Journal of Agricultural Science; Vol. 11, No. 7; 2019
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Crotalaria ochroleuca Susceptibility to *Heterodera glycines* Races

Michelly Ragazzi Cardoso[1], Claudia Regina Dias-Arieira[1], Neucimara Rodrigues Ribeiro[2], Adriély Alves de Almeida[2], Angélica Miamoto[1] & Ana Paula Mendes Lopes[1]

[1] Universidade Estadual de Maringá, Umuarama, Paraná State, Brazil

[2] GDM Seeds-Genética do Brasil, Cambé, Paraná State, Brazil

Correspondence: Angélica Miamoto, Universidade Estadual de Maringá/PGA, Avenida Colombo, n. 5790-Bloco J45, 2° Piso, 87020-900, Maringá, Paraná, Brazil. Tel: 55-449-9904-4320. E-mail: angelicamiamoto@gmail.com

Received: September 11, 2018 Accepted: December 20, 2018 Online Published: May 31, 2019

doi:10.5539/jas.v11n7p205 URL: https://doi.org/10.5539/jas.v11n7p205

Abstract

The aim of the study is to assess *Crotalaria ochroleuca* susceptibility to six *Heterodera glycines* races. To this, *C. ochroleuca* seedlings and two soybean cultivars were transplanted and individually inoculated with nematode races 1, 2, 3, 5, 6 and 14. Plants were collected and assessed 30 and 60 days after inoculation. *Crotalaria ochroleuca* reduced the number of females and the total number of eggs of the races 1, 2, 5 and 6. The reproduction of race 3 in crotalaria was equal to that of soybean cultivars in both assessment periods, whereas race 14 only reduced the reproduction in crotalaria to 60 days. Races 1 and 6 (30th day) and races 6 and 14 (60th day) showed number of eggs female^{-1} smaller than the controls. According to female index (FI), *C. ochroleuca* was resistant to races 1, 2 and 5, susceptible to race 3, and showed varying reactions to races 6 and 14.

Keywords: antagonist plant, crop rotation, crotalaria, soybean, cyst nematode

1. Introduction

The soybean cyst nematode or SCN (*Heterodera glycines* Ichinohe) stands out among the main pests affecting soybean yield in Brazil. It is estimated that such yield may be totally compromised in case of severe infestations (Dias et al., 2009). This pathogen is responsible for causing yellow dwarf disease in soybeans, whose plants present intense yellow shade and reduced size (Dias et al., 2010). It also leads to root system and yield reduction, as well as to premature plant death (Santos-Junior et al., 2002; Juhász et al., 2013).

Heterodera glycines females show lemon-like shape and contain approximately 300 eggs; they remain attached to the root system in the form of small white spots (Schmitt & Riggs 1989; Dias et al., 2010). Dead females detach from the root, acquire dark-brown coriaceous appearance, and are named cysts (Dias et al., 2010). The function of this survival structure lies on protecting the eggs from desiccation, heat and deterioration-fact that keeps them viable for up to eight years, as well as on promoting their dissemination (Schmitt & Noel, 1984; Andrade & Asmus, 1997).

One of the main SCN features lies on the high genetic variability, which derives from sexual reproduction in the species and subdivides it into 16 races (Ross, 1962; Riggs & Schmitt, 1988). Eleven (11) races—1, 2, 3, 4, 4+, 5, 6, 9, 10, 14 and 14+—were recorded in Brazil, distributed in Mato Grosso, Mato Grosso do Sul, Minas Gerais, Goiás, São Paulo, Paraná, Rio Grande do Sul, Bahia, Tocantins and Maranhão states (Cunha et al., 2008; Dias et al., 2009).

The high genetic variability and survival capacity of the SCN are the main features making it difficult to be managed. Among the control measures indicated to reduce the pathogen, it is worth highlighting the use of resistant cultivars and crop rotation using non-host or antagonistic plants (Dias et al., 2009; Santos et al., 2014). The adoption of resistant cultivars is one of the most recommended practices; however, the genetic variability of the pathogen enables other pathogen races to be selected, thus reducing the efficiency of cultivars (Dias et al., 2009).

