Application of herbicides in green cover crops to reduce *Meloidogyne javanica* inoculum in soybean plants

Aplicación de herbicidas en cultivos de cobertura verde para reducir el inóculo de *Meloidogyne javanica* en plantas de soya

Carolina Yumi Futigami[1], Angélica Calandrelli[2*], and Cláudia Regina Dias-Arieira[1]

ABSTRACT

Nematodes are among the main plant parasites affecting Brazilian agriculture. Management practices involving the use of antagonistic or non-host plants are crucial for combating *Meloidogyne javanica* populations in the country. However, there is still limited information on the effects of herbicides on nematode populations. The aim of this research was to evaluate the impact of herbicides applied to crops on *M. javanica* populations in soybean and to examine the direct effect of herbicide products on the hatching of nematode juveniles. We conducted greenhouse and *in vitro* experiments. In the greenhouse experiment, soybean plants were grown in pots with sterile substrate, and a soybean seed was inoculated with 2000 nematodes per plant. The plants grew for 60 d, after which they were cut, and the following cover crops were planted: *Crotalaria spectabilis*, pigeon pea (*Cajanus cajan*), *Stylosanthes*, and buckwheat (*Fagopyrum esculentum*) for 60 d. These cover crops were desiccated with one of the following three herbicides: fomesafen, chlorimuron, or bentazone. Subsequently, soybeans were replanted and cultivated for additional 60 d. *In vitro* assays were used to determine the hatching percentage of *M. javanica* eggs exposed to herbicides. Both tests were repeated at different times of the year (Trials 1 and 2). *Meloidogyne javanica* reproduction was higher on soybean crops grown when buckwheat was a cover crop. Herbicide application reduced total nematode numbers in soybean grown in succession to buckwheat compared with the untreated control. The vegetative development of soybean crops was negatively influenced by herbicide treatment of cover crops, especially with the use of chlorimuron. Bentazone and fomesafen did not affect nematode hatching *in vitro*.

Key words: antagonistic plant, crop management, crop rotation, root-knot nematode.

RESUMEN

Los nematodos se encuentran entre los principales parásitos de las plantas que afectan la agricultura brasileña. Las prácticas de manejo que involucran el uso de plantas antagónicas o no hospederas son cruciales para el combate de las poblaciones de *Meloidogyne javanica* en el país. Todavía hay poca información sobre el efecto de los herbicidas en las poblaciones de nematodos. El objetivo de la presente investigación fue evaluar el efecto de los herbicidas aplicados a los cultivos de cobertura en las poblaciones de *M. javanica* en la soya y examinar el efecto *in vitro* de los herbicidas en la eclosión de los nematodos juveniles. Se realizaron experimentos de invernadero e *in vitro*. En el experimento de invernadero, las plantas de soya se cultivaron en macetas con substrato estéril y luego se sembró una semilla de soya la cual se inoculó con 2000 nematodos por planta. Las plantas crecieron durante 60 d, luego se cortaron y se sembraron los siguientes cultivos de cobertura: *Crotalaria spectabilis*, guandú (*Cajanus cajan*), *Stylosanthes* y trigo sarraceno (*Fagopyrum esculentum*) durante 60 d y se desecaron con uno de los siguientes tres herbicidas: fomesafen, clorimuron o bentazon. Posteriormente, se volvió a sembrar soya y se cultivó durante 60 d adicionales. Se realizaron ensayos *in vitro* para determinar el porcentaje de eclosión de huevos de *M. javanica* expuestos a los herbicidas. Ambas pruebas se repitieron en diferentes épocas del año (ensayos 1 y 2). La reproducción de *M. javanica* fue mayor en los cultivos de soya que crecieron después del trigo sarraceno. La aplicación de herbicidas redujo el número total de nematodos en la soya cultivada en sucesión al trigo sarraceno en comparación con el control no tratado. El desarrollo vegetativo de los cultivos de soya fue influenciado negativamente por el tratamiento herbicida de los cultivos de cobertura, especialmente con el uso de clorimuron. Bentazon y fomesafen no afectaron la eclosión de nematodos *in vitro*.

Palabras clave: planta antagónica, manejo de cultivos, rotación de cultivos, nematodo del nudo radical.

Received for publication: July 6, 2023. Accepted for publication: October 19, 2023.

Doi: 10.15446/agron.colomb.v41n3.109740

[1] Graduate Program in Agricultural Sciences, State University of Maringá, Umuarama (Brazil).
[2] Graduate Program in Agronomy, State University of Maringá, Maringá, (Brazil).
[*] Corresponding author: a.calandrelli@hotmail.com

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Introduction

High host diversity, management complexity, and damage potential of plant-parasitic nematodes place these pests among the major limiting factors affecting agricultural production worldwide. Nematode parasitism causes annual losses of 10-15% of soybean crops (*Glycine max* (L.) Merrill), amounting to more than US $80 billion globally (Lima *et al.*, 2017). In Brazil, the world's largest producer of this oilseed, annual losses can reach US $4.1 billion (Ralmi *et al.*, 2016; Lima *et al.*, 2017; Favoreto *et al.*, 2019).

