HOST STATUS OF ROUGH LEMON TO *ROTYLENCHULUS RENIFORMIS*

R. N. Inserra[1] and L. W. Duncan[2]

Nematologist, Florida Department of Agriculture and Consumer Services, DPI, Nematology Section, P.O. Box 147100, Gainesville, FL 32614-7100,[1] and Associate Professor, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850-2299, U.S.A.[2]

RESUMEN

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Una población de *Rotylenchulus reniformis* Linford y Oliveira del sur de la Florida no pudo infectar ni colonizar plántulas de limón rugoso [*Citrus limon* (L.) Burm.f.] sembradas en porrones con suelo del tipo Rockdale infestado con 1 500 nemátodos/100 cm^3 (P_i), bajo condiciones de invernadero. Entre cinco y nueve meses después del transplante, las densidades del nemátodo en el suelo disminuyeron a 840 y 612 nemátodos/100 cm^3, respectivamente. No se observó ninguna raíz de limón rugoso infectada por el nemátodo. Plantas de guisante [*Vigna unguiculata* (L.) Walp.], susceptibles a este nemátodo, fueron usadas como control. La infección y reproducción del nemátodo en estas plantas alcanzaron valores de P_f de 2 430 y 2 157 nemátodos/100 cm^3 de suelo, después de cinco y nueve meses, respectivamente.

Palabras clave: Citrus limon, patrones de cítrocos, Florida, pruebas de hospederos, nemátodo reniforme, *Rotylenchulus reniformis*.

The reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, has a wide host range which includes numerous tropical and subtropical dicots and monocots. This nematode has been reported in association with citrus in several tropical and subtropical areas (Ayala and Ramirez, 1964). However, there is a lack of information about the ability of *R. reniformis* to reproduce on citrus. The majority of the reports dealing with reniform nematodes (*Rotylenchulus* spp.) on citrus provide information limited to the nematode population densities in soil without any assessment of the nematode densities on citrus roots. Peacock (1956) was the first to report a slight infection by *R. reniformis* on roots of rough lemon [*Citrus limon* (L.) Burm. f.] in Ghana. In spite of this report, the host status of citrus to reniform nematodes remained uncertain (Tarjan, 1964). However, for regulatory purposes in the United States, *R. reniformis* has been included on the quarantine list of nematode pests of citrus by the State of California (Department of Food and Agriculture of California, 1995). The states of Arizona, New Mexico, and some countries such as Chile and Switzerland, also impose quarantine restrictions on *R. reniformis*. In order to provide information about the host status of citrus to a Florida population of *R. reniformis*, we tested the response of rough lemon rootstock to *R. reniformis* from south Florida, where this nematode is often found associated with self-rooted Tahiti lime [*C. aurantifolia* (Christm.) Swing.] (Malo and Tarjan, 1968).

A 25-cm-deep Rockdale loamy soil (Gallatin *et al.*, 1958) with 40% sand, 31% silt, 28% clay and pH = 7.3-7.8 was collected originally in Dade County, Florida, U.S.A. from a snap bean (*Phaseolus vulgaris* L.) field infested with *R. reniformis*. The soil was kept for 3 years in 25-cm-diam plastic pots and sown with successive crops of cowpea [*Vigna unguiculata* (L.) Walp.] to favor nematode population increase. Soil from the pots was bulked and mixed. Nematode densities (P_i) were assessed from a 600-cm^3 sam-

