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O ESTÁGIO DA PLANTA E A POPULAÇÃO INICIAL DO NEMATOIDE DAS GALHAS INFLUENCIAM NO DESENVOLVIMENTO DO PIMENTÃO?

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RESUMO

Buscando alternativa de manejo para áreas produtoras de pimentão infestadas com *Meloidogyne enterolobii*, objetivou-se determinar a patogenicidade dessa espécie em três estádios de mudas de pimentão (*Capsicum annuum*) cv. Orazio (um, três e cinco pares de folhas verdadeiras). O experimento foi conduzido em casa de vegetação e cada parcela foi constituída de uma planta por vaso contendo 3,8 L de substrato. Os tratamentos foram compostos de cinco níveis populacionais (Pi): 0, 300, 1.000, 3.000 e 10.000 ovos e juvenis de *M. enterolobii* para cada tamanho de muda, com delineamento inteiramente casualizado e quatro repetições. A avaliação foi realizada após 60 dias, considerando o desenvolvimento da parte aérea das plantas, peso seco da parte aérea, população final de nematoides e fator de reprodução. Os resultados foram submetidos ao modelo não linear de Seinhorst: $Y = m + (1\text{-}m).\ Z^{Pi - T}$. O limite de tolerância foi de 2.500 nematoides para mudas com um e três pares de folhas e de 8.500 nematoides para a muda com cinco pares de folhas. De acordo com o valor m (peso seco da parte aérea mínimo) obtido na equação, os valores foram de 0,445, 0,809 e 0,965, respectivamente. Portanto, a muda com cinco pares de folhas, denominada de ‘mudão’, mostrou-se mais tolerante, podendo ser considerada no manejo de áreas infestadas com *M. enterolobii*.

Palavras-chave: *Capsicum annuum*. Densidades populacionais. *Meloidogyne enterolobii*. Patogenicidade.

DO THE STAGE OF PLANT AND INITIAL POPULATION OF ROOT KNOT NEMATODE INFLUENCE ON SWEET PEPPER DEVELOPMENT?

ABSTRACT

The aim of this study was to investigate the response of three different stages (one, three and five leaf pairs) of sweet pepper (*Capsicum annuum* cv. Orazio) seedlings to five inoculation levels of *Meloidogyne enterolobii* (zero (control), 300, 1000, 3000 and 10000) under greenhouse conditions. Each plant was cultivated in one pot filled with 3.8 L of substrate. The test was a completely randomized design with four replications. The plants were assessed 60 days after inoculation, plant shoot weight, final population of nematodes, and reproduction factor were measured. The results were fitted to Seinhorst model: Y = m + (1-m). $Z^{Pi\,-T}$. The results showed a tolerance limit (T) of 2,500 nematodes for plants with one and three leaf pair, and 8,500 nematodes for the five-leaf pair plant. The aerial part dry weight minimal, obtained under high population densities of the nematode, was 0.445, 0.809 and 0.965 for the one, three and five leaf pair respectively. The plants with five leaf pair demonstrated a higher tolerance limit to the nematode infection and could be considered in the management in areas infected with *M. enterolobii.*

Keywords*: Capsicum annuum*. *Meloidogyne enterolobii*. Pathogenicity. Population densities

The sweet pepper, *Capsicum annuum* L, is one of the ten most consumed vegetables in Brazil. It is the third most cultivated Solanacea species in the country, with production concentrated in the southeast region. Sweet pepper plants, as all cultivated plants, are subject to several diseases, including those caused by fungi, bacteria, viruses and nematodes (MOURA et al., 2012).

Nematodes of the genus *Meloidogyne*, known as root knot nematodes, are considered the most important plant parasitic nematodes due to the severity of damage they cause on infected plants. *M. enterolobii* has been associated with several phytosanitary problems in Brazil, including problems in sweet pepper. This species was first reported with severe damages in guava trees (*Psidium guajava* L.) in Pernambuco and Bahia states (CARNEIRO et al., 2001).

The presence of *M. enterolobii* in sweet pepper plants was reported in 2006 when 'Silver' sweet pepper rootstock and tomatoes 'Andreia' and 'Debora', both considered resistant to several crucial *Meloidogyne* species (*M. javanica*, *M. incognita* and *M. arenaria*), showed galling symptoms and developmental problems (CARNEIRO et al., 2006). According to Oliveira (2007), *Capsicum frutescens* is the only *Capsicum* considered resistant to *M. enterolobii*. However, this species was incompatible for grafting on susceptible sweet pepper cultivars.

