infestation. Leaf area per plant was measured with a digitizer, and fresh shoot weights were recorded. Two 50-g cores of soil were removed from each pot and placed on screens in Baermann funnels. Roots with adhering soil from each pot were placed on a 1-cm-pore screen and immersed repeatedly in a bucket of distilled water to remove soil without removing nematode egg masses attached to roots. Roots infected with *M. incognita* were given a gall rating of 1 to 5, where 1 = no galls; 2 = 1–25%; 3 = 26–50%; 4 = 51–75%; and 5 = >76% root galling. To collect egg masses, roots were cut into 5 cm segments and macerated in water in a blender with a rubber blade at 20 000 rpm for 40 seconds. After maceration roots segements were removed from the water suspension by filtering through a 710-µm-pore screen and egg masses recovered on a 150-µm-pore screen. Egg masses in 5-ml aqueous aliquots were collected with a small artist brush with the aid of a stereomicroscope and crushed

Table 1. Effects of *Meloidogyne incognita* (Mi) and *Rotylenchulus reniformis* (Rr) alone and in combination on the growth and percentage of chlorotic leaves of cantaloupe cv. Perlita grown for 49 days in two soils infested with 5 Mi and 53 Rr/cm^3 soil.

	Loamy Sand			Sandy Loam		
Treatment	Fresh top weight (g)[z]	Total leaf surface (mm^2)	Chlorotic leaves (%)	Fresh top weight (g)	Total leaf surface (mm^2)	Chlorotic Leaves (%)
Control	132.0 a	2 227 a	10 a	122.7 a	1 966 a	13 a
Mi	76.6 bc	1 213 bc	25 c	64.9 bc	1 129 bc	23 b
Rr	87.8 b	1 506 b	21 b	74.4 b	1 292 b	24 b
Mi + Rr	67.6 c	912 c	20 b	59.3 c	1 032 c	26 b

[z]Values are the mean of six replicates. Column means followed by the same letter are not significantly different according to Fisher's protected LSD test (P = 0.05).

in a glass tube to remove eggs embedded in the gelatinous matrix. Eggs in 1-ml suspensions were counted using a stereomicroscope. Mixed *M. incognita* and *R. reniformis* egg masses in the combination treatments were separated based on the presence of females in the egg masses of *R. reniformis* and on J2 morphology. Data were analyzed by soil type using analysis of variance procedures. Means were separated by Fishers Protected LSD test (5).

Histological observations were made on cantaloupe roots infected concomitantly with *M. incognita* and *R. reniformis*. Five-mm-long root sections infected with *M. incognita* and *R. reniformis* were fixed in FAA, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin. Embedded roots were cut in 5–10 μm sections and stained with safranin-fast green, mounted in Dammar xylene, and examined with a compound microscope (3).

Cantaloupe shoot weight and leaf surface were suppressed by infection with *M. incognita* and *R. reniformis* alone or combined in the two soils (Table 1). The percentage of chlorotic leaves on singularly and

Table 2. Final densities (Pf) and *Meloidogyne incognita* (Mi) and *Rotylenchulus reniformis* (Rr) detected on cantaloupe cv. Perlita grown for 49 days in two soils infested with 5 Mi and 53 Rr/cm^3 of soil alone and in combination.

	Loamy Sand	Sandy Loam
Nematode	Pf[z]	Pf
Mi (single inoculation)	4 a	6 a
Rr (single inoculation)	135 b	322 b
Mi (combined inoculation)	5 a	7 a
Rr (combined inoculation)	160 b	189 b

[z]Values are the mean of six replicates. Column means followed by the same letters are not significantly different according to Fisher's protected LSD test (P = 0.05). Pf values are expressed as J_2 + eggs of Mi and juveniles + preinfective females + eggs of Rr/cm^3 of soil.