ple composed of 15, 40-cm^3 cores. Vermiform nematodes were extracted from soil by centrifugal-flotation (Jenkins, 1964). Nematode population levels were expressed as numbers of vermiform stages per 100 cm^3 of soil. Six-month-old seedlings of rough lemon were planted in 15-cm-diam plastic pots containing the infested Rockdale soil. Cowpea cv. California 5, a good host of the reniform nematode, was grown alone in additional pots containing the nematode-infested soil to serve as a susceptible control. In a third treatment, additional rough lemon seedlings were grown with cowpea in the same pot containing the infested soil to expose the citrus seedlings to continuously high nematode levels that typically are supported by the nematode-susceptible cowpea. Ten replicates of all 3 treatments were distributed randomly on a greenhouse bench and maintained up to 9 months at 15-32°C. Pots containing cowpea were reseeded about every 100 days. Five months after planting, 4 plants from each treatment were harvested and final population densities (P_f) in 300 cm^3 of soil were assessed. Nematode densities in roots were evaluated in 15 g and 0.5 g of rough lemon and cowpea roots, respectively. Roots were separated by plant species, washed gently, and examined with the aid of a stereomicroscope for swollen *R. reniformis* females and egg masses. Nine months after planting, the remaining 6 plants for each treatment were harvested and soil and root nematode densities assessed as described before.

The initial population density in each pot was 1 500 nematodes/100 cm^3 soil. Five months after planting, population densities in pots with only rough lemon declined, whereas those in pots with cowpea alone or in combination with rough lemon increased (Table 1). In all pots containing cowpea, nematode densities were greater than in pots with rough lemon alone. Sedentary females and eggs were observed on cowpea roots, but no evidence of nematode infection and reproduction was observed on rough lemon roots from plants grown alone or in combination with cowpea (Table 2). Similar results were obtained from the plants harvested 9 months after planting (Tables 1 and 2). At this sampling date, soil nematode densities in pots with rough lemon alone were smaller than in those with cowpea. In both treatments, rough lemon roots grown alone or in association with the heavily infected cowpea roots were not parasitized by the *R. reniformis* nematodes. Test results provide evidence that rough lemon is not a suitable host for a population of *R. reniformis* from south Florida. These findings are in agreement with results of surveys conducted in the Rio Grande Valley in Texas, during which microscopic examination of sour orange (*C. aurantium* L.) roots collected from citrus orchards,

Table 1. Number of vermiform *Rotylenchulus reniformis* per 100 cm^3 soil in pots containing rough lemon and cowpea grown alone or in combination for 5 and 9 months in soil infested with 1 500 nematodes per 100 cm^3.

Treatment	5 months*		9 months**	
Rough lemon alone	840 c	(29)	612 b	(49)
Cowpea alone	1 815 b	(95)	2 135 a	(166)
Rough lemon + cowpea	2 430 a	(134)	2 157 a	(75)

Data are means and (standard errors) of 4* and 6** replications. Means in columns followed by the same letter are not different ($P \leq 0.05$) by Tukey's honestly significant difference test.

Table 2. Number of swollen females and eggs of *Rotylenchulus reniformis* per g of fresh roots of rough lemon and cowpea grown alone or in combination for 5 and 9 months in soil infested with 1 500 nematodes per 100 cm^3.

Type of root examined	Sedentary females				Eggs			
	5 months*		9 months**		5 months		9 months	
Rough lemon roots in absence of cowpea	0 b	(0)	0 b	(0)	0 b	(0)	0 b	(0)
Cowpea roots in absence of rough lemon	251 a	(33)	244 a	(23)	1 840 a	(174)	2 369 a	(521)
Rough lemon roots in presence of cowpea	0 b	(0)	0 b	(0)	0 b	(0)	0 b	(0)
Cowpea roots in presence of rough lemon	253 a	(29)	283 a	(32)	1 946 a	(464)	1 788 a	(288)

Data are means and (standard error) of 4* and 6** replications. Means in column followed by the same letter are not different ($P \leq 0.05$) by Tukey's honestly significant difference test.

growing in soil infested with *R. reniformis*, revealed no evidence of infection by *R. reniformis* (C. M. Heald, unpublished). In spite of the fact that both of these citrus rootstocks are not hosts for *R. reniformis* infections in the United States, we cannot exclude the possibility that populations of *R. reniformis* able to infect citrus exist in other areas of the world. Therefore, the ability of *R. reniformis* from Ghana to reproduce on rough lemon (Peacock, 1956) should be confirmed. The report from India of *R. reniformis* root infection on rough lemon and mandarin (*C. reticulata* Blanco) (Ganguly, 1968) also needs verification, since it is based on nematode population densities in the soil rather than on observations of reproduction on citrus roots.

