academicJournals

Vol. 11(25), pp. 2245-2249, 23 June, 2016
DOI: 10.5897/AJAR2016.10947
Article Number: 95CC6EA59118
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

The role of *Crambe abyssinica* in the control of *Heterodera glycines* (Thylenchida: Heteroidae)

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Received 28 February, 2016; Accepted 6 May, 2016

The soybean cyst nematode (SCN), *Heterodera glycines*, is present in more than 150 municipalities in Brazil, and the long persistence of the cysts in the soil combined with the severe degree of parasitism induces high soybean production losses. The objective of this study was to evaluate the biofumigant effect of *Crambe abyssinica* on the SCN population in the second soybean crop season. The experiment was conducted on a rural property whose soil was naturally infested with SCN. *Crambe abyssinica* was planted in the second crop season following harvest, and in the subsequent crop season, 4 soybean varieties, 2 resistant and 2 susceptible to SCN, were planted. The nematode population was evaluated every month for 90 days after planting. In the second crop season, when *C. abyssinica* was in the field, there was a significant decrease in the number of adult SCN females and cysts. During the 90-day period after *C. abyssinica* cultivation, when plant residues were incorporated into the soil and the area was planted with soybeans, both the number of adult SCN females / 10 g roots and the number of cysts decreased. This result indicates that *C. abyssinica* reduced the nematode population during its time in the field.

Key words: Crambe, *Glycine max*, soybean nematode cyst, culture control.

INTRODUCTION

The acreage devoted to soybean [*Glycine max* (L.) Merr.] has grown more than for any other crop in Brazil over recent harvest seasons. However, some factors limit high grain yields in this crop, including the more than 100 nematode species, of approximately 50 genera, that are considered soybean pests worldwide. In Brazil, the most harmful nematodes to this crop are those that form galls (*Meloidogyne* spp.), cysts (*Heterodera glycines*), and root Lesions (*Pratylenchus brachyurus*) as well as reniform nematodes (*Rotylenculus reniformis*) (Dias et al., 2009).

The soybean cyst nematode (SCN), *H. glycines*, is a major soybean pest because of the damage it can cause and the ease of dissemination. It penetrates plant roots and hinders the absorption of water and nutrients, thereby reducing the size and number of pods and causing chlorosis and low productivity (Liu et al., 2012).

The control of phytonematodes over large areas, including those planted with annual crops, is most often

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performed with chemical nematicides, but no chemicals are registered for the control of *H. glycines* in soybean. Despite studies indicating the efficacy of biological agents in the control of other species, no biological nematicides are currently available for the control of SCN in soybeans (MAPA, 2015).

Another management strategy involves the use of resistant plant varieties, but due to the large genetic variability in SCN populations, such plants are not always available for the control of the particular nematode races present in a cultivated area. Therefore, crop succession and rotation are especially important strategies for the control of SCN populations (Franzener et al., 2005).

Crambe abyssinica belongs to the family Brassicacea, and its seed oil concentration is approximately 40%, which has favored its use as a raw material for the production of biodiesel (Lima et al., 2015). After harvest, its crop residues are incorporated into the soil, and because its tissues contain glucosinolates, this incorporation promotes biofumigation of the soil and the consequent reduction in nematode populations (Anita, 2012).

Glucosinolate metabolism results in the production of toxic substances, which act as natural barriers against pathogens and pests (Berbegal et al., 2008; Pitol et al., 2010), and Pal et al. (2009) argue that these compounds, which are primarily found in the Brassicaceae family, have several biological properties, including protection against pathogens and weeds. In the presence of the enzyme myrosinase, such as when plants are cut or chewed, glucosinolates are hydrolyzed to various products, including isothiocyanates, thiocyanates, and indoles.

Isothiocyanates (ITCs) are volatile compounds and toxic by-products of glucosinolate hydrolysis (Wu et al., 2011), and of the various ITCs that have been identified, allyl isocyanate (ITCA) has been found to be the most effective in the control of nematodes such as *Meloidogyne javanica*, *Tylenchulus semipenetrans*, *H. glycines, M. incognita, M. hapla, H. schachtii*, *Pratylenchus penetrans*, and *P. neglectus* (Zasada and Ferris, 2003; Yu et al., 2007).

The objective of this study was to evaluate the effect of *C. abyssinica,* succession culture with soybean in the reduction of SCN under field conditions.