Accordingly, crop rotation or succession using antagonistic plants, mainly the species *Crotalaria* spp., has been recommended to keep nematode populations below the economic damage threshold (Santos et al., 2014). Genus *Crotalaria*, which belongs to family Fabaceae, has natural nematicide action due to the production of pyrrolizidine alkaloids such as monocrotaline, which protect plants from pests and diseases (Wang et al., 2002a;

Silva-López & Pacheco, 2010). Among more than 600 crotalaria species distributed worldwide, the following ones stand out in Brazil: *C. juncea* L., *C. spectabilis* Roth, *C. paulina* Schrank, *C. breviflora* D. C. and *C. ochroleuca* G. Don (Silva et al., 2009).

Crotalaria spectabilis and *C. ochroleuca* are the most used species to control phytonematodes in Brazil. *Crotalaria spectabilis* is efficient in reducing *Meloidogynejavanica* (Treub) Chitwood, *M. incognita* (Kofoid and White) Chitwood, *Pratylenchus brachyurus* (Godfrey) Filipjev and S. Stekhoven, and *H. glycines*, sincethe antagonistic action of the species was confirmed by Kushida et al. (2003), Inomoto (2008), Santana et al. (2012) and Rosa et al. (2013). *Crotalaria ochroleuca* also appeared to be efficient in controlling *P. brachyurus*, *M. incognita*, *M. javanica* and *Rotylenchulus reniformis* Linford and Oliveira (Anwar et al., 1994; Ribeiro et al., 2007; Leandro & Asmus, 2015; Miamoto et al., 2016). On the other hand, Riggs (1992) and Schwan (2003) reported that some crotalaria species may be SCN hosts.

Schwan (2003) assessed *H. glycines* race 10 parasitism in different crotalaria species and observed juvenile penetration, as well as female development in *C. ochroleuca* roots. Thus, the current hypothesis is that the reaction of this plant to different *H. glycines* races may vary, fact that could compromise the efficiency of the crop rotation. In light of the foregoing, the aim of the current study was to assess *C. ochroleuca* susceptibility to *H. glycines* races 1, 2, 3, 5, 6 and 14.

2. Method

The experiments were conducted in a greenhouse located in Cambé, PR, Brazil (latitude 23º16′33″ S and longitude 51º16′42″ W) and adopted a completely randomized design with three treatments and six repetitions, wherein six nematode races were individually assessed 30 and 60 days after inoculation. The treatments comprised the species *C. ochroleuca* and two susceptible soybean cultivars (Lee and Conquista). *Crotalaria ochroleuca* seeds were sownin trays, containing autoclaved sand (for 2 hours, at 120 °C) and remained there for 25 days, before inoculation. Seedlings from the aforementioned soybean cultivars (controls) were also produced in previously autoclaved sand, seven days before inoculation.

The herein assessed *H. glycines* races (1, 2, 3, 5, 6 and 14) were obtained from pure inocula kept in soybean varieties (considered susceptibility patterns of each race), under greenhouse conditions. The inoculum was obtained through the methodology proposed by Dias-Arieira et al. (2003) - the roots of plants infected with the respective nematode races were manually washed (three times each) in running water and the extracted solution was separately deposited in a beaker. Subsequently, the solution was passed through 24 and 60 mesh sieves; cysts and females were collected in the last sieve. Then, the 60-mesh sieve was coupled to a 500 mesh one; the cysts and females were macerated in the first sieve to release eggs and juveniles, which were retained in the second sieve, in order to prepare the inoculum. The inoculum was calibrated into a 4 mL suspension containing 4,000 eggs and eventual second stage juveniles (J2) from each race, separately.

The experiment was carried out in pots containing 300 mL of previously autoclaved (2 hours, at 120 °C) soil-sand mixture (at ratio 1:3). The mixture was subjected to correction and fertilization, according to the chemical analysis of the soil, using 1,200 kg ha^{-1} limestone, 200 kg ha^{-1} potassium chloride and 160 kg ha^{-1} urea, which were converted into the ratio of the pots. The herein used soil was classified as typical Eutroferric Red Latosol (Embrapa, 2013). *Crotalaria ochroleuca* seedlings and soybean cultivars ‘Lee’ and ‘Conquista’ were transplanted to the pots in 10-cm deep holes; subsequently, the inocula from each race were directly deposited on the root system, separately. The experiments, comprising each race were separated by a polyethylene curtain wall in order to prevent contamination at irrigation time. Plants were kept in the greenhouse for 30 and 60 days after inoculation, at temperature varying from 24 to 35 ºC, and under daily irrigation.

