# Interference of *Meloidogyne javanica* in the reproduction of *Pratylenchus brachyurus* in soybean cultivar BRS/MT pintado

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## **ABSTRACT**

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Root-knot nematodes, *Meloidogyne* spp., and the root-lesion nematode, *Pratylenchus brachyurus*, are the most common phytoparasites in soybean crop, which usually occur in association in crop areas. However, few studies were conducted on the interaction between these parasites. Therefore, this study aimed to assess the interference of the increase in *M. javanica* population in the reproduction of *P. brachyurus*, and the effect of mixed populations on soybean (cultivar BRS/MT Pintado) development. The experiment consisted in inoculating a population of 1000 specimens of *P. brachyurus* per plant

and changing the inoculum level of *M. javanica*, using zero, 1000, 2000 and 4000 eggs, in two different periods. After 65 days of inoculation, the increase in the initial population of *M. javanica* reduced by 79 and 73% the final population of *P. brachyurus* in the different experiments. However, both species significantly increased their populations. When subjected to the same inoculum level, *M. javanica* was more efficient in reproducing than *P. brachyurus*. The increase in the number of nematodes reduced the vegetative growth of the plant.

Keywords: Glycine max, interactions, root-knot nematodes, root-lesion nematodes, mixed populations

## **RESUMO**

Fontana, L.F.; Arieira, c.r.d.; Abe1, V.H.F.; Severino, J.J.; Arieira, J.O.; Monteiro, R.N.F. Interferência de *Meloidogyne javanica* na reprodução de *Pratylenchus brachyurus* em soja cultivar BRS/MT pintado. *Summa Phytopathologica*, v.44, n.2, p.143-147, 2018.

Os nematoides das galhas, *Meloidogyne* spp., e o nematoide das lesões radiculares, *Pratylenchus brachyurus*, são os fitoparasitas mais comuns na cultura da soja e comumente ocorrem associados nas áreas de cultivo. Porém, poucos são os estudos da interação existente entre eles. Assim, objetivou-se avaliar a interferência do aumento da população de *M. javanica* sobre a reprodução de *P. brachyurus*, e o efeito das populações mistas no desenvolvimento da soja cv. BRS/MT Pintado. O experimento consistiu em inocular uma população de 1000 espécimes de *P. brachyurus* por planta e variar

o nível de inóculo de *M. javanica*, usando zero, 1000, 2000 e 4000 ovos, e foi conduzido em duas épocas distintas. Após 65 dias da inoculação, observou-se que o aumento na população inicial de *M. javanica* reduziu em 79 e 73 % a população final de *P. brachyurus*, nos diferentes experimentos. Porém, ambas as espécies aumentaram significativamente as populações. Quando submetidos ao mesmo nível de inóculo, *M. javanica* foi mais eficiente em reproduzir-se do que *P. brachyurus*. O aumento no número de nematoides reduziu o desenvolvimento vegetativo da planta.

Palavras-chave: Glycine max, Interações, Nematoides das galhas, Nematoides das lesões radiculares, Populações mistas

Soybean is the most important oilseed crop in the world and has increased in percentage more than the major grain crops, particularly because its market has obtained a solid structure worldwide, the plant has been recognized as an important source of vegetable protein and technology has been generated, allowing the crop expansion to different regions of the world (17).

Although soybean planted areas and productivity have continuously grown, maximum yield potential (4000 kg/ha) is hard to obtain in most countries. The main factors that limit the yield, the profitability and the success of soybean production are diseases, including nematodes which cause significant losses to the Brazilian production, varying from slight to complete (100%) (2, 11).

Species of *Meloidogyne*, particularly *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid and White) Chitwood, represent a serious problem in soybean producing regions (18). Data on specific

yield losses caused by these two species are common and vary among the different crop areas or regions (1), reaching up to 40% in low fertility sandy soils.

Another nematode that has a significant impact on Brazilian soybean production is *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven. It is an aggressive species that heavily infest soybean producing areas (5, 18). Crop losses caused by this nematode are estimated to range from 10 to 30% of the production potential, especially in areas showing sandy soil and irregular rainfall distribution (5). Nevertheless, declines of up to 50% in soybean production have already been recorded for commercial crops infested by *P. brachyurus* in the central-west region of Brazil (14), and losses are greater when the infection is associated with other important nematode species (18).