It is known that the initial nematode population influences damage on plants where, in general, the higher the infestation level is the more severe the damage (JAITEH et al., 2012). Thus, the initial population of root knot nematodes is negatively correlated with the vegetative growth of tomato, pepper and beet (MEKETE et al., 2003).

Temperature, nutritional factors, soil, pH and plant age are important features that may affect the expression of resistance in nematode-infected plants (BRODIE; DUKES, 1972). Root knot nematodes prefer to infect young plant roots, and, for this reason, the host plant age could be considered an important factor for nematode infection and pathogenicity (DROPKIN, 1955). Older transplants sustain less damage by nematode than younger ones do, in these cases the damage decreases to a point where it is not detrimental to its development (BRODIE; COOPER, 1964). Susceptibility of tomato to *Meloidogyne* spp. is known to be reduced with the increase of the age and consequently increase of the roots of the plant, since, with the increase of the amount of the host tissues, the damage decrease (BERGESON, 1968; BRODIE; DUKES, 1972).

In the absence of resistant or tolerant cultivars to *M. enterolobii*, alternative controls deserve attention, such as the usage of developed plants to withstand pathogens. The objective of this study was to determine the importance of sweet pepper plant size on tolerance to different initial population densities of *M. enterolobii*.

Sweet pepper seedlings cv. Orázio were germinated in 3.8 L pots with sterilized substrate and cultivated in a greenhouse at the Laboratory of Nematology of the Experimental Center of the Instituto Biológico, Campinas (SP). The plants were sown in different times so we could work with three different stages of sweet pepper: Seedling one: plants with one pair of true leaves; Seedling three: plants with three pairs of true leaves and Seedling five: plants with five pairs of true leaves (Figure 1). The *M. enterolobii* inoculum was composed of eggs and second stage juveniles retrieved from tomato roots, from Bragança Paulista, SP. The species identification was confirmed by esterase electrophoresis procedure according to Oliveira et al. (2012). The nematode extraction was performed according with Coolen and D'Herde (1972) methods.

The plants inoculation was made by deposition of nematode suspension (eggs and juveniles) in holes made in the substrate around the plants. The inoculum concentration varied to obtain the initial population levels (Pi) according to the five pre-established treatments (0, 300, 1000, 3000 and 10000 nematodes/plant). The experiment was performed in a completely randomized design with four replicates for each seedling stage.

After 60 days, the aerial parts of the plants were removed, stored in paper bags and dried in an oven (60º C) to obtain the aerial part dry weight (APDW). The root system of each plant was washed, and the nematodes were extracted by the Coolen and D'Herde (1972) method.

The population estimation was obtained by counting the nematodes in Peters slides under a light microscope, quantifying the eggs and juveniles of *M. enterolobii*. The final population (FP) corresponds to the number of nematodes extracted from the entire roots of each plant. The reproduction factor (RF) was determined according to Oostenbrink (1966).

The APDW data was analyzed according to the nonlinear model proposed by Seinhorst (1965) based on the equation: $Y = m + (1-m) .Z^{Pi-T}$; in which, Y is the ratio of the estimated variable for plant growth in an initial population density of the nematode (PI) divided by the value obtained in the control plant; m is the minimum yield of the plant obtained under high population densities of the nematode; Z is a constant smaller than 1 ($Z < 1$); Pi is the initial population density of the nematode, and T is the tolerance limit. To perform the Seinhorst analysis we used the SeinFit software, DOS version, developed by Viaene et al. (1997).

The reproductive factor (RF= FP/Pi) for each inoculum density tested in different stages of cv. Orazio seedlings are presented in Table 1. With the increase of nematode density there was a reduction in RF. For young seedlings (seedling 1), the value of RF at the lowest density (300 nematodes) was 244.8, while at the highest nematode density (10000) this value was 7.8; for the seedling 3, RF was 122.8 at lowest density and 8.9 at highest; for seedlings 5, RF was 59.7 with Pi of 300 nematodes and 3.1 with Pi of 10000 nematodes.