Plants were collected after the end of each inoculation period and the root system was carefully separated from the shoot to avoid losing females adhered to it. The shoot was discarded, whereas the root system was subjected to the aforementioned extraction methodology. The number of females per root system was assessed in a stereoscopic microscope equipped with a checkered-bottom acrylic plate. After the number of females was assessed, they were macerated, according to the herein mentioned methodology, in order to assess the total number of eggs and the number of eggs per female using Barlow lens under optical microscope.

The female index from each race was also assessed to allow classifying them as resistant or susceptible. The mean female index (FI) was presented as percentage; it was calculated by dividing the mean number of females found in *C. ochroleuca* by the mean number of females found in cultivar ‘Lee’ multiplied by 100. Thus, if the calculated FI was lower than 10%, the plant was classified as resistant; on the other hand, if the FI was equal to or higher than 10%, was classified as susceptible (Riggs & Schmitt, 1988).

Data were subjected to analysis of variance and compared through Tukey test at 5% probability in the Assistat statistical software (Silva & Azevedo, 2016).

3. Results

According to the herein gathered data, races 1, 2, 5 and 6 showed reduced number of females in *C. ochroleuca* plants, 30 days after inoculation (Table 1). With respect to race 3, *C. ochroleuca* differed from the soybean cultivar 'Conquista', but not from 'Lee'; whereas race 14 presented reduced number of females in comparison to the soybean cultivar 'Lee' (Table 1). All the assessed races showed reduced number of females in *C. ochroleuca* plants 60 days after inoculation, in comparison to the controls (Table 1).

Table 1. Number of females from different *Heterodera glycines* races grown in soybean cultivars 'Lee' and 'Conquista', as well as in *Crotalaria ochroleuca*, 30 and 60 days after inoculation

Females Number						
Treatment	**Race 1**	**Race 2**	**Race 3**	**Race 5**	**Race 6**	**Race 14**
30 days after inoculation						
Lee	343 a	157 a	148 ab	267 a	310 a	156 a
Conquista	240 a	150 a	320 a	317 a	389 a	89 b
C. ochroleuca	24 b	4 b	21 b	2 b	104 b	43 b
MSD	115	124	230	216	132	62
60 days after inoculation						
Lee	478 a	197 a	191 b	648 a	638 a	650 a
Conquista	342 a	288 a	401 a	479 a	554 a	668 a
C. ochroleuca	42 b	19 b	37 c	17 b	61 b	6 b
MSD	202	113	110	254	316	224

Note. Means followed by the same letter did not differ from each other in the Tukey test, at 5% probability. MSD: minimum significant difference.

Crotalaria ochroleuca presented reduction in the total number of eggs from races 1, 2, 5, 6 and 14, when it was compared to the controls 30 days after inoculation, whereas race 3 statistically differed from cultivar 'Conquista' (Table 2). However, *C. ochroleuca* showed reduction in the total number of eggs from all the assessed races, 60 days after inoculation (Table 2).

Table 2. Total number of eggs from different *Heterodera glycines* races grown in soybean cultivars Lee and Conquista, as well as in *Crotalaria ochroleuca*, 30 and 60 days after inoculation

Total number of eggs						
Treatment	**Race 1**	**Race 2**	**Race 3**	**Race 5**	**Race 6**	**Race 14**
30 days after inoculation						
Lee	78440 a	25940 a	16690 ab	31078 a	47406 a	12832 a
Conquista	44294 b	24822 a	37812 a	28970 a	52888 a	7006 b
C. ochroleuca	476 c	593 b	1880 b	444 b	910 b	512 c
MSD	24627	22827	26989	27233	22101	4474
60 days after inoculation						
Lee	104992 a	40070 a	24178 b	121985 a	133916 a	122990 a
Conquista	57352 a	46280 a	46420 a	114757 a	146053 a	130048 a
C. ochroleuca	4566 b	1705 b	968 c	1800 b	1105 b	50 b
MSD	50859	24061	17558	78012	105452	43697

Note. Means followed by the same letter did not differ from each other in the Tukey test, at 5% probability. MSD: minimum significant difference.

