

# DYNAMICS OF CONCOMITANT POPULATIONS OF *PRATYLENCHUS VULNUS* AND *MELOIDOGYNE INCOGNITA* ON PEACH

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## ABSTRACT

Nyczepir, A. P. 2009. Dynamics of concomitant populations of *Pratylenchus vulnus* and *Meloidogyne incognita* on peach. *Nematropica* 39:273-279.

The effect of the interaction between *Meloidogyne incognita* and *Pratylenchus vulnus* on nematode reproduction and vegetative growth of Lovell peach was studied in field microplots. *Pratylenchus vulnus* suppressed the population density of *M. incognita* second-stage juveniles, whereas the presence of *M. incognita* did not affect the population density of *P. vulnus* in soil. Above-ground tree growth, as measured by trunk diameter 12 and 24 months following inoculation, was reduced in the presence of *M. incognita*. Differences in root growth as related to nematode treatment were detected 26 months after inoculation. Root growth was reduced in the presence of the two nematode species together than *P. vulnus* alone, but not when compared to *M. incognita* alone. There was a greater negative impact on vegetative growth of peach seedlings growing in *M. incognita*-infested soil than in *P. vulnus*-infested soil.

*Key words:* Interaction, *Meloidogyne incognita*, microplot, pathogenicity, peach, population dynamics, *Pratylenchus vulnus*, *Prunus persica*, root-knot nematode, root-lesion nematode.

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## RESUMEN

Nyczepir, A. P. 2009. Dinámica de poblaciones concomitantes de *Pratylenchus vulnus* y *Meloidogyne incognita* en duraznero. *Nematropica* 39:273-279.

Se estudió el efecto de la interacción entre *Meloidogyne incognita* y *Pratylenchus vulnus* sobre la reproducción de los nematodos y el crecimiento vegetativo del duraznero Lovell en microparcels en el campo. *Pratylenchus vulnus* redujo la densidad de población de juveniles de segundo estadio de *M. incognita*, mientras que la presencia de *M. incognita* no afectó la densidad de población de *P. vulnus* en el suelo. La presencia de *M. incognita* redujo el crecimiento de la parte aérea del árbol, medida en términos del diámetro del tronco, 12 y 24 meses después de la inoculación. Se detectaron diferencias en el crecimiento de las raíces a los 26 meses después de la inoculación. El crecimiento de raíces se redujo en presencia de ambos nematodos y con sólo *P. vulnus*, pero no con sólo *M. incognita*. Se observó mayor impacto negativo sobre el crecimiento vegetativo de plántulas de duraznero con suelo infestado con *M. incognita* que con suelo infestado con *P. vulnus*.

*Palabras clave:* dinámica de poblaciones, duraznero, interacción, *Meloidogyne incognita*, microparcels, nematodo agallador, nematodo lesionador, patogenicidad, *Pratylenchus vulnus*, *Prunus persica*.

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## INTRODUCTION

Information on interactions between migratory endoparasitic and sedentary endoparasitic nematodes in peach [*Prunus persica* (L.) Batsch] is limited. Research has

focused on a single nematode species and its role in association with the peach disease under study. Information on interactions between different plant-parasitic nematodes coinhabiting the same orchard is essential to understanding their com-

bined impact on disease so that an appropriate management strategy can be implemented. In Georgia and South Carolina it is not uncommon to find several economically important plant-parasitic nematode genera within the same peach orchard, such as root-knot (*Meloidogyne* spp.) and root-lesion (*Pratylenchus* spp.) nematodes (Nyczepir *et al.*, 1985). Root-knot and root-lesion nematodes are important pathogens of peach in the United States and other parts of the world (Nyczepir and Esmenjaud, 2008). In South Carolina peach orchards, *M. incognita* and *M. javanica* were found in 95% and 5% of orchards sampled, respectively (Nyczepir *et al.*, 1997). *Meloidogyne* spp. can cause stunted growth, loss of vigor, and early defoliation of one to two-year-old peach trees when recommended management practices are not followed. At least nine *Pratylenchus* spp. [*P. penetrans*, *P. pratensis*, *P. brachyurus*, *P. zaeae*, *P. convallariae*, *P. neglectus*, *P. thornei*, *P. sefaensis*, and *P. vulnus*] have been found associated with peach throughout the world, but *P. vulnus* is the species of primary concern in California and the southeastern United States (Nyczepir and Esmenjaud; 2008). In California, *P. vulnus* damage to peach rootstocks is estimated to cause approximately 16% reduction in marketable fruit size and yield (McKenry, 1989). In the southeastern United States, limited investigations have been conducted on *P. vulnus*, which until recently was not considered as important a pathogen on peach as *Mesocriconema xenoplax* (Raski) Loof & de Grisse [= *Criconemoides xenoplax* (Raski) Loof and de Grisse] or *Meloidogyne* spp. (Nyczepir and Pinochet, 2001). In Georgia, *P. vulnus* was first reported to be associated with reduced peach tree vigor and rapid deterioration and reduction of feeder roots (Fliegel, 1969), which are characteristic symptoms reported by others (Marull and Pinochet,