The above-mentioned species commonly occur concomitantly in annual crops, which was already reported in Paraná (25), in cities of



Minas Gerais and Goiás (20, 24) and in the states of Mato Grosso and Mato Grosso do Sul (2, 26). Although few studies were conducted on the interaction of these species, *Meloidogyne* spp. have been reported to show competitive advantage over *P. brachyurus* in soybean (10, 16). On the other hand, the intense initial attack by *Pratylenchus* followed by massive root destruction is believed to represent a serious problem to the growth of *Meloidogyne* population (23). This variation occurs because competitive advantage is influenced by different factors, including host susceptibility, host cultivars and nematode population (16, 23). Thus, the present study aimed to assess the influence of the growing population of *M. javanica* on the reproduction of *P. brachyurus*, as well as the effect on soybean cultivar BRS/MT Pintado.

#### MATERIAL AND METHODS

The experiments were conducted in a greenhouse, in the Experimental Farm of "Universidade Estadual de Maringá", Umuarama Regional Campus, Paraná, in completely randomized design, with four treatments and five replicates each, in two different periods: from May to July 2013 (Experiment 1), and from September to November 2013 (Experiment 2).

Soybean seedlings of cultivar BRS/MT Pintado were produced in polystyrene trays and, when the phenological stage V1 was reached, they were transplanted to 2-L plastic pots containing a mixture of soil:sand (2:1) sterilized through a cycle of two hours at 120°C.

Two days after transplanting, the soybean was inoculated with an initial population of 1000 specimens of *P. brachyurus* per plant and with a variable inoculum level of *M. javanica*, using zero, 1000, 2000 and 4000 eggs per plant. The nematodes were obtained from pure populations of *P. brachyurus* and *M. javanica* multiplied in soybean and tomato plants, respectively, in plastic pots kept in a greenhouse for two months. Nematodes were extracted according to the methodology proposed by Hussey and Barker, adapted by Boneti & Ferraz (3).

Sixty-five days after inoculation, soybean plants were collected by separating the roots and the shoot. The root systems were washed and placed on absorbent paper to remove excess water; then, they were weighed and the root fresh weight was determined. Subsequently, the nematodes were extracted from the root system according to the cited methodology.

Nematode suspensions were maintained at 27°C for five days to

allow the hatching of juveniles. Then, samples were examined under an optical microscope at 100 X magnification, and the total number of specimens of *M. javanica* and *P. brachyurus* was counted separately. This value was divided by the root weight to obtain the number of nematodes per gram of root (nematode/g root). The shoot was evaluated for the plant height. Measurements were also taken to obtain shoot fresh and dry weight; the latter was determined after drying in an aircirculation oven for 72 hours at 65°C.

The obtained data were subjected to analysis of variance and means were compared according to regression test at 5% probability, using SISVAR statistical analysis program (12).

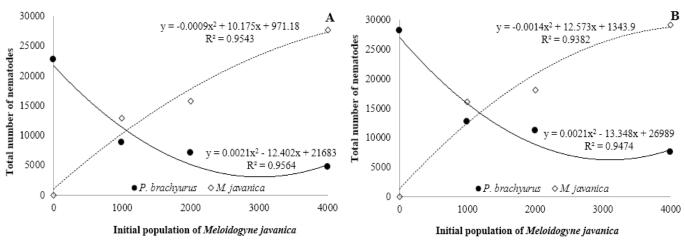
## RESULTS AND DISCUSSION

In both experiments, analysis of variance was significant for nematological and vegetative parameters. In experiment 1, *P. brachyurus* population increased by 2166% when there was no inoculum of *M. javanica* (Figure 1A). On the other hand, inoculating the maximum population of *M. javanica* (4000 eggs), such an increase declined to 369%. The results obtained in experiment 2 confirmed the increase of 2715% in *P. brachyurus* when the initial population of *M. javanica* was zero. However, the increase in *M. javanica* population to 4000 eggs led to an increase of 659% in *P. brachyurus* population (Figure 1B).