Verification of host status of citrus and other fruit trees to *R. reniformis* under field conditions is difficult because *R. reniformis* reproduces on many weed species (Inserra *et al.*, 1989; Quénéhervé *et al.*, 1995) which often grow under the tree canopy. Results based only on the nematological analyses of soil samples collected under the canopy of citrus or other fruit trees can reflect *R. reniformis* originating from weed roots. Only microscopic examination of roots provides certainty of the host status to *R. reniformis*. Often weed roots adhere to citrus roots providing the false impression that *R. reniformis* egg masses are on the citrus roots (Fig. 1). Careful examination and separation of the egg masses can reveal their origin as observed when rough lemon was grown in combination with cowpea.

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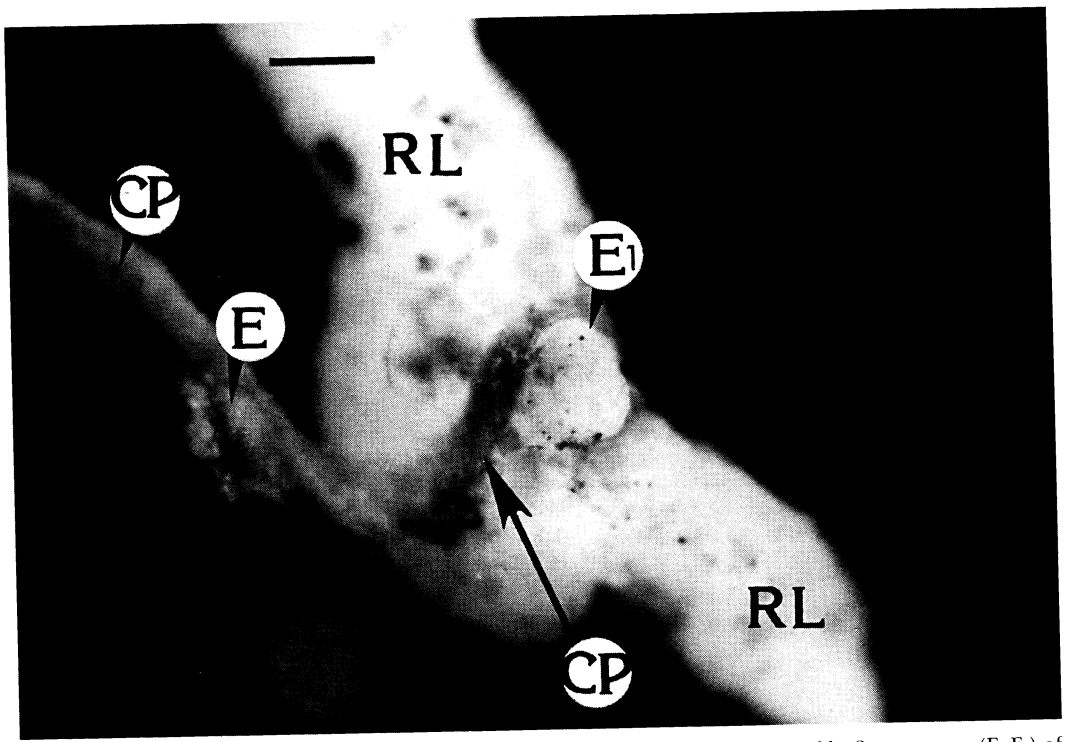

Fig. 1. A rough lemon (RL) fibrous root associated with a cowpea (CP) rootlet infected by 2 egg masses (E, E_1) of *Rotylenchulus reniformis*. Note nematode egg mass (E_1) adhering to both cowpea and rough lemon roots making host identification very difficult without proper root separation. Scale bar = 240 µm.