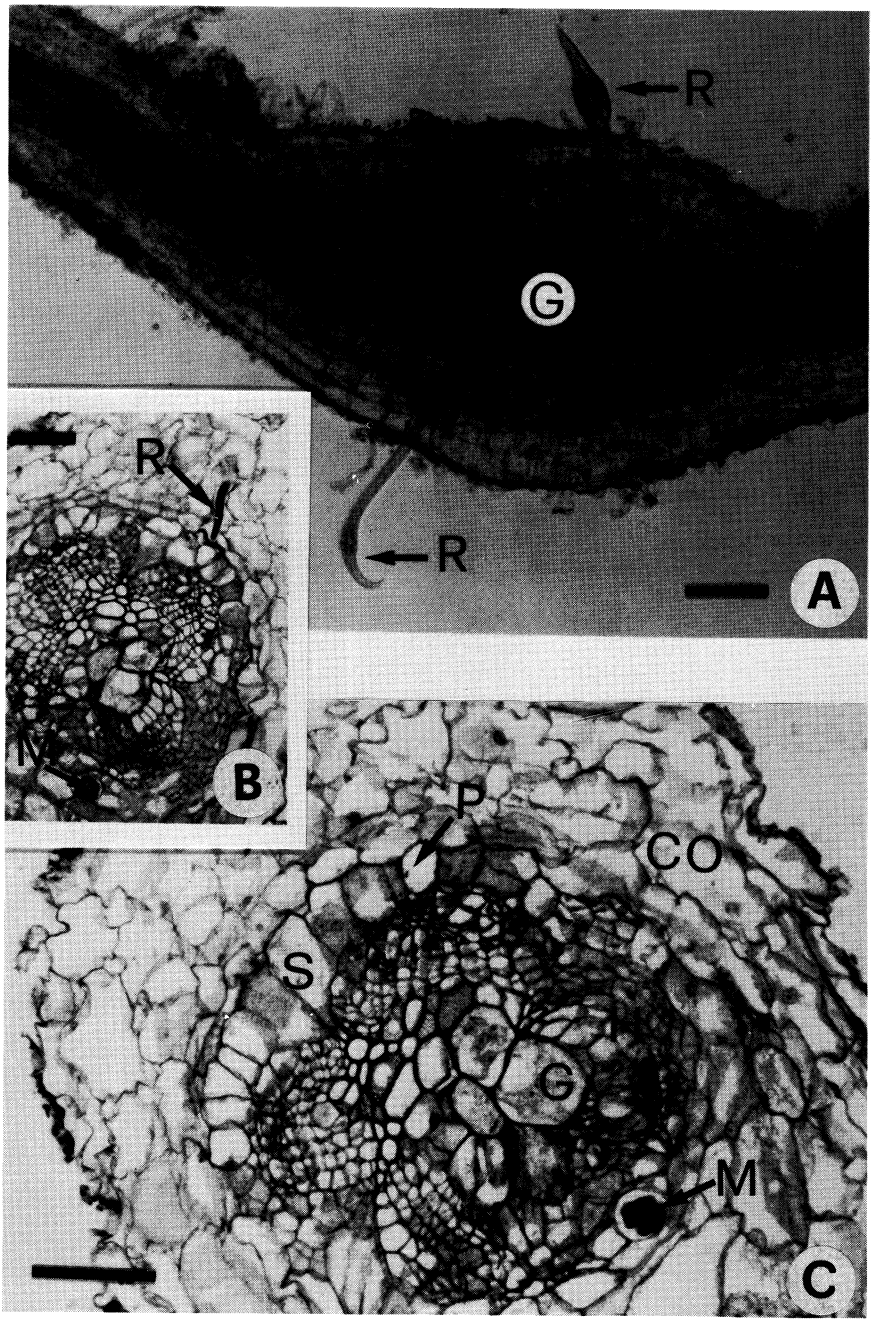
concomitantly inoculated plants was greater ($P = 0.05$) than on noninoculated controls (Table 1).

There were no significant differences in plant parameters among nematode treatments in the two soil types (Table 1). *M. incognita* severely deformed the roots of plants in either single or combined inoculations and a gall index of 5.0 occurred on all single or combined *M. incognita* infected plants in the two soils. However, *M. incognita* final densities (Pf) were less than those of *R. reniformis* (Table 2). The Pf values of each nematode in single and concomitant inoculations did not differ significantly between the two soil types. The smaller Pf of *M. incognita* compared to the Pf for *R. reniformis* were not expected, because *M. incognita* females are more fecund than *R. reniformis* females (300 eggs/female vs 66 eggs/female) (6,7).

The females of *R. reniformis* were observed protruding from the surface of galls induced by *M. incognita* (Fig. 1A). Histological examination of cross sections made through galls infected with both nematodes indicated that both species established feeding sites in different tissues of the stele (Fig. 1B). In galls infected by both nematodes, *R. reniformis* caused the same anatomical changes as reported in single inoculation on cantaloupe roots (1). The nematode induced a syncytium originating from the endodermis and expanding into the pericycle, which showed enlarged cells with dense cytoplasm. Several pericyclic cells were incorporated by successive fusion in the syncytium (Fig. 1B, C). The *R. reniformis* syncytium expanded at the periphery of the stele (Fig. 1B, C). *Meloidogyne incognita* exhibited its characteristic behavior in single inoculations (4) and induced giant cell formation in the stele and hyperplasia of vascular tissue with consequent swelling of the stele that expanded into the cortex (Fig. 1C).

Under field conditions of the Rio Grande Valley, the two soils tested are considered favorable for infection and reproduction by both *M. incognita* and *R. reniformis*.

