

Interaction between *Meloidogyne enterolobii* and *Helicotylenchus dihysteroides* in guava seedlings

Vicente M. Gomes^{a*}, Ricardo M. Souza^a, Thiago F. Ferreira^a, Guilherme B. Miranda^a, Alexandre M. Almeida^a and Renata R. Robaina^a

^aLaboratório de Entomologia e Fitopatologia (LEF), Centro de Ciências e Tecnologias Agropecuárias (CCTA), Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes (RJ) Brazil
*vanssan523@yahoo.com.br

HIGHLIGHTS

- Individual infection by *H. dihysteroides* alone or together with the other pathogen did not cause damage in guava plants.
- The joint infection by *H. dihysteroides* and *M. enterolobii* resulted in a reduction in the final population of *H. dihysteroides*.
- Guava decline only occurs in orchards infested with *M. enterolobii* with the associated presence of *F. solani*.

ABSTRACT: In order to evaluate the effect of joint infection by *Meloidogyne enterolobii* and *Helicotylenchus dihysteroides* on vegetative growth of guava seedlings (*Psidium guajava* L.), as well as to observe symptoms caused by pathogens in host plants, an experiment was conducted in microplots. In these experimental units, suspensions were used containing *H. dihysteroides* and *M. enterolobii*. The plants were separately inoculated with *H. dihysteroides* and jointly inoculated with *H. dihysteroides* and *M. enterolobii*, and the blank controls were represented by non-inoculated plants. No significant differences were observed between treatments related to morpho-physiological variables of guava seedlings, but joint inoculation resulted in a reduced final population of *H. dihysteroides*. It was also concluded that neither infection by *H. dihysteroides* alone nor a joint infection by the two pathogens caused any damage to guava seedlings.

Keywords: complex disease, guava root-knot nematode, spiral nematode, *Psidium guajava*.

Cite as

Gomes VM, Souza RM, Ferreira TF, Miranda GB, Almeida AM, Robaina RR. Interaction between *Meloidogyne enterolobii* and *Helicotylenchus dihysteroides* in guava seedlings. *Nematoda*. 2014;1:e04014. <http://dx.doi.org/10.4322/nematoda.04014>

Received: July 10, 2013 **Accepted:** July 15, 2014

INTRODUCTION

Diseases caused by nematodes in guava trees only recently became known by producers. The first report in Brazil was written by Moura & Moura^[1] in the state of Pernambuco. Since then, various genera and species of phytonematodes have been identified in association with the guava crop^[2, 3, 4, 5, 6, 7, 8]. However, there are few articles that report the comparative evaluation of pathogenic actions by species of nematodes when these occur in isolated or joint infestations.

Phytonematodes generally occur in polyspecific communities, interacting in a dynamic way with the host plant, the environment and the other organisms present in the rhizosphere^[9]. Studies demonstrate that sites of infection from ecto and endoparasitic nematodes are different. The two types may coexist on the plant without any interaction occurring^[10]. However, interactions can occur between the species and these may be mutually antagonistic or suppressive for one of the species involved^[11].

In a study carried out by Moreira et al.^[5] to evaluate the phytonematodes associated with guava in the lower-middle São Francisco River valley, soil and root samples were collected in orchards of the cultivar 'Paluma', and the following phytopathogenic genera were noted: *Meloidogyne* Cobb, *Xiphinema*

Cobb, *Hemicycliophora* De Man, *Pratylenchus* Filipjev, *Aorolaimus* Sher, *Rotylenchulus* Linford & Oliveira, *Helicotylenchus* Steiner, *Belonolaimus* Steiner and *Ditylenchus* Filipjev.

Currently, in Brazilian conditions and particularly for the guava (*Psidium guajava* L.), *Meloidogyne enterolobii* Yang and Eisenback is the nematode species that has most harmed crops, when it is associated with *Fusarium solani* (Mart.) Sacc^[12]. In São João da Barra County, Rio de Janeiro state, this nematode has limited production of the guava crop, leading to losses of up to 100%^[13]. In work carried out by Almeida et al.^[8], high population densities of *H. dihysteroides* Siddiqi and *M. enterolobii* were found concomitantly in areas with dwindling productivity.

Species of *Helicotylenchus* are globally distributed, ranging across many climate types, and they are associated with the root system of various crops of agricultural importance. Although existing data do not allow us to characterize this genus as a severe parasite for many crops, suppression of plant growth has been consistently associated with some cosmopolite species of *Helicotylenchus*^[14].

The objective of this work was to use microplots to evaluate the effect of *H. dihysteroides*, alone and in conjunction with *M. enterolobii*, on the vegetative development of guava seedlings, and to observe symptoms caused by the pathogens in the host plant.

MATERIAL AND METHODS

To this end, seedlings of 'Paluma' guava, which is highly susceptible to *M. enterolobii*, were taken at the six-leaf-pair stage and transplanted into microplots, constituted of plastic pots filled with 70 liters of washed river sand and kept in the open air. The plants received monthly fertilizations of NPK and Mg, S, B, Cl, Cu and Zn with the commercial product Biofert® Jardim 4-14-8+6, at a dose of 5mL/L, via foliar application, in accordance with the recommendations of Pereira^[15], and irrigated whenever necessary. The plants were submitted to three treatments, as follows: without nematode (control - T1), inoculated with 50 females of *H. dihysteroides* (T2) or co-inoculated with 50 females of *H. dihysteroides* and 500 eggs of *M. enterolobii* (T3). The inoculum of *H. dihysteroides* was obtained by processing soil Jenkins^[16] from highly infested commercial guava orchards in the county of São João da Barra, Rio de Janeiro state. After processing, the females of *H. dihysteroides* were collected individually under a stereoscopic microscope with the aid of a fine bamboo stick, and then transferred to 10 mL of water and inoculated into four points in the pot. The *M. enterolobii* inoculum was obtained from tomato plants cultivated in a greenhouse, and applied in a suspension of 15 ml, which was mixed with sand in four points in the pot, 30 days after inoculation with *H. dihysteroides*.

The treatments were arranged in a completely randomized design, with eight repetitions (pot with one plant) per treatment. Twelve months after inoculation (M.A.I.), evaluation was made of the population of nematodes in simple samples of 100 cc of soil from the pots, removed with a probe at a depth of 20 cm, as well as of plant height, leaf chlorophyll content, obtained with a portable measurer SPAD - 502® (Soil Plant Analysis Development) from five leaves per plant, and the total number of leaves. At 22 M.A.I., the same variables were reevaluated, as well as biomass of the aerial part and fresh roots, total root volume, calculated by displacement of water in a test-tube, and the final nematode population/100 cc of soil removed with a probe at a depth of 20 cm. The data were submitted to analysis of variance and the means were compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

Significant differences were not observed in the morpho-physiological variables at 12 months (data not shown) and at 22 months (Table 1), which indicates that *H. dihysteroides* alone or *H. dihysteroides* associated with *M. enterolobii* were not pathogenic to guava seedlings in the conditions found in this experiment. These results corroborated those obtained by Khan et al.^[17]. Those authors suspected that the participation of *Helicotylenchus dihystra* (Cobb) Sher could be involved in the decline of guava, but did not observe damage. Gomes et al.^[12] reported in their studies that *M. enterolobii* is not an aggressive pathogen and that guava decline only occurs in the presence of *Fusarium solani*.

However, the joint infection (T3) resulted in a reduction in the final population of *H. dihysteroides* in comparison with T2, at the two evaluation times (Table 1). It was concluded that individual infection by *H. dihysteroides* alone or together with the other pathogen did not cause damage in guava plants. It is possible that both species occurring on the same plant may have caused competition for or destruction of the infection sites of *H. dihysteroides*, and this species was suppressed. It may be that

Table 1. Variables of the aerial part and root system of guava seedlings, at 22 months, and the populations of *Helicotylenchus dihysseroides* and *Meloidogyne enterolobii* in the soil at 12 and 22 months after inoculation (M.A.I.).

Treatment	H (cm)	APM (g)	CC (ng/cm ²)	FRM (g)	RV (ml)	NL	12 M.A.I.		22 M.A.I.	
							PMe	PHd	FPMc	FPHd
T1	151b	5133	40	923	656	493	0	0	0	0
T2	138	3881	39	838	729	419	0	171	0	409
T3	141	4478	37	868	553	411	244	62	6018	15
CV (%)	17.1	44.5	8.79	32.6	29.6	52.4	-	-	-	-

*The means of all the variables did not differ significantly among themselves, at 5% of probability, by Tukey test.

*T1: non-inoculated control; T2: inoculation with *H. dihysseroides*; T3: inoculation with *H. dihysseroides* and *M. enterolobii*. H = height; APM = Aerial part mass; CC = chlorophyll content; FRM = fresh root mass; RV = root volume; NL = number of leaves; PMc = population of *M. enterolobii* / 100 cc of soil; PHd = population of *H. dihysseroides* / 100 cc of soil; FPMc = final population of *M. enterolobii* / 100 cc of soil; FPHd = final population of *H. dihysseroides* / 100 cc of soil.

^aValues are mean of eight repetitions.

the proliferating capacity of *M. enterolobii* is greater than that of *H. dihysseroides* and the initial level of inoculum used for *H. dihysseroides* was lower, by a ratio of 1:10. Research has shown that the effect of the interaction between different nematode species can depend on diverse factors, including the initial inoculum density, exposure time, host plant and inoculation method^[11, 18, 19].

Although *Helicotylenchus* species have been reported causing damage to guava seedlings^[17], research in healthy and diseased orchards and in controlled experiments in India^[20], did not obtain convincing proof that *Helicotylenchus* sp. was involved in guava decline. The results obtained in the work presented here corroborate those of other researchers. Although other associations were not tested here, our findings reinforce reports that guava decline only occurs in orchards infested with *M. enterolobii* with the associated presence of *F. solani*^[8, 21].

CONCLUSIONS

No significant differences were observed between treatments related to morpho-physiological variables of guava seedlings, but joint inoculation resulted in a reduced final population of *H. dihysseroides*.

It was also concluded that neither infection by *H. dihysseroides* alone nor a joint infection by the two pathogens caused any damage to guava seedlings.

REFERENCES

- [1] Moura RM, Moura AM. 1989. Meloidoginose da goiabeira: doença de alta severidade no Estado de Pernambuco, Brasil. Nematologia Brasileira, 13: 13-19.
- [2] Rammah A, Hirschmann H. 1988. *Meloidogyne enterolobii* n.sp. (*Meloidogynidae*) a root-knot nematode from Puerto Rico. Journal of Nematology, 20: 58-69.
- [3] Ferreira NC Fo, Santos JM, Silveira SF. 2000. Caracterização morfológica e bioquímica de uma nova espécie de *Meloidogyne* parasita da goiabeira no Brasil. Nematologia Brasileira, 24: 121-125.
- [4] Barbosa FR. 2001. Goiaba: fitossanidade. Embrapa-Semi árido, Petrolina, 63 pp.
- [5] Moreira WA, Barbosa FR, Moura AO. 2001. Distribuição populacional de fitonematóides em goiabeira no submédio São Francisco. Nematologia Brasileira, 25: 124-125.
- [6] Kimati H, Amorin L, Rezende JAM, Bergamin A Fo, Camargo LEA. 2005. Manual de Fitopatologia. Doenças das plantas cultivadas. Agronômica Ceres, São Paulo.
- [7] El Borai FE, Duncan LW. 2005. Nematodes parasites of subtropical in tropical fruits tree crops. In: Luc M, Bridge J (eds.). Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Cambridge, p. 467-492.
- [8] Almeida AM, Souza RM, Gomes VM, Miranda GB. 2012. Greenhouse and field assessment of different organic compounds against guava-parasitic *Meloidogyne enterolobii*. Bragantia, 71: 67-74.
- [9] Eisenback JD. 1985. Interactions among concomitant populations of nematodes. In: Sasser JN, Carter CC (eds.), An advanced treatise on *Meloidogyne*, North Carolina State University Graphics, Raleigh. p. 193-213.
- [10] Eisenback JD. 1993. Interactions between nematodes in cohabitation. In: Khan, WM. (ed.). Nematode interactions. Chapman and Hall, London, p. 134-174.

-
- [11] Khan RM, Khan MW, Khan AM. 1985. Cohabitation of *Meloidogyne incognita* and *Rotylenchulus reniformis* in tomato roots and effect on multiplication and plant growth. *Nematologia Mediterranea*, 13: 153-159.
- [12] Gomes VM, Souza RM, Mussi-Dias V, Silveira SF, Dolinski C. 2011. Guava decline: a complex disease involving *Meloidogyne mayaguensis* and *Fusarium solani*. *Journal of Phytopathology*, 159: 45- 50.
- [13] Gomes VM, Souza RM, Midorikawa G, Miller R, Almeida AM. 2012. Guava decline: evidence of nationwide incidence in Brazil. *Nematropica*, 42: 153-162.
- [14] Subbotin SA, Inserra RN, Marais M, Mullin P, Powers TO, Roberts PA, Berg EV, Yeates GW, Baldwin JG. 2011. Diversity and phylogenetic relationships within the spiral nematodes of *Helicotylenchus* Steiner, 1945 (Tylenchida: Hoplolaimidae) as inferred from analysis of the D2-D3 expansion segments of 28S rRNA gene sequences. *Nematology*, 13: 333-345.
- [15] Pereira FM. 1995. Cultura da goiabeira. FUNEP, Jaboticabal, Brazil, 47 pp.
- [16] Jenkins WR. 1964. A rapid centrifugal flotation technique for separating nematode from soil. *Plant Disease Reporter*, 48: 692.
- [17] Khan RM, Kumar S, Reddy PP. 2001. Role of plant parasitic nematode (s) and fungi in guava wilt. *Pest Management Horticulture Ecosystem*, 7: 152-161.
- [18] Stetina SR, Russin JS, McGawley EC. 1997. Replacement series: a tool for characterizing competition between phytoparasitic nematodes. *Journal of Nematology*, 29: 35-42.
- [19] Koenning SR, Waters SA, Barker KR. 1996. Impact of soil texture on the reproductive and damage potential of *Rotylenchulus reniformis* and *Meloidogyne incognita* on cotton. *Journal of Nematology*, 28: 527-536.
- [20] Willers P, Gretch NM. 1986. Pathogenicity of the spiral nematode *Helicotylenchus dihystra* to guava. *Plant Disease*, 70: 352.
- [21] Gomes VM, Souza RM, Silveira SF, Almeida AM. 2013. Guava decline: effect of root exudates from *Meloidogyne enterolobii*-parasitized plants on *Fusarium solani* in vitro and on growth and development of guava seedlings under controlled conditions. *European Journal of Plant Pathology*, 137: 393-401.