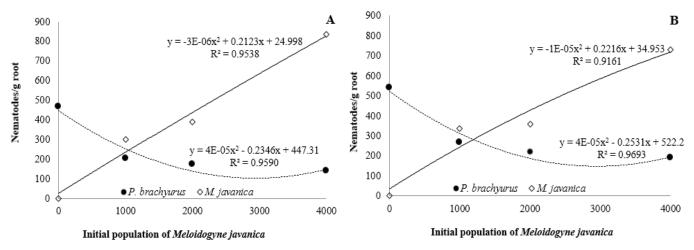
Inoculating both species with the same concentration of nematodes (1000), *M. javanica* population was greater than *P. brachyurus* population, showing increases of 1183 and 771% in experiment 1, for the respective nematodes (Figure 1A), and 1506 and 1168% in experiment 2 (Figure 1B).

The results obtained for the parameter number of nematodes/g root were similar to those obtained for the total number of nematodes. In experiment 1, when the initial population of *M. javanica* was zero, the number of *P. brachyurus*/g root was equivalent to 465.4, but for an initial population of 4000 eggs of *M. javanica*, the number of *P. brachyurus*/g root was 141.6, which is equivalent to a reduction of 69.0% (Figure 2A). Likewise, in experiment 2, the increase in the initial population of *M. javanica* from zero to 4000 eggs decreased *P. brachyurus* population by 65 % (Figure 2B).

All vegetative parameters reduced following the increase in the initial population of the root-knot nematode. The increase in *M. javanica* 



**Figure 1.** Total number of *M. javanica* and *P. brachyurus* per root system of soybean 65 days after inoculation with 1000 specimens of *P. brachyurus* and *M. javanica* population ranging from zero to 4000 eggs (A: Experiment 1; B: Experiment 2).



**Figure 2.** Nematode/g soybean root 65 days after inoculation with 1000 specimens of *P. brachyurus* and *M. javanica* population ranging from zero to 4000 eggs (A: Experiment 1; B: Experiment 2).

population from zero to 4000 eggs reduced the soybean height by 38.0 and 41.0% in experiments 1 and 2, respectively (Figure 3A). Similar results were obtained for root weight (g): reductions of 31.8 and 23.9% were observed after the increase in *M. javanica* population from zero to 4000 eggs in experiments 1 and 2, respectively (Figure 3B).

Decreases in the shoot fresh weight were 48.0 and 38.0% (experiments 1 and 2, respectively) when *M. javanica* population increased from zero to 4000 eggs (Figure 4A). The results obtained for shoot dry weight were also similar to those of the other vegetative parameters, showing decreases of 58.0 and 54.0% in experiments 1 and 2, respectively (Figure 4B).

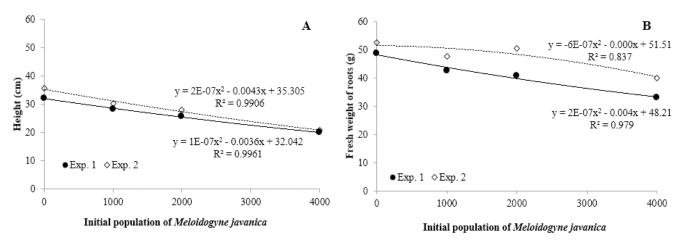
The present study demonstrated that the increase in the initial population of *M. javanica* dramatically reduced *P. brachyurus* reproduction in soybean cv. BRS/MT Pintado. Nevertheless, both species managed to parasitize the crop and significantly increased their populations 65 days after inoculation.

Inoculation of 4000 eggs of *M. javanica* reduced by 79 and 73% (experiments 1 and 2, respectively) the total number of *P. brachyurus*, compared to the treatment that did not involve inoculation of *M. javanica*. Similarly, the number of *P. brachyurus*/g root reduced by 69 and 65%, respectively. These results corroborate the study carried out by Herman et al. (16), who assessed the effects of separate or combined

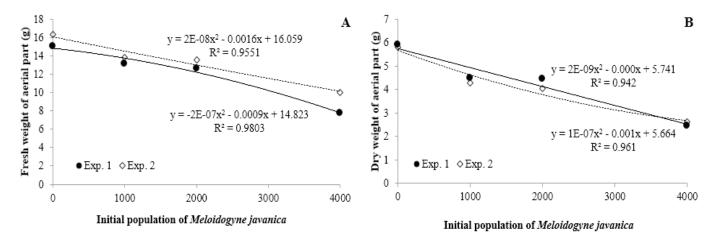
inoculations of *M. javanica* and *P. brachyurus* at growing population levels in soybean varieties and found that, in the interaction, *M. javanica* reduced by 80 to 85% the reproductive potential of *P. brachyurus*, while the reduction observed for *M. javanica* was only 30%. Another study involving the same pathosystem showed that both species parasitized soybean, but the nematodes, especially *P. brachyurus*, were negatively affected by the competition, compared to simple infestations (9).