Races 1 and 6 produced fewer eggs per female (Table 3); 18 and 19 eggs per female were found in *C. ochroleuca*, whereas 235 and 150 eggs per female were found in the soybean cultivar 'Lee', respectively. The other races did

not present significant statistical differences (Table 3). The number of eggs per female in races 1 and 5 did not statistically differ from the controls 60 days after inoculation, whereas races 2 and 3 only differed from each other in the cultivar 'Lee', since they produced, respectively, 74 and 30 eggs per female in *C. ochroleuca*, as well as 207 and 150 eggs per female in the soybean cultivar 'Lee' (Table 3). Races 6 and 14 presented reduced number of eggs per female in *C. ochroleuca*, in comparison to the controls; 30 and 36 eggs per female in *C. ochroleuca,* and 204 and 195 eggs per female in the soybean cultivar 'Lee', respectively (Table 3).

Table 3. Number of eggs female^{-1} from different *Heterodera glycines* races grown in soybean cultivars 'Lee' and 'Conquista', as well as in *Crotalaria ochroleuca*, 30 and 60 days after inoculation

Number of egg female^{-1}						
Treatment	**Race 1**	**Race 2**	**Race 3**	**Race 5**	**Race 6**	**Race 14**
30 days after inoculation						
Lee	235 a	156^{ns}	106^{ns}	131^{ns}	150 a	84^{ns}
Conquista	186 a	158	121	92	136 a	117
C. ochroleuca	18 b	135	150	148	19 b	50
MSD	70	154	99	277	40	139
60 days after inoculation						
Lee	212^{ns}	207 a	150 a	185^{ns}	204 a	195 a
Conquista	187	159 ab	114 ab	246	257 a	193 a
C. ochroleuca	172	74 b	30 b	281	30 b	36 b
MSD	168	81	98	467	79	71

Note. Means followed by the same letter did not differ from each other in the Tukey test, at 5% probability. ns = not significant. MSD: minimum significant difference.

Crotalaria ochroleuca was resistant (FI < 10) to races 1, 2 and 5 and susceptible (FI > 10) to race 3, regardless of the assessment period (Table 4). On the other hand, there was FI variation in races 6 and 14. Race 6 presented FI > 10 (33.5), when it was assessed 30 days after inoculation, as well as FI close to 10 (9.5) 60 days after inoculation (Table 4). Similar result was recorded for race 14, since *C. ochroleuca* presented FI (27.5) 30 days after inoculation, and FI (0.9) 60 days after inoculation (Table 4).

Table 4. Female Index-FI (%) in *Crotalaria ochroleuca* roots applied to the classification as to the susceptibility of different *Heterodera glycines* races, 30 and 60 days after inoculation

Races	**30 days after inoculation**		**60 days after inoculation**	
	FI	**Reaction**	**FI**	**Reaction**
1	7.0	Resistant	8.7	Resistant
2	2.5	Resistant	9.6	Resistant
3	14.1	Susceptible	19.4	Susceptible
5	0.7	Resistant	2.6	Resistant
6	33.5	Susceptible	9.5	Resistant
14	27.6	Susceptible	0.9	Resistant

4. Discussion

The present study confirmed that *C. ochroleuca* allowed the development of all the SCN races, which completed the cycle and formed females in all the assessed incubation periods. Schwan (2003) assessed *H. glycines* race 10 development 30 and 45 days after inoculation and found females in *C. ochroleuca* plants, in both periods. The reaction of *C. ochroleuca* to the SCN differed from that recorded for other species, since the nematode did not reach maturity in *C. spectabilis* and *C. juncea*; only J3 and J4 stages were recorded 28 days after inoculation (Kushida et al., 2003). Valle et al. (1996) assessed the reaction of different legumes to *H. glycines* and did not find female in *C. striata*, *C. paulina* and *C. spectabilis*; however, male formation was recorded in *C. striata*. *Crotalaria juncea* was classified as resistant to SCN (race 3), whose number of cysts was 0.05 and parasitism index was zero (Rossi & Ferraz, 2001).

Studies focused on assessing the relation between crotalaria and *H. glycines* species are scarce in the literature.On the other hand, the potential of *Crotalaria* spp. to control nematodes in several species was confirmed, mainly in studies using *C. spectabilis* and *C. juncea* against *Meloidogyne* spp. (Inomoto et al., 2006; Santana et al., 2012; Rosa et al., 2013; Miamoto et al., 2016) and *P. brachyurus* (Inomoto et al., 2006; Dias et al., 2012; Vedoveto et al., 2013; Braz et al., 2016). Silva et al. (1989) assessed *M. javanica* development in *C. spectabilis*, *C. juncea*, *C. retusa* and *C. paulina*; however, they did not record formation of adult individuals 45 days after inoculation. *Crotalaria juncea* impaired the life cycle of the *R. reniformis* species—although it did not prevent egg formation, it delayed female development—in comparison to favorable hosts such as cowpea (Wang et al., 2002b).