Fig. 1. Symptoms induced by concomitant infection of *Meloidogyne incognita* and *Rotylenchulus reniformis* in cantaloupe cv. Perlita roots. Scale bars = 100 μm. A) *R. reniformis* swollen females (R) in a gall (G) induced by *M. incognita*. B) Cross section of an *M. incognita* gall infected simultaneously with *R. reniformis*. *M. incognita* (M) and *R. reniformis* (R) have established their feeding sites in different parts of the stele. C) Cross section of an *M. incognita* gall infected simultaneously with *R. reniformis*. Note the syncytium (S) of *R. reniformis* at the periphery of the stele involving the endodermis and enlarged pericycle cells (P) with dense cytoplasm. The giant cells (G) induced by *M. incognita* occupy the central part of the stele. Hyperplasia (H) of vascular parenchyma and asymmetry of the stele is evident at *M. incognita* feeding site.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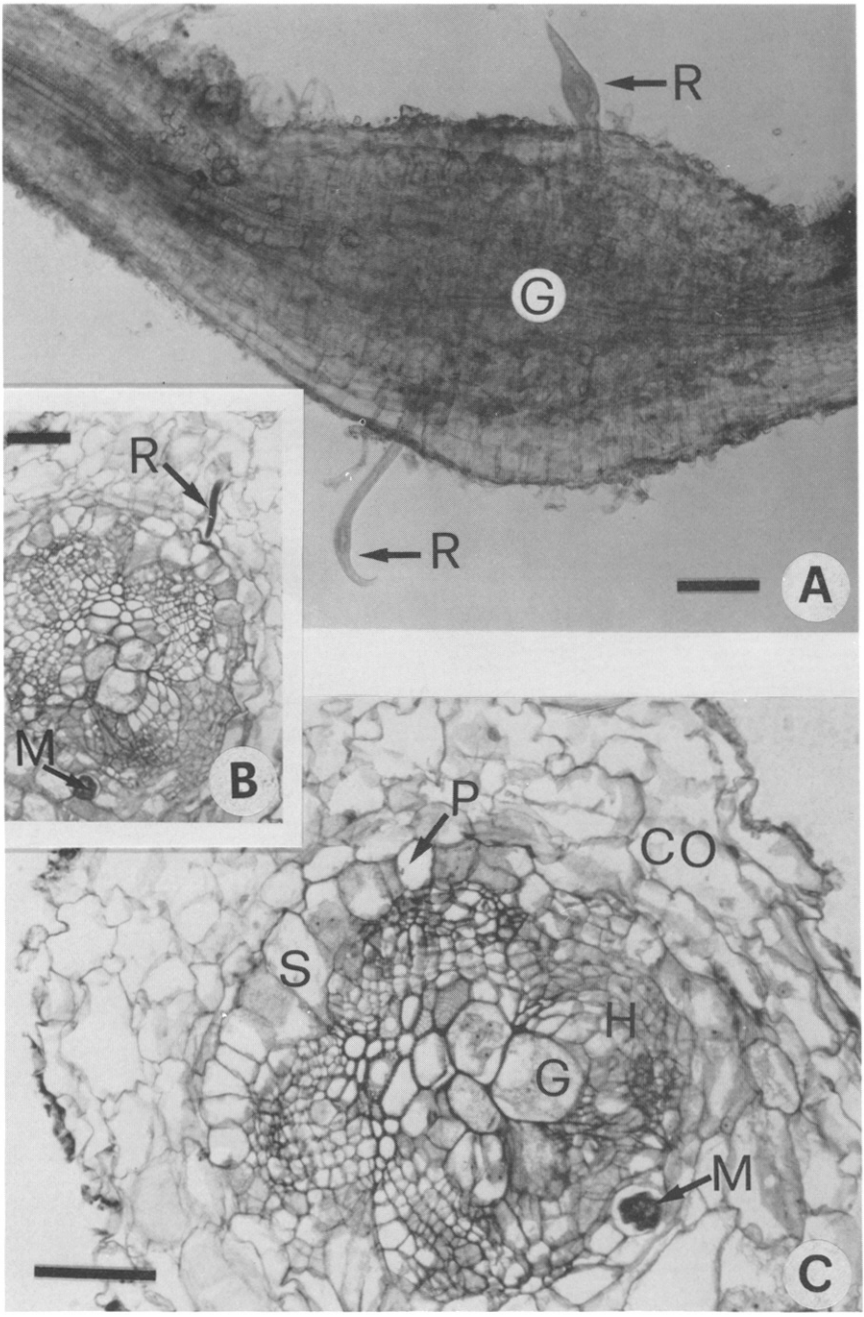

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LITERATURE CITED

1. HEALD, C. M. 1975. Pathogenicity and histopathology of *Rotylenchulus reniformis* infecting cantaloupe. Journal of Nematology 7:149–152.
2. HEALD, C. M. 1980. Effect of reniform nematode on cantaloupe yield. Plant Disease 64:282–283.
3. JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill: New York.
4. JONES, M. G. K. 1981. The development and function of plant cells modified by endoparasitic nematodes. Pp. 155–279 *in* B. M. Zuckerman and R. A. Rohde, eds. Plant Parasitic Nematodes, Vol. 3. Academic Press: New York.
5. SNEDECOR, G. W., and W. G. COCHRAN. 1980. Statistical Methods. Iowa State University Press: Ames, Iowa.
6. SIDDIQI, M. R. 1972. *Rotylenchulus reniformis*. C.I.H. Descriptions of plant-parasitic nematodes, Set 1, No. 5. Commonwealth Institute of Helminthology: St. Albans.
7. TAYLOR, A. L. and J. N. SASSER. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Department of Plant Pathology, North Carolina State University and U. S. Agency for International Development: Raleigh, North Carolina.

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