COMPETITION BETWEEN *PRATYLENCHUS ZEAE* AND *MELOIDOGYNE INCOGNITA* ON SUGARCANE

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ABSTRACT

Fontana, L. F., C. R. Dias-Arieira, D. Mattei, J. J. Severino, F. Biela, and J. O. Arieira. 2015. Competition between *Pratylenchus zeae* and *Meloidogyne incognita* on sugarcane. Nematropica 45:1-8.

Mixed populations of nematodes involving sedentary endoparasites (*Meloidogyne* spp.) and the migratory endoparasite *Pratylenchus zeae* are frequent in sugarcane production areas, but details about the competition between these nematodes is not well known. This study aimed to evaluate the competition between *M. incognita* and *P. zeae*, as well as the effect of mixed populations on sugarcane. The study was divided into two experiments with four treatments. One experiment used an initial population of 1,000 *P. zeae* per plant and varied *M. incognita* inoculum levels from 0 to 4,000 eggs per plant. The other experiment consisted of an initial population density of 2,000 eggs of *M. incognita* and *P. zeae* levels ranging from 0 to 2,000 nematodes per plant. Ninety days after inoculation the increase in the initial population of one of the species caused a reduction in the reproduction of the other species although both species significantly increased their populations on sugarcane. With the same inoculum level, *P. zeae* showed greater reproductive capacity than *M. incognita*. Sugarcane growth was not affected by the nematodes at the levels studied. This research indicates that *P. zeae* is more competitive than *M. incognita* on sugarcane.

Key words: interactions, mixed populations, reproductive capacity, root lesion nematodes, root-knot nematodes, *Saccharum officinarum*.

RESUMO

Fontana, L. F., C. R. Dias-Arieira, D. Mattei, J. J. Severino, F. Biela, e J. O. Arieira. 2015. Competição entre *Pratylenchus zeae* e *Meloidogyne incognita* em cana-de-acúcar. Nematropica 45:1-8.

Populações mistas de nematoides envolvendo endoparasitos sedentários do gênero *Meloidogyne* e o endoparasita migrador *Pratylenchus zeae* são frequentes em áreas de produção de cana-de-açúcar, mas detalhes da interação entre estes nematoides são pouco conhecidos. Assim, o trabalho teve como objetivo avaliar a competição entre *M. incognita* e *P. zeae*, bem como o efeito das populações mistas sobre a cultura da cana-de-açúcar. O trabalho foi dividido em dois experimentos, com quatro tratamentos e cinco repetições. Um dos experimentos consistiu em fixar a população inicial de *P. zeae* em 1000 espécimes por planta e variar o nível de inóculo de *M. incognita*, usando de 0 a 4000 ovos. O outro, em fixar a população inicial de *M. incognita* em 2000 ovos e aumentar a população de *P. zeae*, inoculando de 0 a 2000 espécimes. Após 90 dias da inoculação, observou-se que o aumento na população inicial de uma das espécies, sempre ocasionou a redução na reprodução da outra. Contudo, ambas as espécies aumentaram significativamente suas populações. Quando submetidos ao mesmo nível de inóculo, *P. zeae* foi mais eficiente em reproduzir-se do que *M. incognita*. O desenvolvimento vegetativo da cana-de-açúcar não foi afetado pelas infecções mistas ou pelo aumento na concentração do inóculo de um dos nematoides.

Palavras chave: interações, populações mistas, capacidade reprodutiva, nematoides das lesões radiculares, nematoides das galhas, *Saccharum officinarum*.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) production is an expanding crop in Brazil. The area planted with sugarcane in the 2014-2015 season is estimated at 9 million hectares, an increase of 286.6 thousand hectares over the previous season. Production in 2014-2015 is also expected to increase, reaching 659.10 million tons (Conab, 2014).

The Brazilian agro-industry is based on the latest technological advances, and the production of sugarcane is increasing each year to meet market demands. This has led to the planting of new areas in poor and sandy soils. Thus, some plant diseases have become persistent, especially the damage caused by plant-parasitic nematodes (Moura *et al.*, 2000; Dinardo-Miranda, 2005).

In Brazil three species of nematodes are known to be important on sugarcane because of the damage that they cause. These include the sedentary endoparasitic root-knot nematodes [*Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949], and the migratory endoparasitic lesion nematode, *Pratylenchus zeae* Graham, 1951 (Moura *et al.*, 2000; Dinardo-Miranda, 2005; Severino *et al.*, 2010).

Pratylenchus zeae is the most common, although *M. incognita* is thought to be the most aggressive, causing the most severe damage to the crop (Dinardo-Miranda, 2005). The problems caused by nematodes vary according to the size of the population, type of soil, and sugarcane variety that is grown. On average, *M. javanica* and *P. zeae* suppress yield by 20 to 30% in the first harvest in susceptible varieties. *Meloidogyne incognita* may cause higher losses, nearly 40%. For very susceptible varieties and high nematode population densities, the losses caused by nematodes may reach 50% on sugarcane crop. Damage to the ratoon crop must also be included. Although smaller yield losses occur, they are also significant, and may range between 10 and 20 tons per ha per harvest, which drastically reduces ratoon longevity (Dinardo-Miranda and Garcia, 2002).

There are few studies on the population levels of *Meloidogyne* spp. that cause damage to sugarcane. According to Novaretti (1997), population densities of more than 400 juveniles per 50 g of roots justify the use of management strategies. Regarding *P. zeae*, Dinardo-Miranda *et al.* (1996) affirm that 2,500 eggs per 50 g of roots reduce yield in susceptible varieties.

Damage caused to the crop has been enhanced by the occurrence of mixed populations composed of root-knot and lesion nematodes in sugarcane plantations in the states of Paraná (Severino *et al.*, 2010) and São Paulo (Novaretti *et al.*, 1998; Dinardo-Miranda *et al.*, 2003) and in the northeast region of Brazil (Moura *et al.*, 1999; Moura *et al.*, 2000).

Studies indicated that mixed populations of migratory and sedentary endoparasites during plant growth usually has a negative impact, but do not always result in greater damage than that caused by a single species (Ogbuji, 1978; Herman *et al.*, 1988) and competition between nematode species is a factor that explains the results (Ferraz, 1995).

Santos (1996) suggested two possible advantages in the competition involving *Meloidogyne* spp. and *Pratylenchus* spp. The female of *Meloidogyne* produces, on average, four to six times the number of eggs produced by *Pratylenchus* females, which could be a significant advantage. The eggs of *Meloidogyne* are protected from natural enemies and from dessication by the egg mass secreted by the female. This does not occur with the eggs of *Pratylenchus*, which deposits eggs in the soil or plant tissue. On the other hand, in the event of high initial infestation by *Pratylenchus*, many roots will suffer necrosis and *Meloidogyne* will have difficulty in establishing a functional feeding site, which is necessary to complete the life cycle.

This generates the hypothesis that, if in a given crop situation there is an initial predominance of one species over the other, the species with the highest population will tend to prevail. If there is a balance in the population levels, then both species can successfully establish in the host plant, provoke their characteristic symptoms, and cause damage. However, over time, one species may become more prevalent than the other. The present study was aimed to assess the competition between *M. incognita* and *P. zeae*, and to determine the effect of their concomitant populations on reproductive success by both nematodes and the growth of sugarcane.

MATERIALS AND METHODS

Two experiments were conducted in the greenhouse at the experimental farm of the State University of Maringá, Regional Campus of Umuarama, Paraná. The experiments were both arranged in a completely randomized design, and each had four treatments and five replications. In one experiment, the initial population of *P. zeae* was established at 1,000 specimens per plant and the *M. incognita* population was varied to include densities of 0, 1,000, 2,000 and 4,000 eggs per plant. In the second experiment, the initial population of *M. incognita* was 2,000 eggs, and the population of *P. zeae*, was varied from 0, 500, 1,000, and 2,000 vermiform nematodes per plant. These experiments were conducted from October 2012 to January 2013. Both experiments were repeated during the period of February to May, 2013. For the production of sugarcane seedlings, billets of cultivar RB 867515 were cut 7 cm from each side of the bud and planted in pots containing sterilized sand. Twenty days after sprouting, the plants were transplanted to 3-L pots containing sandy soil (81.9% sand; 3.4% silt; 14.7% clay) that had been sterilized in an autoclave for 2 hr at 120°C.

Two days after transplanting, the plants were simultaneously inoculated with the respective treatments. The nematodes were obtained from pure populations of *M. incognita* and *P. zeae*, multiplied on tomato (*Solanum lycopersicum* L.) and maize (*Zea mays* L.), respectively, in pots containing sterilized soil and maintained in the greenhouse for 2 mon. The nematodes used for inoculum were extracted according to the method of Hussey and Barker (1973), adapted by Boneti and Ferraz (1981), in which the roots were triturated and passed through sieves of 100 mesh and 500 mesh. Suspensions were calibrated and inoculated in three 3- to 5-cm-deep open holes dug into the soil around of the plant.

Ninety days after inoculation, the plants were harvested by separating the roots from the tops. The roots were washed and weighed to determine fresh root weight. Subsequently, the nematodes were extracted from the root system as cited earlier. The suspensions obtained were placed on petri dishes and kept at 28ºC for 5 d to allow the eggs to hatch. The samples were examined with an optical microscope and the total number of individuals of each genus was counted. This number was divided by the root weight to calculate the number of nematodes per gram of root.

The top of the plant was measured for the height in meters, from the base of the stem to the largest leaf. Measurements were also made of the fresh weight and dry weight after drying in oven with circulating air for 72 hr at 65°C.

The data obtained were analyzed with analysis of variance and the means were compared by regression at 5% probability using the Sisvar statistical analysis software (Ferreira, 2011).

RESULTS

Analysis of variance of the means was significant ($P = 0.05$) for the following nematological parameters in all the experiments: total number of nematodes and nematodes per gram of root. When the population of *P. zeae* was held constant at established 1,000 individuals and the population of *M. incognita* was increased from 0 to 4,000 eggs, *P. zeae* was suppressed by the competition, in both experiments (Fig. 1A and 1B). In the first experiment (Fig. 1A), when only *P. zeae* was inoculated, the population of this nematode increased 46-fold, 90 d after inoculation. On the other hand, where 4,000 eggs of *M. incognita* were also added, the population of *P. zeae* rate of increase was lower (17.8-fold). When both species were inoculated with the same number of nematodes (1,000), the increase in the population of *P. zeae* was greater than that of *M. incognita* (Fig. 1A).

The results obtained in experiment 2 confirmed competition between nematodes in the parasitizing sugarcane. When the initial population of *M. incognita* was 0, the population of *P. zeae* increased 50.36-fold; but after raising the *M. incognita* initial population to 4,000 eggs per plant, the increase in the *P. zeae* population was considerably lower (Fig. 1B).

Comparisons of the means of the number of nematodes per gram of root were similar to those obtained for the total number of nematodes. With the population of *P. zeae* kept constant, when the initial population of *M. incognita* was 0, the number of *P. zeae* per gram of root was 1,324, but with an initial population of 4,000 *M. incognita*, the number of *P. zeae* reduced to 485 nematodes per gram of root, a decrease of 63% (Fig. 2A). Similarly, in experiment 2, the increase in the initial population of *M. incognita* from 0 to 4,000, led to a 65% decrease in the number of *P. zeae* per gram of root (Fig. 2B).

Competition between nematode genera was also evident where the population of *M. incognita* was kept constant at 2,000 eggs per pot and changing the number of *P. zeae*. In experiment 1, in the treatment where there was zero *P. zeae*, *M. incognita* increased significantly, but when the two genera were combined at 2,000 nematodes per pot, the increase in population of both was considerably lower and roughly equivalent (Fig. 3A). Repeating the experiment (experiment 2) confirmed the results obtained in experiment 1 (Fig. 3B). When the initial population of the two nematode species was 2,000 individuals to *P. zeae* or eggs to *M. incognita*, the population of *P. zeae* out-numbered *M. incognita* by 26%.

With a constant population of *M. incognita* in the absence of *P. zeae*, an average population of 3,714 eggs of *M. incognita* per gram of root was obtained. With the increase of *P. zeae* to 2,000 nematodes, the number of eggs of *M. incognita* was reduced to 653 per gram of root, a decrease of 82% (Fig. 4A). In experiment 2, when the population of *P. zeae* increased from 0 to 2,000, the number of *M. incognita* per gram of root was reduced by 76% (Fig. 4B). The number of *P. zeae* per gram of root was reduced by 63 and 71%, in experiments 1 and 2, respectively, when the population of *M. incognita* increased from 0 to 4,000. The reduction was 82 and 76% for *M. incognita*, when the population of *P. zeae* was increased from 0 to 2,000.

The vegetative growth of sugarcane was not affected by mixed infections or by increasing the

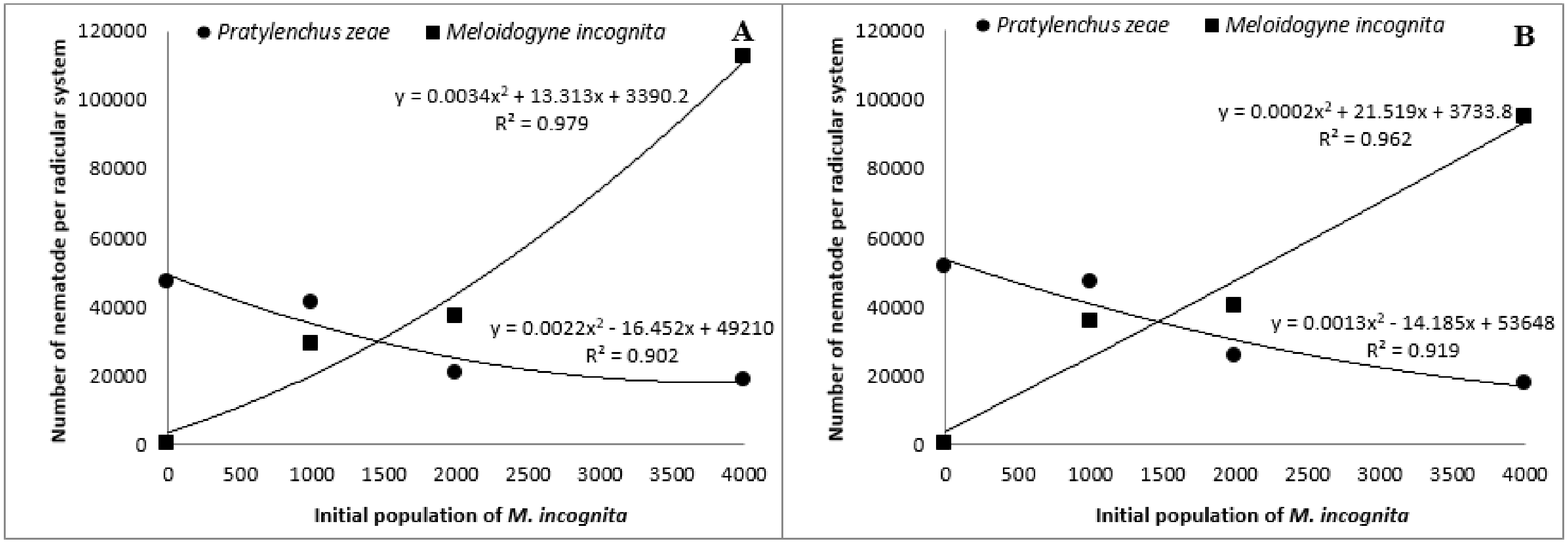

Fig. 1. Total number of *Pratylenchus zeae* and *Meloidogyne incognita* per radicular system of sugarcane 90 d after inoculation with 1,000 *P. zeae* and increasing populations of *M. incognita*. A: Experiment 1; B: Experiment 2.

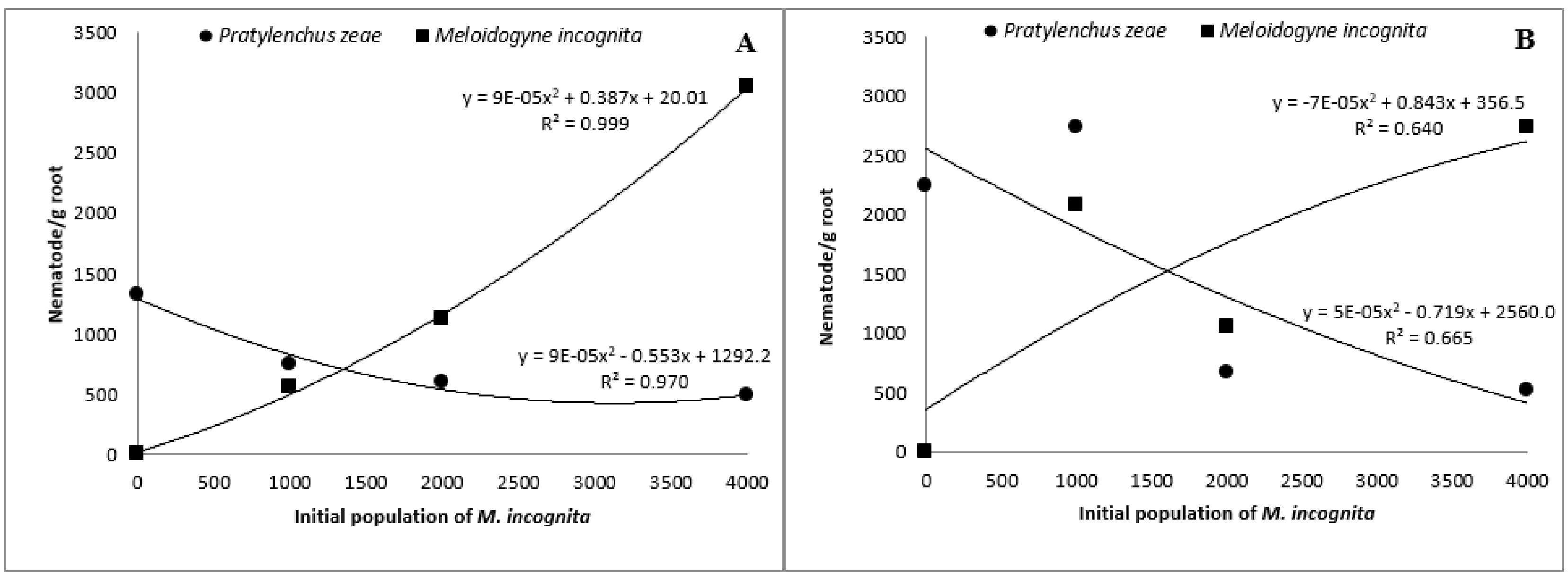

Fig. 2. Number of nematodes per gram of root of sugarcane 90 d after inoculation with 1,000 of *Pratylenchus zeae* and increasing populations of *Meloidogyne incognita*. A: Experiment 1; B: Experiment 2.

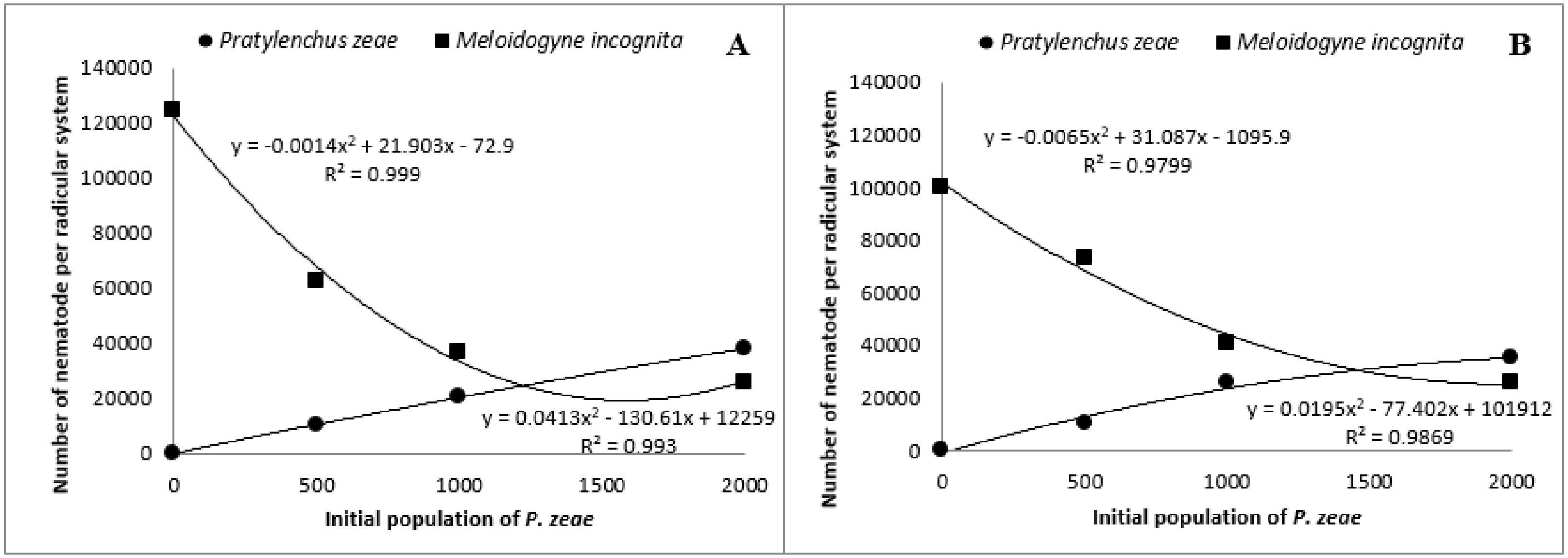

Fig. 3. Total number of *Pratylenchus zeae* and *Meloidogyne incognita* per radicular system of sugarcane 90 d after inoculation with 2,000 *M. incognita* eggs and increasing populations of *P. zeae*. A: Experiment 1; B: Experiment 2.

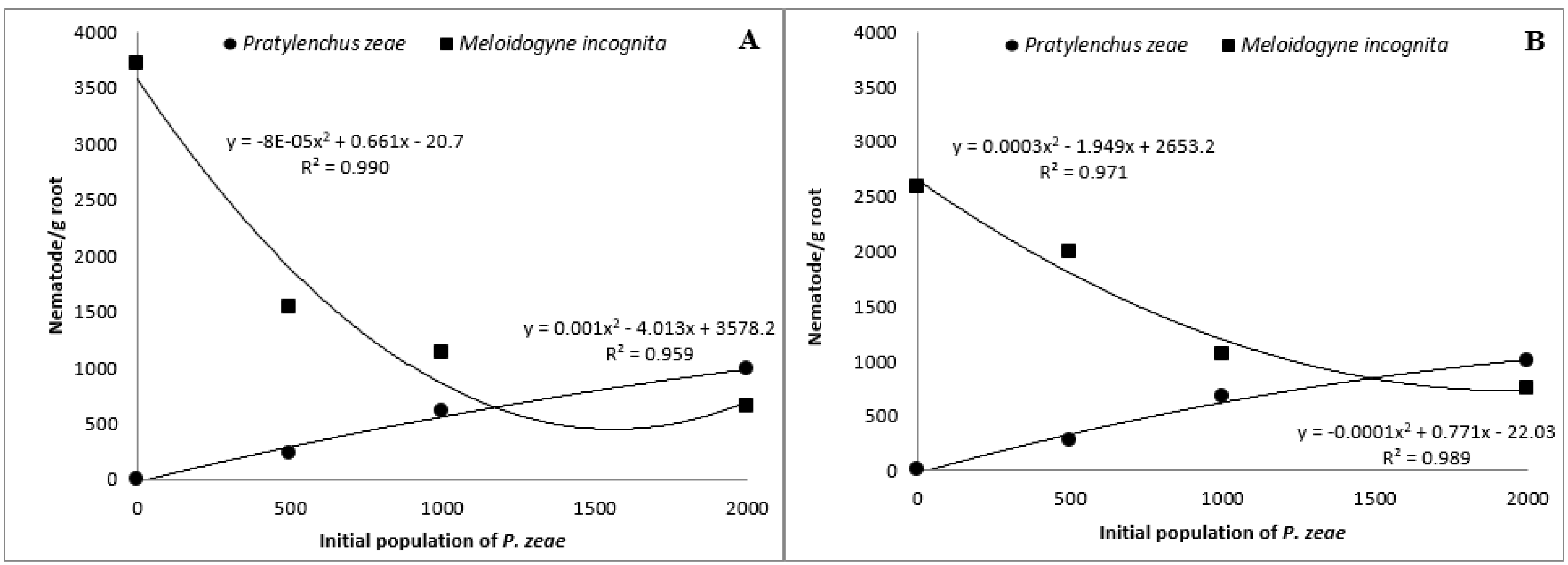

Fig. 4. Number of nematodes per gram of root of sugarcane 90 d after inoculation with 2,000 *Meloidogyne incognita* eggs and increasing populations of *Pratylenchus zeae*. A: Experiment 1; B: Experiment 2.

population densities number of either one of the nematode species when compared with single population.