The action mechanism of *Crotalaria* species has been mainly investigated in studies about root-knot nematodes. Accordingly, studies indicated that *M. incognita* penetrated the root system of crotalaria plants, although it did not fully develop (Jaehn & Mendes, 1979). Similar result was recorded for *M. javanica* in *C. spectabilis*, *C. ochroleuca* and in *C. juncea* (Miamoto et al., 2016). According to reports, the ability of crotalaria plants to interfere in certain nematode development stages results from the production of pyrrolizidine alkaloids (PA), mainly the monocrotaline, which suppresses pathogen development (Calegari et al., 1993; Wang et al., 2002a; Lopes et al., 2008).

Studies about the action of PAs showed that *M. incognita* mobility was affected, although it did not prevent nematode penetration, when concentration 10 μg mL^{-1} PA was used (Fassuliotis & Skucas, 1969). In addition, shoot and root extracts from different crotalaria species—such as *C. pallida*, *C. goreensis* and *C. retusa*—used separately, showed nematosthetic potential against second stage *M. javanica*, *M. incognita* and *M. enterolobii* Yang and Eisenback (= *M. mayaguensis*) juveniles (Jourand et al., 2004). According to Danahap and Wonang (2016), root exudates from *C. retusa*, *C. breviflora*, *C. spectabilis* and *C. juncea* have nematicidal properties that may kill *M. incognita* or inhibit its development.

Crotalaria ochroleuca did not affect the full SCN development in the current study; however, races 1, 2, 5 and 6 showed reduced number of females in the root system 30 days after inoculation, whereas all the races showed reduction in such parameter 60 days after inoculation. Thus, feeding sites may have limited functionality in female feeding; therefore, in addition to reducing the number of nematodes, these sites may also affect females' reproduction capacity (Wang et al., 2002a).

Histopathological studies confirmed that the *M. javanica* feeding site was modified in *C. spectabilis* and *C. juncea* roots, since the giant cells formed therein showed dense, granular, poorly-nucleated cytoplasm without large vacuoles, thus limiting nematode feeding and, consequently, its development (Silva et al., 1990). Studies using *Mucuna pruriens* (L.) DC. as *H. glycines* race 3 antagonist plant showed that the syncytial cells degenerated and were inefficient in feeding the nematode. The syncytia observed 15 and 20 days after inoculation, in the current study, were necrotic or poorly functional, showed dense cytoplasm, ruptured cell walls and hypertrophied nuclei, fact that did not allow the pathogen to become fully grown (Sanches, 2001). However, it is necessary conducting histopathological studies about the crotalaria and SCN pathosystems in order to confirm such action mechanism.

Reduced number of *H. glycines* eggs was recorded in both assessments. This nematode, which is able to produce 300 eggs per female, on average (Schmitt & Riggs, 1989), showed production lower than 40 female $eggs^{-1}$ in races 1 and 6 (30 days after inoculation) and in races 6 and 14 (60 days after inoculation). Similar results were recorded when *C. spectabilis*, *C. juncea*, *C. mucronata* Desv., *C. breviflora* and *C. ochroleuca* were used to reduce the egg mass of *M. javanica* and *M. enterolobii*, in comparison to results recorded for Rutgers tomatoes, which were used as control (Rosa et al., 2013; Rosa et al., 2015). Data concerning the number of eggs per female did not statistically differ between the other races and the controls, in both assessments.

The female index assessment confirmed *C. ochroleuca* variation against different *H. glycines* races; it was susceptible to race 3, resistant to races 1, 2 and 5, and showed varying reactions to races 6 and 14. Soares et al. (2015) confirmed *C. ochroleuca* susceptibility to *H. glycines* race 3, when they cultivated this plant species in the field; they observed symptoms such as intense yellow shade and smaller plant size in subjected to laboratory analysis, which confirmed pathogen presence in the root system; more than 9,000 eggs and J2 were found in 10 g root.