MATERIALS AND METHODS

The experiment was conducted in the municipality of Ipameri in the state of Goias, Brazil (latitude: 17°43'19" S, longitude: 48° 09'35" W, altitude: 764 m). In the previous crop season, soybean that was susceptible to *H. glycines* was grown in an area naturally infested with the species (890 cysts / 100 cm^3). The area was sampled to quantify the initial nematode population, after which *C. abyssinica* cv. FMS Brilhante was sown and variety of soybean NA7337RR (susceptible to SCN). During the crop cycle, soil and root samples were collected at 30, 60, and 90 days after planting (DAP), placed In labeled plastic bags and transferred to the Nematology Laboratory of the Federal Institute of Goias, Urutai Campus.

Female nematodes were collected by filtering the roots through 60 mesh sieves and washing them with a strong jet of water. They were subsequently transferred to beakers and counted using a stereoscopic microscope. To collect cysts from the soil, a 100-cm^3 aliquot was obtained and transferred to a 1-L becker, and the volume was completed with water. After 30 s, the supernatant was filtered using 20 mesh sieves about 60 mesh. This procedure was repeated 3 times, and after collection, the cysts were quantified using a stereoscopic microscope. The parameters evaluated were the number of adult females in the roots and the number of cysts in the soil.

Once sampling was complete, the reproduction factor (RF) of the SCN in *C. abyssinica* plants and for soybean variety NA7337 was calculated as RF = fp / ip, where RF is the reproduction factor, fp is the final population of the nematode, and ip is the initial population of the nematode (previous sampling). After harvest, the *C. abyssinica* plant residue was incorporated into the soil, and the second part of the experiment was performed. Four treatments were adopted: Soybean AS3730 and NS5959 (both without information on the SCN behavior), NA7337RR (susceptible to SCN), and P98Y51 (resistant to SCN races 1 and 3) in 6 replicates, totaling 24 plots, in a randomized block design. These treatments were adopted for to check if crambe had nematicide effect on the population of *H. glycines* or just nematostatic effect. Using variety with different behaviors in relation to *H. glycines*, the influence of root exudates in the population of this nematode was observed. This exudation favors the outbreak of juvenile when using susceptible variety.

Each plot consisted of 6.6 m long rows. The 2 end rows served as borders; the 2 central rows were used to evaluate productivity, and the 2 remaining rows were used to evaluate the SCN population. Two subsamples were collected from each row, totaling 4 subsamples per plot, and each sample contained both soil and roots.

The evaluations were performed 30, 60, and 90 days after sowing, and the samples were placed in labeled plastic bags and transferred to the Nematology Laboratory of the Federal Institute of Goias, Urutai Campus. The evaluated parameters were the number of female nematodes in the roots and the number of viable cysts in the soil, as previously detailed.

The data were subjected to analysis of deviance (ANODEV), and regression models were fit with predictors containing a simple linear effect. The Poisson distribution (Poisson regression) was assumed for the variables of number of female nematodes, eggs per female, and number of cysts, and the nominal level of significance was set at 5%. All analyses were performed using R software version 3.0.3 (Team RC, 2014).

RESULTS AND DISCUSSION

There was a linear effect ($p < 0.001$) of the duration of the plant in the field on the number of female SCNs in the roots (Figure 1A). After 30 days, the mean number of female nematodes was approximately 9-fold greater than at 90 days, indicating that the SCN population decreased over time.

In contrast to crambe, the population of *H. glycines* females in soybeans remained high in the three evaluation periods. It is higher at 60 days after planting. The number of cysts significantly increased over time. Going from 300 cysts/100 cm^3 soil at 30 days after planting to 907 cysts/100 cm^3 soil at 90 days after planting (Table 1).

Table 1. Population of females de *H. glycines* and cysts in crambe crop and soybeans at 30, 60 and 90 days after planting.

Assessment	Crambe			Soybean		
	Days after planting			Days after planting		
	30	60	90	30	60	90
Females (10 g/root)	68	13	7	590	775	580
Cysts (100 cm^3)	121	91	19	300	501	907