In general, seedlings with five pairs of true leaves (seedling five) had lower RF at all inoculum densities when compared to younger seedlings with one and three true leaf pairs. Thus, younger plants were more susceptible comparing to older plants, and the higher Pi density is the lower the multiplication rate is.

The results obtained with the Seinhorst nonlinear model (Figure 2), showed that plants with one pair of true leaves (seedling one), adjusted the equation as follow: $Y = 0.445 + (0.555).1^{Pi-2500}$. The value found for T was 2500, therefore, plants began to suffer damage caused by nematodes at this population density. There was also a reduction of 55.5% of the APDW, demonstrating the severity of damages caused by *M. enterolobii* in sweet pepper development.

For seedlings with three pairs of true leaves (seedling three), the equation was as follow: $Y = 0.809 + (0.191).1^{Pi-2500}$, where the tolerance limit, T, was the same as observed in seedlings 1, T = 2500, but the m value was 0.809, indicating that plant growth was only reduced by an average of 19.1% in response to nematode infection.

For higher plants (seedling five) the equation that best fit the relationship between APDW and population density of *M. enterolobii* was $Y = 0.965 + (0.035).0.993^{Pi-8500}$, demonstrating that the older seedlings were the more tolerant to nematode attack, since the T value was 8500, which means that a high infestation is necessary for the plant to start showing damage caused by *M. enterolobii*. Analyzing the APDW in relation to the control plant, the infected plants showed an average reduction of only 3.5% of their weight, which did not seem to compromise the development of the plant.

Considering the severe damages caused by *M. enterolobii* on sweet pepper plants and the difficulty of its management, different cultivation options are being adopted by producers from São Paulo State in order to minimize losses of plant production. One of the alternatives is the use of greater sweet pepper seedlings, with five pairs of leaves (Ag. Eng. Junior Basseto, personal communication). However, to date there was no study linking the age of these plants with the damage caused by the root knot nematode.

The results of the present study showed that the oldest sweet pepper seedlings (seedling five) were more tolerant to *M. enterolobii* attack, compared to younger plants. Seedlings five demonstrated a tolerance limit of 8500 nematodes, while in younger seedlings (seedlings one and three) the tolerance limit was 2500 nematodes per plant. Older plants withstood nematode attack better than younger plants (Figure 3). In a similar study, Huang and Ploeg (2001) observed reductions of 81-85% in carrot and lettuce growth when plants were inoculated at an early stage.

The effect of plant age on nematode tolerance has been observed in several crops. Fernandez et al. (1995) studied the influence of the age of eight *Prunus* grafts on resistance to *M. incognita*. Three studied plants [GF 677 (*P. persica* x *P. dulcis*), Montclar (*P. persica*) and Barrier (*P. persica* x *P. davidiana*)], inoculated at 12-month age, showed lower number of galls and nematodes when compared to plants inoculated at two months old. It was also observed that Barrier grafts presented moderate resistance to *M. incognita* at two months old, and that the same plant variety showed signs of resistant at 12 months old, suggesting that the plant age may affect the resistance to *Meloidogyne* spp. in some species of *Prunus*. A similar result was described by Canals et al. (1992), in which *M. javanica*-resistant G x N No.1 (*P. persica* x *P. amygadalus*) grafts were susceptible at 30 days of age and highly resistant at 13 months of age. Apparently, this type of resistance also requires physiological maturation of the plant before resistance is expressed against the nematode. In the case of sweet pepper, a decrease in nematode number and in its reproduction rate was shown in plants inoculated at a higher stage of vegetative development.

Khan et al. (2000) studied the effect of *M. incognita* population densities (2000, 4000 and 6000) on different ages of tomato plants (three, four and five weeks). They observed that, with the increase of initial population of the nematode, there was a significant reduction of plant height, number of leaves, fresh and dry weight of leaves and stems and roots. It was also observed an increase in the number of galls and egg masses. Less damage was detected in seedlings that were 5 weeks old compared to plants that were 3 and 4 weeks old.

Huang and Ploeg (2001) studied the effect of different initial densities of *Longidorus africanus* in lettuce and carrot seedlings at different inoculation times. Through the analysis performed by the Seinhorst model, the authors observed that the development of plants was affected in all studied plant ages. However, the minimum yield increased significantly in plants inoculated ten days after sowing when comparing to direct inoculation on seeds.