Although *C. ochroleuca* was susceptible to races 6 and 14, 30 days after inoculation (according to FI), it was resistant to them 60 days after it; it showed that the plant did not prevent nematode penetration; however, it may have developed some late resistance mechanism, since it reduced the number of females 60 days after inoculation. Despite the scarcity of studies focused on investigating *C. ochroleuca* susceptibility to SCN, it was

already proved that the use of this plant efficiently reduced the number of *H. glycines* individuals from races 1, 2 and 5, as well as the number of other nematode species such as *P. brachyurus*, *M. incognita*, *M. javani*ca and *R. reniformis* (Anwar et al., 1994; Dias et al., 2012; Leandro & Asmus, 2015; Miamoto et al., 2016).

5. Conclusion

Crotalaria ochroleuca presents varying susceptibility to *H. glycines*; it is resistant to races 1, 2 and 5, susceptible to race 3, and shows varying reactions to races 6 and 14.

References

Andrade, P. J. M., & Asmus, G. L. (1997). Disseminação do nematoide de cisto da soja (Heterodera glycines) pelo vento durante o preparo do solo. *Nematologia Brasileira, 21*(79), 98-100.

Anwar, S. A., Trudgill, D. I., & Phillips, M. S. (1994). The contribution of variation in invasion and development rates of *Meloidogyne incognita* to host status differences. *Nematologica, 40*(1), 579-586. https://doi.org/10.1163/003525994X00436

Braz, G. B. P., Oliveira Junior, R. S., Constantin, J., Raimondi, R. T., Ribeiro, L. M., Gemelli, A., & Takano, H. K. (2016). Plantas daninhas como hospedeiras alternativas para *Pratylenchus brachyurus*. *Summa Phytopathologica, 42*(3), 233-238. https://doi.org/10.1590/0100-5405/2129

Calegari, A., Alcântara, P. B., Miyasaka, S., & Amado, T. J. C. (1993). Caracterização das principais espécies de adubo verde. *Adubação Verde no Sul do Brasil*. Rio de Janeiro (RJ): AS-PTA.

Cunha, R. P., Maia, G. L., Rodacki, M. E. P., Silva, G. S., & Meyer, M. C. (2008). Ciclo de vida de *Heterodera glycines* raça 9 em soja no Estado do Maranhão. *Summa Phytopatologica, 34*(3), 262-264. https://doi.org/10.1590/S0100-54052008000300012

Danahap, L. S., & Wonang, D. L. (2016). Antinematicidal efficacy nof root exudates of some *Crotalaria* species on *Meloidogyne incognita* (root-knot nematode) (Kofoid and White) Chitwood isolated from infected *Lycopersicum esculentum* L. (Tomato) plant. *International Journal of Scientific and Technology Research, 5*(1), 79-84.

Dias, W. P., Garcia, A., Silva, J. F. V., & Carneiro, G. E. S. (2010). *Nematoides em Soja: Identificação e Controle*. Londrina (PR): Embrapa Soja.

Dias, W. P., Orsini, I. P., Ribeiro, N. R., Parpinelli, N. M. B., & Freire, L. L. (2012). Efeito do cultivo de espécies vegetais sobre a população de *Pratylenchus brachyurus* na soja. *Proceedings* of VI Congresso Brasileiro de Soja, 2012, Cuiabá.

Dias, W. P., Silva, J. F. V., Carneiro, A. G., Arias, C. A. A. (2009). Nematoide de Cisto da Soja: Biologia e Manejo pelo uso da resistência genética. *Nematologia Brasileira, 33*(1), 1-16.

Dias-Arieira, C. R., Ferraz, S., Freitas, L. G., & Demuner, A. J. (2003). Efeito de lixiviados de raízes de gramíneas forrageiras na eclosão de juvenis de *Meloidogyne incognita*, *M. javanica* e *Heterodera glycines*. *Nematologia Brasileira, 27*(1), 23-28.

EMBRAPA. (2013). *Sistema Brasileiro de Classificação de Solos*. Brasília (DF): Embrapa Solos.

Fassuliotis, G., & Skucas, G. P. (1969). Effect of a pyrrolizidine alkaloid ester and plants containing pyrrolizidine on *Meloidogyne incognita* acrita. *Journal of Nematology, 65*(7), 287-288.