1991; McKenry, 1989). Furthermore, in field microplots, *P. vulnus* (GA-peach isolate) was associated with reduced peach tree growth of 'Guardian®', 'Lovell', and 'Nemaguard' rootstocks (Nyczepir and Pinochet, 2001). *Pratylenchus vulnus*, which destroys the root cortical parenchyma cells, is also known for producing avenues for secondary infection by bacteria and fungi (Marull and Pinochet, 1991). Therefore, managing *Meloidogyne* spp. and *P. vulnus* is essential for establishment and optimizing yield of a peach orchard. The current pre-plant nematicide recommendation for managing these two plant-parasitic nematodes in the southeastern United States includes fumigation with Telone II (1,3-D) or Vapam (metam-sodium) (Horton *et al.*, 2009).

The combined impact of parasitism by a sedentary endoparasitic and a migratory endoparasitic nematode on growth of peach is unknown. This study assesses the effects and interactions between *M. incognita* and *P. vulnus* on peach vegetative growth and nematode reproduction.

## MATERIALS AND METHODS

### *Nematode source and inoculum*

*Pratylenchus vulnus*, which originated from a peach orchard in Byron, Georgia was reared monoxenically on carrot (*Daucus carota* L.) disk cultures (Moody *et al.*, 1973) and incubated at 22°C for multiplication. The *Meloidogyne incognita* isolate, originating from a commercial peach orchard in Warner Robins, Georgia, was cultured on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in the greenhouse. Root-lesion nematode inoculum was prepared by macerating the nematode-infested carrot disks in water in a commercial blender for four times at 5-s intervals. The nematode/carrot suspension was then concen-

trated using a 250- $\mu\text{m}$  sieve nested on a 38- $\mu\text{m}$  sieve (60 and 400-mesh, respectively). The carrot debris collected on the 250- $\mu\text{m}$  sieve was discarded, whereas the content on the 38- $\mu\text{m}$  sieve was placed on a Baermann funnel, from which the nematode inoculum was obtained. Root-knot nematode eggs were extracted from the tomato roots using the NaOCl method described by Hussey and Barker (1973).

#### *Field microplot experiment*

The effects of the interactions between *M. incognita* and *P. vulnus* on peach vegetative growth and nematode reproduction were evaluated in field microplots. Approximately 3-month old Lovell peach seedlings were planted singly in bucket microplots (Barker, 1985) (25-cm-diam  $\times$  31-cm-deep) containing 15,000  $\text{cm}^3$  of steam pasteurized soil (86% sand, 10% silt, 4% clay, pH 6.1, 0.54% OM) in February 2003. Microplots were established in a shaded area (30% shade) in the field at the USDA, ARS Southeastern Fruit and Tree Nut Research Laboratory, Byron, Georgia.

In March 2003, one month after seedling survival was evident, the following nematode treatments were added per microplot: i) 2,500 *M. incognita* eggs (Mi); ii) 2,500 *P. vulnus* adults and juveniles (Pv); iii) 2,500 *P. vulnus* adults and juveniles + 2,500 *M. incognita* eggs (Pv + Mi); and iv) a nontreated control. The initial population density of 2,500 *M. incognita* or 2,500 *P. vulnus* per microplot is equivalent to 17 *M. incognita* eggs/100  $\text{cm}^3$  soil or 17 *P. vulnus* juveniles or adults/100  $\text{cm}^3$  soil, respectively. The soil in each microplot was infested with the respective nematode inoculum in 40 ml total solution added to two furrows (10 cm long  $\times$  3 cm wide  $\times$  7 cm deep) around each seedling. The experiment consisted of a 2  $\times$  2 factorial with single tree replications per treatment

arranged as 10 randomized complete blocks. Tree-trunk diameters were measured 7.5 cm above the soil line in March 2004 and 2005. Plants were watered as needed and fertilized with Osmocote [14-14-14 (N-P-K)]. The study was terminated approximately 26 months (May 2005) after soil infestation and nematode population densities in roots and soil were quantified. Nematode population density in soil was determined from five cores (2.5-cm-diam  $\times$  23-cm deep) that were collected from each microplot. Nematodes were counted following extraction from a 100- $\text{cm}^3$  subsample with a semi-automatic elutriator (Byrd *et al.*, 1976) and centrifugal-flotation (Jenkins, 1964). *Pratylenchus vulnus* in roots were extracted by randomly cutting a 5 gram fresh weight part of the root system and placing it on a fine screen in a Seinhorst mistifier chamber (Hopper, 1970) for 9 days at 23°C. After extracting the nematodes from the roots, the dry root weight (dried at 70°C in aluminum foil until no more loss in weight occurred) of each tissue extraction sample was determined. *Meloidogyne incognita* eggs in roots were estimated by randomly cutting a 5-gram fresh weight part of the root system and extracting eggs with a NaOCl solution as mentioned above. After extracting the eggs from the roots, the dry root weight of each tissue extraction sample was determined. The remaining root systems were dried on greenhouse benches to a constant weight and then combined with the tissue extraction sample weights for total dry weight.