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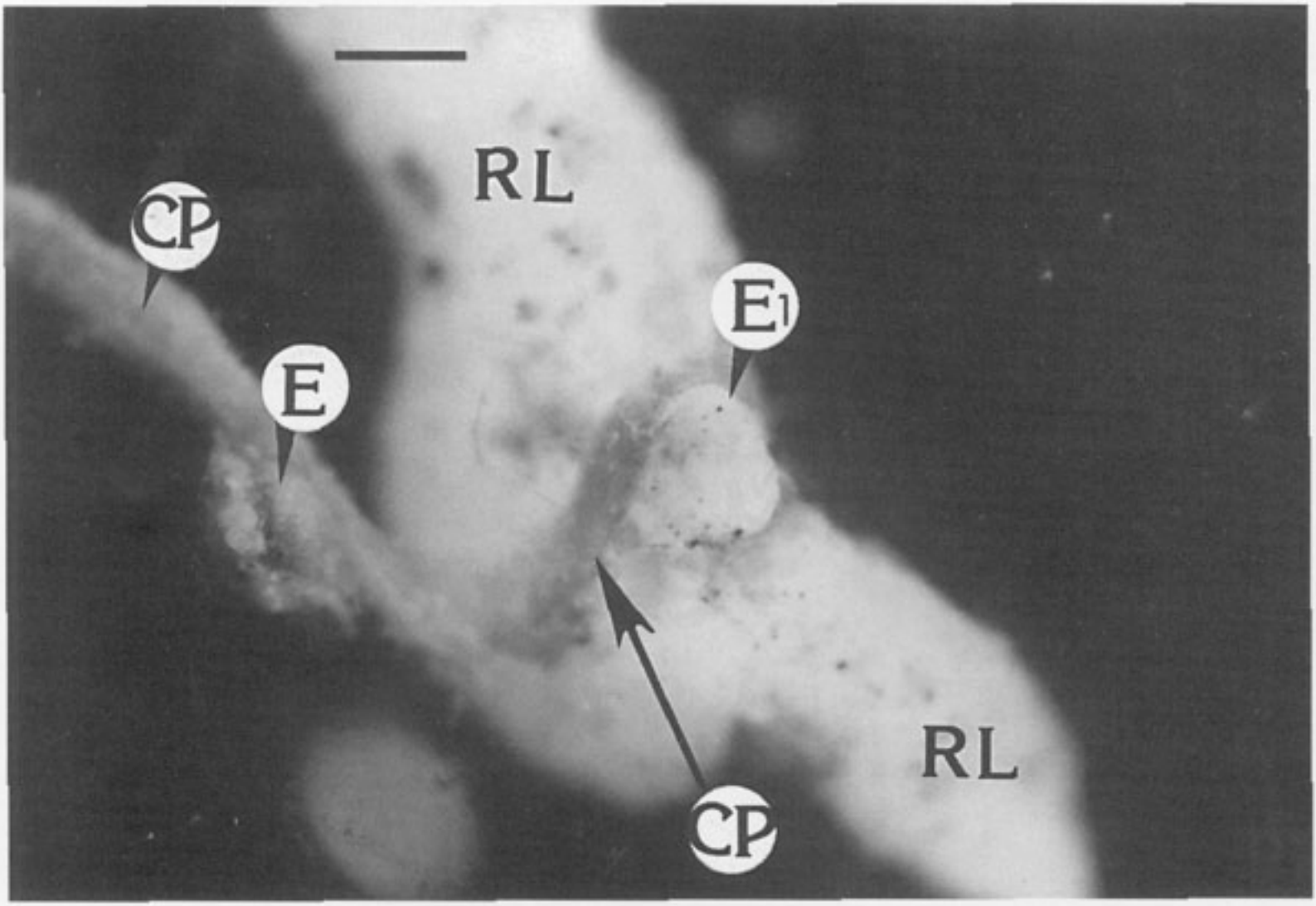

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