Inomoto, M. M. (2008). Importância e manejo de *Pratylenchus brachyurus*. *Revista Plantio Direto, 108*(1), 4-9.

Inomoto, M. M., Motta, L. C. C., Beluti, D. B., & Machado, A. C. Z. (2006). Reação de seis adubos verdes a *Meloidogyne javanica* e *Pratylenchus brachyurus*. *Nematologia Brasileira, 30*(1), 39-44.

Jaehn, A., & Mendes, B. V. (1979). Avaliação da Penetração e Desenvolvimento de *Meloidogyne incognita* em *Crotalaria spectabilis*. Proceedings of Congresso Brasileiro de Pesquisas Cafeeiras, 1979, Araxá, IBC.

Jourand, P., Rapior, S., Fargette, M., & Mateille, T. (2004). Nematostatic activity of aqueous extracts of West African *Crotalaria* species. *Nematology, 6*(1), 765-771. https://doi.org/10.1163/1568541042843513

Juhász, A. C. P., Pádua, G. P., Wruck, D. S. M., Favoreto, L., & Ribeiro, N. R. (2013). Desafios fitossanitários para a produção de soja. *Informe Agropecuário, 34*(276), 66-75.

Kushida, A., Suwa, N., Ueda, Y., & Momota, Y. (2003). Effects of *Crotalaria juncea* and *C. spectabilis* on hatching and population density of the soybean cyst nematode, *Heterodera glycines* (Tylenchida: Heteroderidae). *Applied Entomology and Zoology, 38*(3), 393-399. https://doi.org/10.1303/aez.2003.393

Leandro, H. M., & Asmus, G. L. (2015). Rotação e sucessão de culturas para o manejo do nematoide reniforme em área de produção de soja.. *Ciência Rural, 45*(6), 945-950. https://doi.org/10.1590/0103-8478cr20130526

Lopes, E. A., Ferraz, S., Ferreira, P. A., Freitas, L. G., Gardiano, C. G., Dhingra, O. D., & Dallemole-Giaretta, R. (2008). Efeito da incorporação da parte aérea de quatro espécies vegetais sobre *Meloidogyne javanica*. *Nematologia Brasileira, 32*(4), 76-80.

Miamoto, A., Dias-Arieira, C. R., Cardoso, M. R., & Puerari, H. H. (2016). Penetration and reproduction of *Meloidogyne javanica* on leguminous crops. *Journal of Phytopathology, 164*(11-12), 890-895. https://doi.org/10.1111/jph.12508

Ribeiro, N. R., Dias, W. P., Homechin, M., Silva, J. F. V., & Francisco, A. (2007). *Avaliação da reação de espécies vegetais ao nematoide das lesões radiculares*. Proceedings of XXIX Reunião de Pesquisa de Soja da Região Central do Brasil, 2007, Campo Grande, Embrapa Soja.

Riggs, R. D. (1992). Host Range. In R. D. Riggs, & J. A. Wrather (Eds.), *Biology and Management of the Soybean Cyst Nematode* (pp. 107-114). St. Paul: The American Phytopathology Society.

Riggs, R. D., & Schmitt, D. P. (1988). Complete characterization of the race scheme for *Heterodera glycines*. *Journal of Nematology, 20*(3), 392-395.

Rosa, J. M. O., Westerich, J. N., & Wilcken, S. R. (2013). Reprodução de *Meloidogyne javanica* em olerícolas e em plantas utilizadas na adubação verde. *Tropical Plant Pathology, 38*(2), 133-141. https://doi.org/10.1590/S1982-56762013000200007

Rosa, J. M. O., Westerich, J. N., & Wilcken, S. R. S. (2015). Reprodução de *Meloidogyne enterolobii* em olerícolas e plantas utilizadas na adubação verde. *Revista Ciência Agrônomica, 46*(4), 826-835. https://doi.org/10.5935/1806-6690.20150071

Ross, J. P. (1962). Physiological strains of *Heterodera glycines*. *Plant Disease Reporter, 46*(1), 766-799.

Rossi, C. E., & Ferraz, L. C. C. B. (2001). Efeito de diferentes plantas hospedeiras sobre a morfometria de uma população brasileira de *Heterodera glycines*. *Arquivos do Instituto Biológico, 68*(1), 95-102.