The negative effect of the genus *Meloidogyne* on *Pratylenchus* was investigated and demonstrated in other crops. For example, in alfalfa and red clover subjected to two treatments: 1) simultaneous inoculation of *M. incognita* and *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven, and 2) inoculation of *M. incognita* six days earlier, *M. incognita* reduced the reproductive potential of *P. penetrans*, regardless of the time of inoculation (4). Similarly, *M. incognita* reduced the penetration and the reproduction of *Pratylenchus coffeae* Zimmermann in mung bean (*Vigna radiata* L.), regardless of the time of inoculation and inoculum level (27). Advantage of sedentary endoparasites over migratory endoparasites was also observed in the reduction of *Belonolaimus longicaudatus* Raup by *Heterodera glycines* Ichinohe and *M. incognita* eleven weeks after inoculation in soybean (6).

Interaction tests involving M. incognita and P. penetrans, using



**Figure 3.** Height (A) and root fresh weight (B) of soybean 65 days after inoculation with 1000 specimens of *P. brachyurus* and *M. javanica* population ranging from zero to 4000 eggs.



**Figure 4.** Shoot fresh (A) and dry weight (B) of soybean 65 days after inoculation with 1000 specimens of *P. brachyurus* and *M. javanica* population ranging from zero to 4000 eggs.

split root of tomato, showed that the inhibition was not associated only with competition for feeding sites, but also with the change in the concentration of plant growth regulators (8). However, this was not observed for other associations involving the genera *Meloidogyne* and *Pratylenchus* (28).

In the present study, *P. brachyurus* was put in an advantageous position as it was inoculated at the infective stages, while *M. javanica* was inoculated in form of eggs. In addition, the root-knot nematode was more competitive than the root-lesion nematode since, with the same initial inoculum level (1000 nematodes), *M. javanica* population was greater. Similarly, inoculation of *P. coffeae* two or four days before *M. incognita* did not result in statistical difference in the penetration of the first (27).

However, competitive advantage of sedentary endoparasites over migratory endoparasites is not a rule, and there are reports of suppression of *M. incognita* and *M. arenaria* (Neal) Chitwood populations in the presence of *P. brachyurus* in cotton plant roots (13). Besides, infection by *M. incognita* has stimulated the penetration of *Hoplolaimus columbus* Sherem soybean cv. Davis (15) and reproduction in cotton plant (19). In a study conducted by Melakerbehan & Dey (21), *P. penetrans* was negatively affected by *H. glycines* only when the inoculum level of the sedentary endoparasite was 75% higher than that of the migratory endoparasite.

According to Santos (23), the predominance of one species during parasitism of *Meloidogyne* and *Pratylenchus* can be considered under different aspects because *Meloidogyne* females produce, on average, four to six times more eggs than *Pratylenchus* females, which is a competitive advantage. Moreover, the eggs of *Meloidogyne* are protected from natural enemies and lack of humidity inside the egg masses secreted by the females, which does not happen with the eggs of *Pratylenchus* deposited on the soil. On the other hand, if the initial infestation of *Pratylenchus* is heavy, there will be extensive root necrosis and, since *Meloidogyne* is a sedentary nematode that needs active feeding sites, parasitism may not progress.

Regarding the vegetative parameters, joint infestation has caused greater reduction in soybean growth than simple infestation by *P. brachyurus*, which is consistent with previous evidence involving the association between *M. incognita* and *P. brachyurus* in soybean (16). However, there are also contrasting findings, as tobacco growth has already been observed to be more severely affected by simple

infestations of *P. brachyurus* than when inoculated with mixed populations of *P. brachyurus* and *M. incognita* (22). The impact of this type of infection on plant growth has been hypothesized to be generally negative, though not always resulting in greater damage than in isolated infections (7). According to the above-mentioned author, this can be explained because the result depends on the involved nematode species, on the host susceptibility of and on the initial nematode population, leading to significant differences in results in the literature.

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