#### *Statistical analysis*

All data were subjected to a general linear model procedure of SAS (SAS Institute, Cary, NC). An analysis of variance was performed on the final soil population density (Pf) of *P. vulnus* in the two treatments that initially received *P. vulnus* and *P. vulnus* +

*M. incognita*. A similar analysis was also performed on the Pf density of *M. incognita*. Nematode data were transformed to  $\log_{10}(x + 1)$  values and only non-transformed means are reported in tables. Additionally, an ANOVA using a factorial design was performed to determine main nematode effects and interactions for trunk diameter and dry root weight. Only significant differences ( $P \leq 0.05$ ) will be discussed unless stated otherwise.

## RESULTS AND DISCUSSION

The presence of *P. vulnus* contributed to the suppression ( $P \leq 0.05$ ) in population density of *M. incognita* second-stage juveniles (J2) on Lovell peach 26 months after inoculation (Table 1), but did not affect the reproduction potential of *M. incognita* in peach as measured by number of eggs per plant or number of eggs per gram dry root. In contrast, the presence of *M. incognita* did not detectably

affect the population density of *P. vulnus* in soil and/or roots.

Three explanations for the suppression in nematode reproduction by one nematode species on another may be attributed to i) a reduction or alteration of suitable feeding sites on the root; ii) other factors than availability of feeding sites (e.g., plant growth regulators), or iii) environmental factors (e.g., soil temperature). Nematode feeding sites on roots differ between a sedentary endoparasite, such as the root-knot nematode, and a migratory endoparasite, such as the root-lesion nematode. *Meloidogyne* spp. penetrate at the root tip, establish themselves, and feed within the vascular cylinder region for the remainder of their life cycle (de Guiran and Ritter, 1979). In contrast, the root-lesion nematode feeds in the root cortex, moving through and between the parenchyma cells which result in visible necrotic lesions (Castillo and Vovlas, 2007). The visible necrotic lesions are believed to result from

Table 1. Population densities and/or reproduction of *Meloidogyne incognita* and *Pratylenchus vulnus* (all vermiform stages) alone and combined on Lovell peach in field microplots 26 months after soil infestation.

Treatment*	Nematode		
	<i>M. incognita</i>		
	J2/100 cm <sup>3</sup> soil	Eggs/plant	Eggs/g dry root
<i>M. incognita</i> (Mi)	128 a <sup>z</sup>	88 ns	33 ns
Mi + Pv	20 b	80	74
	<i>P. vulnus</i>		
	Nematodes/100 cm <sup>3</sup> soil	Soil + roots	Nematodes/g dry root
<i>P. vulnus</i> (Pv)	4,269 ns <sup>z</sup>	4,456 ns	91 ns
Mi + Pv	3,312	3,517	175

Data are means of 10 replications, except for *M. incognita* which had four replications.

<sup>z</sup>Initial population density of *M. incognita* = 17 eggs/100 cm<sup>3</sup> soil, *P. vulnus* = 17 juveniles or adults/100cm<sup>3</sup> soil, and Mi + Pv = 17 Mi + 17 Pv/100 cm<sup>3</sup> soil.

<sup>y</sup>Significant at  $P \leq 0.05$ ; ns =  $P > 0.05$  according to ANOVA.

<sup>x</sup>Data were transformed [ $\log_{10}(x + 1)$ ] before analysis and nontransformed data are shown in table.