Sanches, J. B. (2001). *Histopatologia de raízes de Mucuna pruriens (L.) DC infectadas pelo nematoide de cistos da soja, Heterodera glycines*. Viçosa, MG: Universidade Federal de Viçosa.

Santana, S. M., Dias-Arieira, C. R., Biela, F., Cunha, T. P. L., Chiamolera, F. M., Roldi, M., & Abe, V. H. F. (2012). Antagonistic plants in the management of *Meloidogyne incognita*, in sandy soil of vegetables growing areas. *Nematropica, 42*(2), 287-294.

Santos, P., Rebelato, G., Favera, D. D., Sotto, R. D., Balardin, R., & Madalosso, M. G. (2014). Mapa dos nematoides. *Revista Cultivar, 187*(1), 12-15.

Santos-Junior, R. F., Santos, J. M., Rudorff, B. F., & Marchiorato, I. A. (2002). Detecção de *Heterodera glycines* em plantios de soja mediante espectrorradiometria no visível e infravermelho próximo. *Fitopatologia Brasileira, 27*(4), 355-360. https://doi.org/10.1590/S0100-41582002000400004

Schmitt, D. P., & Noel, G. R. (1984). Nematodes parasites of soybean. In W. R. Nickle (Ed.), *Plant and Insect Nematodes*. New York (NY): Marcel Dekker.

Schmitt, D. P., & Riggs, R. D. (1989). Population dynamics of *Heterodera glcyicnes* in the Southeastern United States. *Variability and Population Dynamics of Roots-Knot and Cyst in the Southern Region of the United State*. Texas (TX): The Texas A & M University System.

Schwan, A. V. (2003). *Antagonismo de espécies de crotalária ao nematoide de cisto da soja (Heterodera glycines Ichinohe)* (Master's thesis, Universidade Federal de Mato Grosso do Sul, Dourados, MS).

Silva, B. B., Mendes, F. B. G., & Kageyama, P. Y. (2009). *Crotalárias*. Piracicaba (SP): USP-Escola Superior de Agricultura "Luiz de Queiroz".

Silva, F. A. Z., & Azevedo, C. A. V. (2016). The Assistat Software Version 7.7 and its use in the analysis of experimental data. *African Journal Agricultural Research, 11*(39), 3733-3740. https://doi.org/10.5897/AJAR2016.11522

Silva, G. S., Ferraz, S., & Santos, J. M. (1989). Atração, penetração e desenvolvimento de larvas de *Meloidogyne javanica* em raízes de *Crotalaria* spp. *Nematologia Brasileira, 13*(1), 151-163.

Silva, G. S., Ferraz, S., & Santos, J. M. (1990). Histopatologia de raízes de *Crotalaria* parasitadas por *Meloidogyne javanica*. *Fitopatologia Brasileira, 15*(1), 46-48.

Silva-López, R. E. D., & Pacheco, J. D. S. (2010). Genus *Crotalaria* L. (Leguminoseae). *Revista Fitos, 5*(3), 43-52.

Soares, P. L. M., Carvalho, R. B., Ferreira, R. J., Santos, J. M., Santos, T. F. S., Passos, A. D., & Neto, J. A. D. (2015). *Primeira ocorrência de Heterodera glycines R3 em Crotalaria ochroleuca*. Proceedings of Congresso Brasileiro de Nematologia, 2015, Londrina, Sociedade Brasileira de Nematologia.

Valle, L. A. C., Dias, W. P., & Ferraz, S. (1996). Reação de algumas espécies vegetais, principalmente leguminosas, ao nematoide de cisto da soja, *Heterodera glycines* Ichinohe. *Nematologia Brasileira, 20*(1), 30-40.

Vedoveto, M. V. V., Dias-Arieira, C. R., Rodrigues, D. B., Arieira, J. O., Roldi, M., & Severino, J. J. (2013). Adubos verdes no manejo de *Pratylenchus brachyurus* em soja. *Nematropica, 43*(1), 226-232.

Wang, K. H., Sipes, B. S., & Schmitt, D. P. (2002a). Crotalaria as a cover crop for nematoide management: A review. *Nematropica, 32*(1), 35-57.

Wang, K., Sipes, B. S., & Schmitt, D. P. (2002b). Suppression of *Rotylenchulus reniformis* by *Crotalaria juncea*, *Brassica napus* and *Tagetes erecta*. *Nematropica, 31*(2), 237-251.