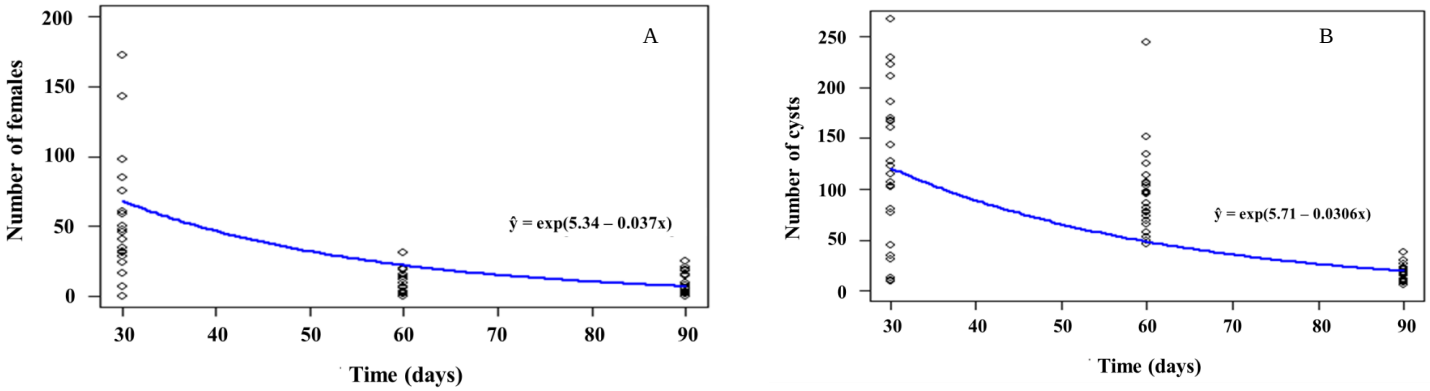

Figure 1. Analysis of deviance. (A) Number of female *H. glycines* as a function of the duration of *C. abyssinica* in the field. (B) Number of cysts as a function of the duration of *C. abyssinica* in the field.

A linear effect ($p < 0.001$) of the duration of the crops in the field on the number of cysts was also observed, but this relationship was inversely proportional (Figure 1B). After 30 days, the mean number of cysts was approximately 6-fold greater than at 90 days, following the same trend as the number of female nematodes.

This result demonstrates the activity of *C. abyssinica* against SCN because the number of viable cysts decreased from 890 cysts / 100 cm^3 to approximately 25 cysts / 100 cm^3 during the 90 days of cultivation. Similar results were found by Zasada and Ferris (2003), who observed a lethal effect of this crop on *H. schachtii* and *Globodera rostochiensis*, which was associated with compounds originating from the isothiocyanates.

After the crop residues were incorporated into the soil, the effect of the interaction ($p < 0.001$) between crop duration and variety on the number of adult females was evaluated in 10 g of roots (Figure 2). At 30 days, the mean number of female nematodes in variety AS3730 IPRO was lower ($p < 0.05$) than that in the other varieties, but no significant differences ($p > 0.05$) were observed between varieties NS5909 (without information on the SCN behavior), NA7337RR (susceptible on the SCN behavior), and P98Y51 (resistant on the SCN behavior). At 60 days, there was a significant difference ($p < 0.05$) among the varieties, and the number of female SCNs was higher in variety NA7337RR (susceptible). At 90 days, there were no significant differences ($p > 0.05$) among varieties NS 5959, AS3730, and NA7337RR, which indicates their relative susceptibility to SCN. However, the number of adult females remained low in all cultivars and times evaluated, indicating the harmful effect of *C. abyssinica* on female SCNs.

Wu et al. (2011) emphasize that the disadvantage of performing crop rotation with *Brassica* to control nematodes is that the levels of isothiocyanates released into the soil are unknown. In contrast, Zasada and Ferris (2003) evaluated the effects of various isothiocyanates on *Meloidogyne javanica* and *Tylenchulus semipenetrans* and found that low concentrations of these compounds (0.025 µmol / mL to 0.045 µmol / ml) decreased nematode populations. Zasada and Ferris (2009) worked with *M. incognit*a and managed to reduce the number of second stage juveniles, the egg mass, and the gall index using a concentration of 0.03 mmol / mL of isothiocyanate.

Our results indicate that *C. abyssinica* decreased the nematode population for up to 30 days after its cultivation and that the use of resistant plant varieties maintained the number of adult females at low levels. This result confirms the resistance of variety D (P98Y51), which linearly decreased the female population for 90 days. Poromarto and Nelson (2010) evaluated the invasion potential of *H. glycines* in several plant crops and found that SCN populations did not grow in *C. maritima*. This result corroborates the findings of the present study by inferring that *Crambe* sp. are poor hosts of and active against *H. glycines*. A similar result was observed by Wu