Several studies reported that nematode tolerance is directly related to plant age. For example, studies with *M. hapla* in alfalfa by Griffin and Hunt (1972), *M. incognita* in *Prunus* spp. (FERNANDEZ et al., 1995), *M. incognita* in melon (PLOEG; PHILLIPS, 2001), *M. javanica* in lettuce (SANTOS et al., 2006), *M. arenaria* in peanuts (DONG et al., 2007), *M. incognita* in cardamom (EAPEN, 1992) and *M. graminicola* in rice (Pokharel et al., 2012). In all the above studies, there was an observed decrease in the severity of damage to plants at a later stage of development when compared to younger plants inoculated with the same density of nematodes, which correlates with the results obtained in the present study.

The results obtained in this study support the observations made by growers in Sao Paulo state. Older (5 leave pairs) sweet pepper plants demonstrate a higher tolerance limit to *M. enterolobii.* It is not known if even older plants would have a better tolerance limit, but considering the growers needs we believe that 5 leave pairs is the best option due to cultivation practices and production.

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Table 1. Aerial part dry weight (APDW), final nematode population (FP) and reproduction factor (RF) of *Meloidogyne enterolobii* on sweet pepper cv. Orazio seedlings in different ages.

Treatment	Initial population (Pi)	APDW	FP	RF
Seedling 1	0	11.1	0	-
(one pair of true leaves)	300	12.3	73,450	244.8
	1000	12.5	135,150	135.1
	3000	11.3	40,300	13.4
	10000	6.6	77,925	7.8
Seedling 3	0	10.5	0	-
(three pairs of true leaves)	300	8.6	36,837	122.8
	1000	8.9	113,675	113.7
	3000	8.4	111,975	37.3
	10000	7.1	88,750	8.9
Seedling 5	0	10.7	0	-
(five pairs of true leaves)	300	11.5	17,900	59.7
	1000	10.4	21,012	21.0
	3000	12.1	50,850	16.9
	10000	11.8	30,575	3.1

Figure 1. Sweet pepper seedlings cv. Orazio with one (A), three (B) and five (C) pairs of real leaves.

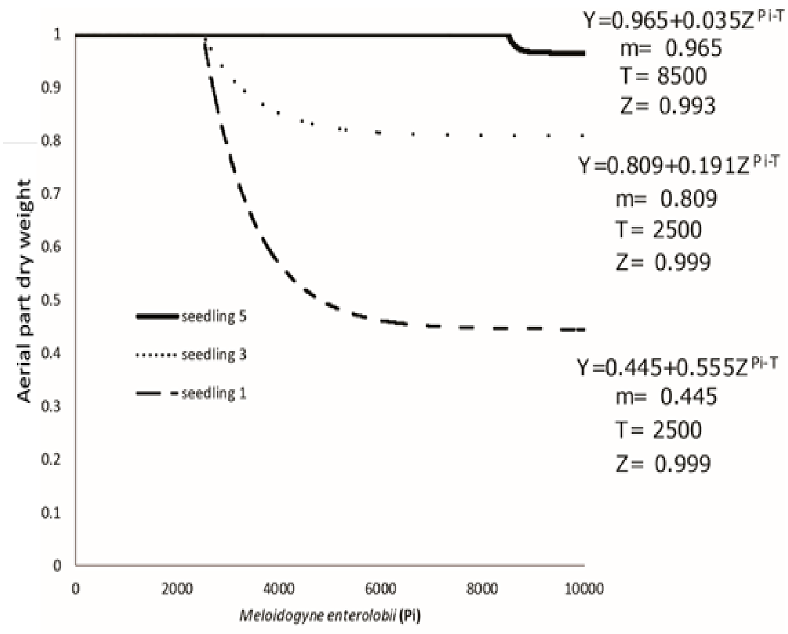

Figure 2. Relationship between *Meloidogyne enterolobii* initial population (Pi) and the top dry weight of sweet pepper plants, 60 days after inoculation. In accordance with Seinhorst model, m is the minimum relative top dry weight; T is the tolerance limit of the crop to the nematode; and Z is a constant < 1.

Figure 3. Symptoms of parasitism in different population densities of *Meloidogyne enterolobii* in sweet pepper seedlings cv. Orazio with one (A), three (B) and five (C) pairs of real leaves, 60 days after inoculation.

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