a combination of both secretion of cell-wall degrading enzymes and mechanical force from the stylet (i.e., pressure from labial region and thrusting) of *Pratylenchus* spp. It seems that, as a result of direct or indirect competition for feeding sites, the more aggressive nematode may influence reproduction of the cohabiting nematode. On soybean and olive, *M. incognita* suppressed reproduction of *P. brachyurus* and *P. vulnus*, respectively (Herman *et al.*, 1988; Lamberti *et al.*, 2001). However, on tomato *P. penetrans* and *M. incognita* mutually suppressed reproduction when they both were present (Estores and Chen, 1972). It appears that the inhibitory effects of *P. penetrans* on *M. incognita* in tomato were due to competition for feeding sites, whereas suppression of *P. penetrans* by *M. incognita* implicated factors other than feeding sites; such as production or translocation of plant-growth regulators in response to parasitism by root-knot nematode. Similarly, *M. chitwoodi* populations were influenced in the presence of *P. neglectus*, but the outcome differed with soil temperature and host (Umesh and Ferris, 1994). The presence of *P. neglectus* suppressed *M. chitwoodi* populations in barley at 15°C, but not 20 or 25°C, whereas in potato *P. neglectus* suppressed *M. chitwoodi* populations at 25°C, but not 15 or 20°C. In the present study, *P. vulnus* suppressed the population density of *M. incognita* J2 in peach and appears to be the more aggressive nematode specie and competitor in this nematode-nematode host-parasite relationship.

Differences in Lovell tree growth as related to nematode treatment were detected 12, 24, and 26 MAI (Table 2). Main nematode treatment effects for above-ground differences (i.e., trunk diameter) indicated that the presence of *M. incognita* reduced ( $P \leq 0.05$ ) mean trunk diameter at 12 and 24 MAI. The presence of *P. vulnus* had no effect on above-ground

tree growth. The interaction between *M. incognita* and *P. vulnus* was also significant ( $P \leq 0.05$ ) for trunk diameter on both sampling dates. At 12 MAI, the combined nematode treatment (Pv + Mi) was less than *P. vulnus* alone, but it was not less than *M. incognita* alone, which resulted in a significant interaction for trunk diameter. At 24 MAI, the combined treatment (Pv + Mi) was greater than *P. vulnus* alone, but again not less than *M. incognita* alone.

Differences in peach root growth (i.e., dry root weight) as related to nematode treatment were detected 26 months after inoculation (Table 2). Main treatment effects indicate that the presence of *M. incognita* or *P. vulnus* reduced ( $P \leq 0.01$ ) root growth (Table 2). The interaction between *M. incognita* and *P. vulnus* was also significant ( $P < 0.01$ ) for dry root weight. Although the combined nematode treatment (Pv + Mi) was less than *P. vulnus* alone, it was not less than *M. incognita* alone, which resulted in a significant interaction for dry root weight. Our results indicate that above-ground tree growth is less with trees growing in the presence of *M. incognita* than *P. vulnus*, even though *P. vulnus* reduced peach root growth.

In summary, there appears to be a greater negative impact on vegetative growth of peach seedlings growing in *M. incognita*-infested soil than in *P. vulnus*-infested soil. However, even though *M. incognita* affected peach tree growth more than *P. vulnus*, both nematodes are still considered economically important pathogens to the peach industry in the southeastern United States and that preplant nematode samples need to be collected and analyzed for their presence prior to orchard establishment. Such a basic and important practice will allow growers to make the proper nematode management decisions so that they can obtain a well-established and profitable orchard.

Table 2. Trunk diameter and dry root weight of Lovell peach seedlings grown in field microplots with *Meloidogyne incognita* and *Pratylenchus vulnus* alone and in combination.

Factors	Trunk diameter (mm)		Dry root weight (g)
	12 MAI <sup>x</sup>	24 MAI	26 MAI
Treatment mean			
Control	13.89	20.16	218.33
<i>P. vulnus</i> (Pv) <sup>y</sup>	11.97	16.36	133.51
<i>M. incognita</i> (Mi) <sup>y</sup>	11.32	13.91	79.98
Pv + Mi <sup>y</sup>	11.91	16.68	108.33
Main effect mean			
Pv -	12.60	18.07	175.76
+	11.94	16.52	120.92
Mi -	12.93	18.26	173.69
+	11.61	15.75	100.23
Significance for:			
Pv (+) vs. Pv (-)	ns <sup>z</sup>	ns	**
Mi (+) vs. Mi (-)	**	*	**
Pv × Mi	*	**	**

Data are means of 10 replications, except for *M. incognita* which had five and four replications at 24 and 26 MAI, respectively.

<sup>x</sup>MAI = Months after inoculation.

<sup>y</sup>Initial population density of *M. incognita* = 17 eggs/100 cm<sup>3</sup> soil, *P. vulnus* = 17 juveniles or adults/100cm<sup>3</sup> soil, and Mi + Pv = 17 Mi + 17 Pv/100 cm<sup>3</sup> soil.

<sup>z</sup>Significant at  $P \leq 0.05$ ; \* =  $P \leq 0.01$ ; ns =  $P > 0.05$  according to ANOVA.

## ACKNOWLEDGEMENTS

The author thanks W.T. Taylor, Jr. for technical assistance.

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Received:

19/VIII/2009

Accepted for publication:

3/XXI/2009

Recibido:

Aceptado para publicacion: