

Population dynamics of the nematodes *Heterodera glycines* and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea

Dinámica poblacional de los nemátodos *Heterodera glycines* y *Pratylenchus brachyurus* en un cultivo de sucesión de soya y garbanzo

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ABSTRACT

The objective of this research was to evaluate the population dynamics of the nematodes *Heterodera glycines* race 5 and *Pratylenchus brachyurus* in a succession crop of soybeans and chickpeas. The experiment was carried out in Campos Novos dos Parecis, MT State, Brazil, from February to May 2017. Six chickpea cultivars were planted in February and evaluated in a naturally infested area of 60 ha. Five soil samples were collected at random in georeferenced and equidistant locations, forming a composite sample by replication. Five plants per georeferenced point and new soil samples were collected at regular intervals of about 30 d. The nematodes were extracted, identified, and counted. Data were subjected to variance and regression analyses. A reduction in the population levels of *H. glycines* race 5 was observed throughout the chickpea cycle, indicating that this species can be cultivated in succession with soybeans in the presence of this nematode. However, due to the maintenance in the population of *P. brachyurus* in the roots, rotation of soybean with chickpeas is not recommended in fields naturally infested with this nematode.

Key words: *Cicer arietinum* L., *Glycine max* (L.) Merrill., cyst nematode, root-lesion nematode.

RESUMEN

El objetivo de esta investigación fue evaluar la dinámica poblacional de los nematodos *Heterodera glycines* raza 5 y *Pratylenchus brachyurus* en cultivos sucesivos de soya y garbanzo. El experimento se llevó a cabo en Campos Novos dos Parecis, estado de MT, Brasil, de febrero a mayo de 2017. Se sembraron seis cultivares de garbanzo en febrero y se evaluaron en un área naturalmente infestada de 60 ha. Se recolectaron cinco muestras de suelo al azar en ubicaciones georreferenciadas y equidistantes, formando una muestra compuesta por replicación. Se recolectaron cinco plantas por punto georreferenciado y también nuevas muestras de suelo a intervalos regulares de aproximadamente 30 d. Los nematodos se extrajeron, identificaron y contaron. Los datos fueron sometidos a análisis de varianza y regresión. Se observó una reducción en los niveles poblacionales de *H. glycines* raza 5 a lo largo del ciclo del garbanzo, lo que indica que es posible cultivar esta especie en sucesión con soya en presencia de este nematodo. Sin embargo, debido al mantenimiento en la población de *P. brachyurus* en las raíces, no se recomienda la rotación de soya con garbanzos en campos naturalmente infestados con este nematodo.

Palabras clave: *Cicer arietinum* L., *Glycine max* (L.) Merrill., nematodo quístico, nematodo lesionador de raíces.

Introduction

Chickpea (*Cicer arietinum* L.) is the fourth most important legume grown in the world after soybeans, peanuts, and beans (FAOSTAT, 2021). Its grains contain fibers, proteins, vitamins, carbohydrates, mineral salts, unsaturated fatty acids, and β -carotene and are a source of protein in human and animal food supplementation (Souza, 2019). There are reports of cultivation in 55 countries, with a

total production of 17.19 million t (FAOSTAT, 2021). The world average yield is approximately 1.2 t ha⁻¹; however, some countries reach higher values, such as Israel (6.04 t ha⁻¹), China (5.2 t ha⁻¹), and Moldova (3.9 t ha⁻¹) (Avelar *et al.*, 2018).

In Brazil, domestic consumption and production are considered low, but in recent years, due to its nutritional characteristics and changes/trends in the patterns of food

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consumption, the demand for chickpea grains has grown. However, it is still necessary to import a large part of the quantity consumed in the domestic market, most of which comes from Mexico and Argentina (Artiaga *et al.*, 2015), with amounts close to 8,000 t per year (Avelar *et al.*, 2018). This new scenario gives chickpea an important role as an economic option for planting in the second summer harvest (known as *safrinha* in Portuguese and meaning “little harvest”) in the Brazilian Cerrado biome, which takes advantage of the drought tolerance ability of this species to grow despite little rainfall (Artiaga *et al.*, 2015).

The many species of plant-parasitic nematodes (PPN) are among the constraints that can cause impairments to chickpea production; some of these species include root-knot nematodes (*Meloidogyne javanica*, *M. incognita*, and *M. artiella*), cyst nematode (*Heterodera ciceri*), and the root-lesion nematode (*Pratylenchus* spp.) (Castillo *et al.*, 2008; Zwart *et al.*, 2019). Characterization studies in various parts of the world show that most chickpea genotypes are normally susceptible (Zwart *et al.*, 2019). However, there are few reports of chickpea genotypes with nematode tolerance (Ali & Ahmad, 2000; Ansari *et al.*, 2004; Hassan & Devi, 2004; Chakraborty *et al.*, 2016; Sumita, 2017), making more studies in this area necessary.

PPN also damage soybeans, with *Heterodera glycines* as one of the most harmful species due to its wide geographic distribution. However, in recent years, mainly in the Midwest region of Brazil, as well as in other regions, damages were registered and also attributed to the root-lesion nematode, *Pratylenchus brachyurus*, a polyphagous species (Cruz *et al.*, 2020) that colonizes even grasses such as maize and pastures.

Heterodera glycines can also colonize other plants like sunn hemp (*Crotalaria juncea*), pigeon pea (*Cajanus cajan*) (Valle *et al.*, 1996), common bean (*Phaseolus vulgaris*), azuki bean (*Vigna angularis*), and mung bean (*Vigna radiata*) (Rossi & Ferraz, 2001). Poromarto and Nelson (2010) verified that chickpea was a poor host of *H. glycines* race 3; however, there are no studies on *H. glycines* race 5. Cardoso *et al.* (2019) evaluated the resistance of *Crotalaria ochroleuca* to races 1, 2, 3, 5, 6, and 14 of *H. glycines*, and verified that it was resistant to races 1, 2 and 5, but was susceptible to race 3 and showed varied reactions to races 6 and 14. Due to the increase in the cultivated areas of chickpea in succession to soybeans in the Brazilian Midwest, research on these two PPN species becomes important. Thus, the objective of this study was to evaluate the population dynamics of

Heterodera glycines race 5 and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea.

Materials and methods

The experiment was carried out in Campos Novos dos Parecis, a municipality in the state of Mato Grosso, Brazil, from February to May 2017, in a field area naturally infested with the species *Heterodera glycines* race 5 and *Pratylenchus brachyurus*. The regional climate according to the Köppen classification is tropical with a dry winter and rainy summer, CWB type. The weather of the summer season in the Cerrado biome is characterized by a semi-humid tropical climate, with average temperatures of 25°C and annual precipitation of 1,677 mm. Six chickpea cultivars, BRS Aleppo, BRS Cícero, Jamu 96, BRS Cristalino, BRS Toro, and BRS Kalifa, were evaluated. The sowing occurred on February 24, 2017.

The experiment was conducted in an area of 60 ha (10 ha per cultivar), previously cultivated with a susceptible soybean cultivar, in a completely randomized design with three replicates. Soil and plant samples were collected at regular intervals of about 30 d on March 24, April 24, and May 25. For each replicate, five soil samples of 500-600 g and roots were collected using equidistant georeferenced points. All the samples were identified, extracted, and quantified in the Nematology Laboratory of Embrapa Vegetables, Brasília - DF.

The *H. glycines* and *P. brachyurus* species were identified according to their morphologic characteristics (Gonzaga, 2006; Mekete *et al.*, 2012). The identification of *H. glycines* race 5 was performed with the differentiating host test according to Riggs and Schmitt (1988).

The extraction of second-stage juveniles and cysts of *H. glycines*, as well as juveniles and adults of *P. brachyurus* from the soil samples, was performed using the rapid centrifugal-flotation technique, employing a sucrose solution, in accordance with Jenkins (1964). The extraction of PPN associated with roots was performed using the method of Hussey and Barker, modified by Bonetti and Ferraz in accordance with Wilcken *et al.* (2010). After the extractions, the quantification of the nematodes was performed with a Peters chamber and the visualization was made under a trinocular stereoscopic microscope (model Eclipse 80i, Nikon, Melville, NY, USA) in accordance with Bellé *et al.* (2017).

The following response variables were evaluated: number of viable cysts of *H. glycines*/150 cm³ of soil (NVCS), number of nonviable cysts of *H. glycines*/150 cm³ of soil (NNVCS), number of juveniles of second stage (J2) of *H. glycines*/150 cm³ of soil (NJ2HS), number of juveniles and adults of *P. brachyurus*/150 cm³ of soil (NJAPS), and number of juveniles and adults of *P. brachyurus*/g of fresh roots (NJAPR). The data were subjected to variance and regression analyses using the Genes statistical program (Cruz, 2016).

Results and discussion

There was no effect of the cultivars on the response variables evaluated. On the contrary, statistical differences were observed for the source of variation - date of collection or sampling time for all the evaluated variables, except for the number of juveniles and adults of *Pratylenchus* in roots (NJAPR). Table 1 indicates that the population levels of *P. brachyurus* per g of roots remained constant. There was also no interaction between the cultivars and sampling times - collection (CxCL) (Tab. 1), indicating that the population

dynamics of the studied nematode species was not affected by any of these factors (cultivars and times).

Regarding the number of viable cysts of *H. glycines*/150 cm³ of soil (NVCS), a significant reduction in the population was observed during the chickpea crop cycle (Fig. 1), regardless of the cultivar used.

An increase in the number of nonviable cysts of *H. glycines* /150 cm³ of soil (NNVCS) was observed according to the sampling times (CL) (Fig. 2), regardless of the chickpea cultivar.

As for the number of juveniles of second stage (J2) of *H. glycines*/150 cm³ of soil (NJ2HS), among all the evaluated cultivars, there was a reduction of population levels from 57.64 to 16.67 (Fig. 3).

The increase in the number of nonviable cysts, the reduction of viable cysts, and the reduction in the number of these PPN in the soil may be due to the hatching of

TABLE 1. Summary of the joint analysis of variance of the population dynamics of the soybean cyst nematode (*Heterodera glycines* race 5) and the root-lesion nematode (*Pratylenchus brachyurus*) in a chickpea crop.

Source of variation	Mean square				
	NCVS	NNVCS	NJ2HS	NJAPS	NJAPR
Cultivar (C)	79.13 ^{ns}	220.71 ^{ns}	912.37 ^{ns}	446.82 ^{ns}	31411.15 ^{ns}
Collection (CL)	211.19*	1772.54*	5245.22*	2779.94*	272338.9 ^{ns}
CxCL	46.95 ^{ns}	195.15 ^{ns}	920.01 ^{ns}	890.57 ^{ns}	43086.66 ^{ns}
Residue	62.21	160.17	1629.16	955.03	94252.54
General mean	10.24	15.01	34.20	28.64	339.56
CV (%)	36.42	29.95	59.56	41.35	23.20

*Significant at 5% of probability by F test. ns - Not significant at 5% probability by F test. NCVS - Number of viable cysts of *H. glycines*/150 cm³ of soil; NNVCS - number of nonviable cysts of *H. glycines*/150 cm³ of soil; NJ2HS - number of second-stage juveniles (J2) of *H. glycines*/150 cm³ of soil; NJAPS - number of juveniles and adults of *Pratylenchus brachyurus*/150 cm³ soil; NJAPR - number of juveniles and adults of *P. brachyurus*/g of roots (fresh weight). CV - coefficient of variation.

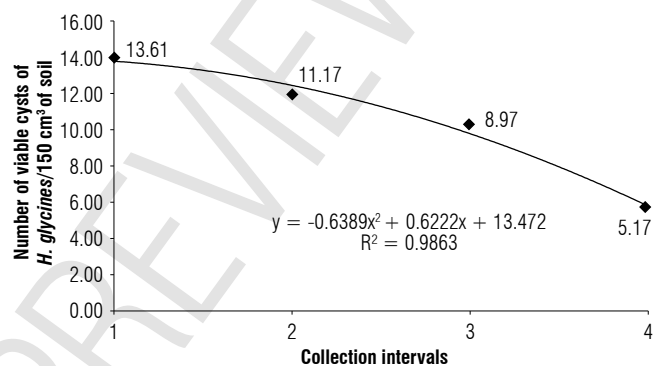


FIGURE 1. Number of viable cysts of *Heterodera glycines*/150 cm³ of soil according to the collection intervals (30, 60, and 90 days after sowing).

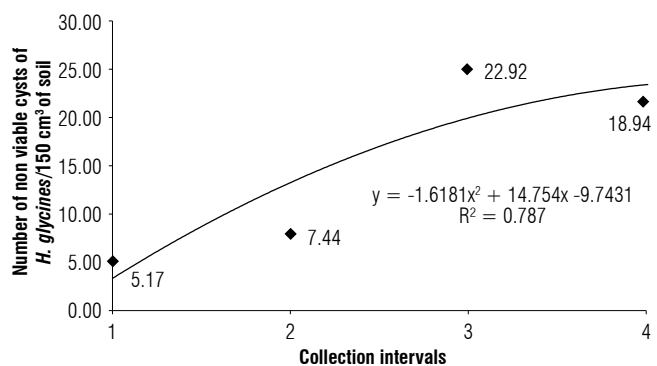


FIGURE 2. Number of nonviable cysts of *Heterodera glycines*/150 cm³ of soil according to the collection intervals (30, 60, and 90 days after sowing).

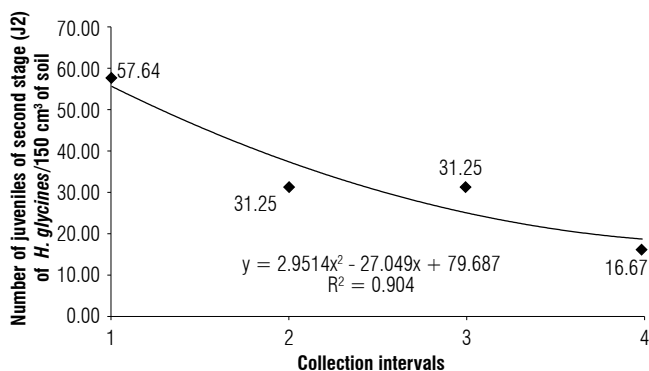


FIGURE 3. Number of juveniles of second stage (J2) of *Heterodera glycines*/150 cm³ of soil according to the collection intervals (30, 60, and 90 days after sowing).

juveniles of *H. glycines* race 5 and the absence of a host plant in the area for a period that prevented the reinfection and continuity of its life cycle (Cardoso *et al.*, 2019). This result shows that chickpea is not a host of *H. glycines* race 5 and that it can be indicated for crop rotation or succession with soybeans in the areas infested with this race. This is a significant finding, as there are no similar studies in the literature.

The number of juveniles and adults of *P. brachyurus*/150 cm³ of soil (NJAPS) was maintained during the first 60 d of the cycle, with a subsequent sharp reduction observed from 35.42 to 10.42 between the samplings collected at 60 and 90 d of the cycle (Fig. 4). However, as previously verified, the population per g of roots remained high in all the samples. This reduction in the population of *P. brachyurus* in the soil, considering that the chickpea vegetative cycle is close to 110 d, was probably because this nematode is a migrating endoparasite that prefers roots to soil. When plants are expanding their canopy in an area with a large initial population of nematodes in the soil, it is usual for migration to occur with more intensity as the roots are also developing. At the end of the crop cycle, there will be a new migration to the soil, with a subsequent search for host plants (Puerari *et al.*, 2020).

Santos (2019) evaluated these same six cultivars in a naturally infested field with the presence of *M. javanica* for their resistance to *P. brachyurus*, finding a marked differentiation in terms of their susceptibility. Nevertheless, this author estimated only the reproduction factor (RF) based on the rate between the initial population at the time of the sowing and the final nematode population in the soil. That study verified that the cultivar Jamu 96 maintained the same population of *P. brachyurus* in both periods, *i.e.*, showing a reproduction factor equal to 1; BRS Cicero

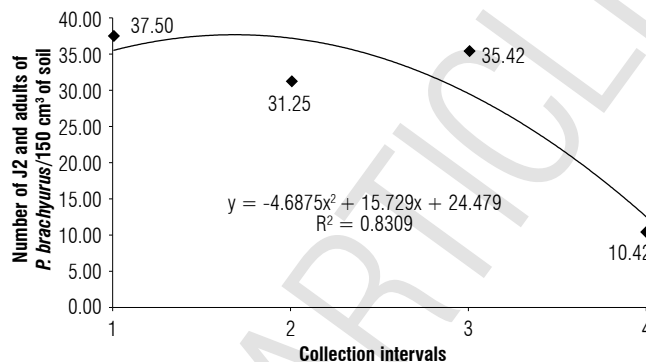


FIGURE 4. Number of juveniles of second stage (J2) and adults of *Pratylenchus brachyurus*/150 cm³ of soil according to the collection intervals (30, 60, and 90 days after sowing).

showed a reproduction factor of 0.95 and all the others had RFs ranging from 0.43 to 0.65. The initial population level of *P. brachyurus* in the soil may be a determinant factor to the degree of resistance of the cultivars; since the population in this study was considered very high, it may have resulted in a greater nematode pressure, making it impossible to verify differences in the resistance levels of the cultivars.