DISCUSSION

Results suggested that *P. zeae* inhibited *M. incognita* population by greater magnitude than *M. incognita* inhibited *P. zeae*. However, both nematodes inhibited each other and the outcome depended on which nematode was initially present at higher ratio, showing that the species with the highest level of parasitism was the most competitive. When the population of *P. zeae* was kept constant, the suppression of reproduction in the presence of the highest rate of *M. incognita* was 60 and 65%, respectively, in experiments 1 and 2. However, when the initial population of *M. incognita* was kept constant, reproduction of the root-knot nematode was suppressed by 80 and 74%, respectively, in experiments 1 and 2 at the highest rate of *P. zeae*.

These results are consistent with a study realized by Gay and Bird (1973), who reported that populations of *M. incognita* were suppressed by the presence of *Pratylenchus brachyurus* (Godfrey, 1929) and Filipjev and Schuurmans Steckhoven, 1941 in cotton (*Gossypium hirsutum* L.) roots. Recently, Chiamolera *et al.* (2012) found that the population of *M. incognita* was low at the beginning of a soybean and corn crop rotation study, and declined to non-detectable levels after 3 mon, while the population density of *P. brachyurus* increased constantly, but there are no reports of susceptibility of the maize hybrid used to *Meloidogyne* spp. It has been suggested that migratory endoparasites such as *Pratylenchus* spp. penetrate the host quickly and do not allow sedentary species to infect and establish permanent feeding sites efficiently (Gay and Bird, 1973; Chapman and Turner, 1975). In the present work, *P. zeae* inoculum consisted of juveniles and adults, while *M. incognita* consisted of eggs, which may have given a competitive advantage to *P. zeae*.

However, in a study with alfalfa and red clover, Chapman and Turner (1975) found that *M. incognita* suppressed the reproductive capacity of *P. penetrans* both with simultaneous inoculations of the two nematodes and when *M. incognita* preceded *P. penetrans* by 6 d. It is important to note, however, that interactions between sedentary and migratory endoparasitic species are not always negative, and sometimes they can be neutral or stimulating (Griffin, 1983). In studies with tobacco, *M. incognita* increased the reproductive capacity of *P. brachyurus* (Johnson and Nusbaum, 1970), and on *Agrostis palustris* Huds, *Meloidogyne naasi* Franklin, 1965 stimulated the reproduction of *P. penetrans* (Sikora *et al.*, 1972). In red clover 3 mon after inoculation, *Meloidogyne hapla* Chitwood, 1949 did not show any effect on *P. penetrans*; but 5 mon later, *M. hapla* suppressed *P. penetrans* (Amosu and Taylor, 1975). So, interactions vary between migratory and sedentary endoparasites, depending on the nematode species involved, the initial population of the nematode, and the suitability of the host, which may explain different results that have been reported (Eisenback, 1985).

Despite the proven pathogenicity of the nematodes to sugarcane (Sundararaj and Mehta, 1994; Dinardo-Miranda *et al.*, 2004; Barros *et al.*, 2005; Moura and Oliveira, 2009), in the present study, the occurrence of mixed populations did not affect vegetative growth when compared to single nematode inoculations. Similarly, Ogbuji (1978) observed that the growth of tobacco was more severely affected with *P. brachyurus* alone than when plants were inoculated with populations composed of *P. brachyurus* and *M. incognita*. However, it is emphasized that the experiment was conducted in pots and, in the field, other stresses associated with crop production are constant and can result in higher nematode reproduction and economic impact. In addition, exposure of sugarcane to nematodes for prolonged periods may result in the vegetative growth reductions, as noted in another pathosystem (Sikora *et al.*, 1972).

Our results showed that, in the interaction involving *M. incognita* and *P. zeae* in sugarcane, the increase in the initial population of one of the genera always caused a reduction in the reproductive capacity of the other, but the populations of both increased significantly after 90 d. The vegetative growth of the sugarcane plants was not affected by any rate or combination of nematodes, corroborating the hypothesis that concomitant populations involving migratory and sedentary endoparasites do not always result in more damage than that seen with a single species. The data refer to the experiment in the greenhouse, but in the field may be different since additional variables such as soil type, temperature, humidity, organic matter and other microbes, may affect the competitive ability of the organisms especially over several years.

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