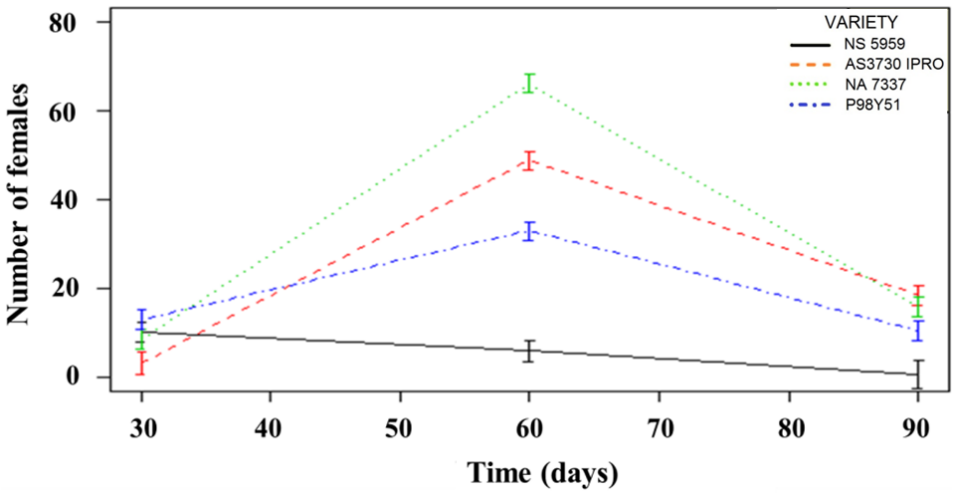

Figure 2. The number of female SCNs and the 95% confidence intervals for each combination of plant variety and duration in the field. Soybean varieties: NS5959; AS3730; NA7337RR; and P98Y51.

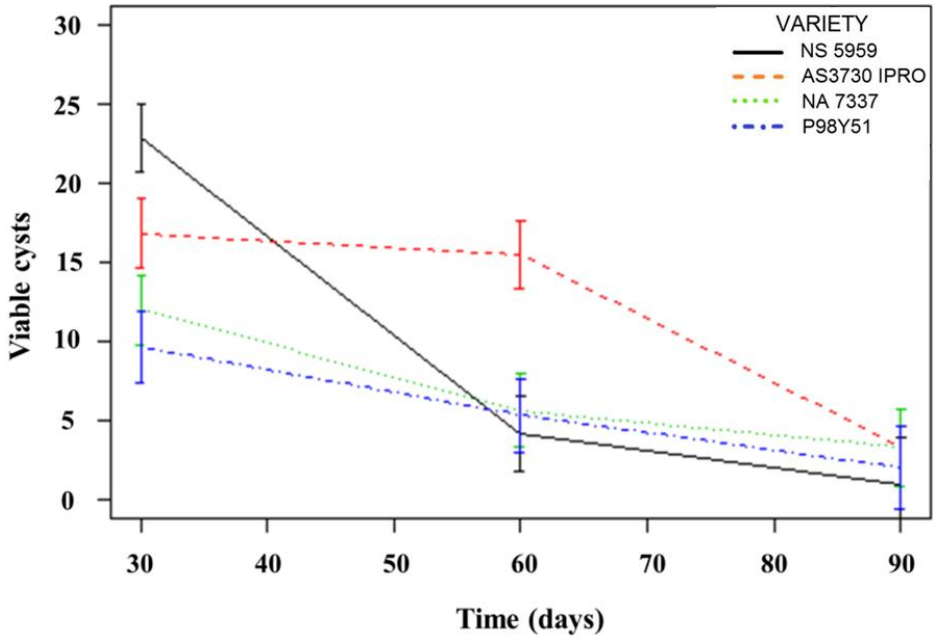

Figure 3. 95% confidence interval for each combination of plant variety and duration in the field. (A) Number of viable cysts. NS5959; AS3730; NA7337RR; and, P98Y51.

et al. (2011), who evaluated the mortality rate of second-stage juvenile *M. javanica* and found that it increased with the duration of exposure to *C. abyssinica* metabolites. The authors attributed this mortality to the presence of aliphatic isothiocyanates formed by the hydrolysis of glucosinolates.

Our results indicated a decrease in the number of viable cysts in all plant varieties from 30 to 90 days (Figure 3A). At 30 days, resistant variety D (P98Y51) and susceptible variety C (NA7337RR) had the lowest number of viable cysts ($p > 0.05$), while the varieties A (NS5959) and B (AS3730 IPRO) had the highest.

After 60 days, variety B (AS3730) differed significantly from the remaining varieties and had the greatest number of viable cysts, and at 90 days, the total population of viable SCN cysts was low, with no significant difference among the crop varieties. This result emphasizes the value of *C. abyssinica* in the control of *H. glycines*

because prior sampling indicated a population of 890 viable cysts / 100 cm^3. The number of female nematodes and cysts decreased in the presence of *C. abyssinica* in the field and in the subsequent soybean.

The harmful effect of *C. abyssinica* against many pathogens has been reported in the literature, and this effect has always been associated with the presence of isothiocyanates. Schroeder and MacGuidwin (2010) evaluated the LD_{50} and LD_{95} of isothiocyanates for juvenile *H. glycines* and found that even low concentrations are toxic to SCN populations.

Conclusion

The cultivation of *C. abyssinica* and the incorporation of its crop residues into the soil promotes biofumigation, which decreases the population of *H. glycines*.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors are grateful to farmers Sonia Bonato and Nilton Bonato for providing the field for the experiment.

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