Santos (2019) demonstrated that these chickpea cultivars showed resistance to *M. javanica* in a naturally infested field with a mixed population of *M. javanica* and *P. brachyurus*. However, Bernardes Neto *et al.* (2019) evaluated the same cultivars in a greenhouse trial and considered them susceptible to *M. incognita* and *M. enterolobii*, which are also important for both chickpeas and soybeans.

Among the most widespread control methods for PPN are the use of nematicides, crop rotation, antagonistic plants, organic matter, fallowing, soil fumigation, solarization, and use of genetic resistance (Ravichandra, 2014; Cruz *et al.*, 2020). Crop rotation with non-host plant species for a sufficient period to allow the decomposition of infested residues reduces the viability and association of the pathogen, especially in the no-tillage system (Cruz *et al.*, 2020). It also increases beneficial soil organisms and minimizes the impact of root diseases (Cruz *et al.*, 2020).

To evaluate the response of different plant species to *H. glycines* and to better understand what crops could be recommended in rotation or succession or as green manure for soybeans, Sanches (2001) evaluated two species of velvet bean (*Mucuna pruriens* and *Mucuna utilis*) for reaction to races 2, 3, 4, 5 and 14 of *H. glycines* and verified that both are not hosts of this nematode. Evaluating the response of several species used as green manure or cover crops to

race 3 of this nematode, Valle *et al.* (1996) found that sunn hemp (*Crotalaria juncea*), pigeon pea (*Cajanus cajan*), azuki beans (*Vigna angularis*), and mungo beans (*Vigna radiata*) are hosts that can multiply these PPN. The susceptibility of azuki and mungo beans was also verified by Rossi and Ferraz (2001), who also found susceptibility in common beans (*Phaseolus vulgaris*) and white lupine (*Lupinus albus* L.) to race 3 of this nematode.

In addition to cultivated plants, *H. glycines* can also be found in weeds. Venkatesh *et al.* (2000) evaluated 22 weed species from 13 families of dicotyledonous as alternative hosts of *H. glycines* in a greenhouse in Ohio, USA, and found that the red dead-nettle (*Lamium purpureum*), common henbit (*Lamium amplexicaule*), field pennycress (*Thlaspi arvense*) and shepherd purse (*Capsella bursa-pastoris*) were hosts. A greater reproduction of race 1 and a low reproduction of race 6 were found in red dead-nettle. For race 3, the highest level of reproduction occurred in red dead-nettle, field pennycress, and common henbit (Venkatesh *et al.*, 2000).

Poromarto and Nelson (2010) found that the chickpea was not an efficient host of *H. glycines* race 3, although it allowed the multiplication of a small number of this nematode. However, Cardoso *et al.* (2019), who evaluated the resistance of rattlepod (*Crotalaria ochroleuca*) to races 1, 2, 3, 5, 6, and 14 of *H. glycines*, found that this plant species was resistant to races 1, 2, and 5 but was susceptible to race 3 and showed varied reactions to races 6 and 14.

The identification of these *H. glycines* races is important since prior to this research, no study had identified resistance of this plant to race 5 of this nematode.

Conclusions

There was a reduction in the population levels of *Heterodera glycines* race 5 throughout the cycle of chickpeas, indicating that it is possible to grow this species in a succession crop with soybeans in the presence of this nematode.

Due to the maintenance of the population of *Pratylenchus brachyurus* in the roots throughout the chickpea cycle, crop rotation of soybean with chickpeas would not be recommended for fields infested with this nematode.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

JBP, AGM, and DB designed the experiments. JBP, AGM, JGJ, LR, CCM, and DB carried out the field and laboratory experiments. GOS and RACM contributed to the data analysis. JBP, GOS, AGM, RACM, PPS, and WMN wrote the article. All authors reviewed the manuscript.

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