Root-knot nematodes (*Meloidogyne* spp.) are obligate biotrophs. The most frequent and aggressive species associated with soybean crops is *Meloidogyne javanica* (Treub, 1885), widely distributed in areas where they are grown (Jones *et al.*, 2013; Mattos *et al.*, 2016; Favoreto *et al.*, 2019; Mazzetti *et al.*, 2019). Because of the complexity of interactions between root-knot nematodes and their hosts, managing these pathogens requires integrated strategies. Crop rotation with non-host, bad host, resistant, or antagonistic crops is highly recommended (Fileti *et al.*, 2011; Favoreto *et al.*, 2019). Furthermore, many plants used in crop rotation have the added benefits of adding organic matter to the soil, increasing soil microbial activity, and improving physical, chemical, and biological properties of the soil (Oka, 2010; Debiasi *et al.*, 2016; Franchini *et al.*, 2018).

For the success of pest management, it is necessary to carefully select the most adequate plant species for each crop rotation, as crop rotations can reduce nematode populations. However, in view of the polyphagous nature of *M. javanica*, it is important to bear in mind that some plant species may be suitable hosts for plant-parasitic nematodes. Another potential problem is that rotation crops may act as weeds for the next crop, serving as a nematode reservoir in the off-season (Braz *et al.*, 2016; Matias *et al.*, 2018; Favoreto *et al.*, 2019).

Herbicides may indirectly influence nematode management by controlling weeds or rotation species that serve as alternative hosts (Werle *et al.*, 2013; Matias *et al.*, 2018). Herbicide treatment may also have direct effects by reducing juvenile hatching (Wong *et al.*, 1993; Levene, 1995), soil populations (Castro-Carvajal *et al.*, 2015), and nematode development and reproduction (Nelson *et al.*, 2006; Riboldi *et al.*, 2013; Barbosa *et al.*, 2014; Kashiwaki *et al.*, 2020). Because root-knot nematodes depend on host plants, whether rotation, weed, or volunteer plants (Jones *et al.*, 2013; Rodiuc *et al.*, 2014; Ferraz & Brown, 2016), these parasites may die of starvation in case of rapid plant death and degradation of root tissues. Despite this, information on interactions between herbicides, cover crops, and nematode management in crop rotations is virtually non-existent.

The aims of this study were to assess the effect of herbicide application to cover crops on *M. javanica* populations in soybean and evaluate the direct effect of herbicide products on the hatching of second-stage juveniles (J2) *in vitro*.

Materials and methods

Meloidogyne javanica reproduction and soybean development in succession to cover crops subjected to herbicide treatment

This study was conducted in a greenhouse (23°47'34.5" S; 53°15'22.1" W) according to a completely randomized design with a 4×4 (herbicide treatment × cover plant) factorial arrangement, seven replicates for each treatment, and two experimental trials. Trial 1 lasted from November 6, 2020 to May 20, 2021. The mean daily minimum and maximum temperatures during this period were 20°C and 32°C. Trial 2 was conducted from December 14, 2020 to June 27, 2021, when the mean daily minimum and maximum temperatures were 19°C and 29°C, respectively.

Each experimental unit consisted of a polystyrene cup containing 500 cm^3 of substrate (soil (Tab. 1) and sand at a volumetric ratio of 2:1, autoclaved for 2 h at 120°C), and planted with one seed of soybean M6410 IPRO (seed provided by Cocamar, Maringá, Brazil).

Five days after emergence, each seedling was inoculated with 2000 eggs and J2 of *M. javanica* by pipetting a nematode suspension into a 2 cm deep hole in the soil close to the plant root. The nematode inoculum was extracted from pure populations maintained on soybean plants in a greenhouse. Nematodes were extracted from the root system by the method of Hussey and Barker (1973) as adapted by Bonetti and Ferraz (1981). The suspension was adjusted to 2000 eggs + J2 ml^{-1} in a Peters chamber under an optical microscope. Plants were maintained in the greenhouse (12 h photoperiod) and irrigated daily as needed. Plants were fertilized once with 3 g of Osmocote® (15% N, 9% P_2O_5, 12% K_2O, 1% Mg, 2.3% S, 0.05% Cu, 0.45% Fe, 0.06% Mn, 0.02% Mo).

At 60 d after inoculation, the aerial part of the soybean plants was cut and discarded. Then, the following cover crop treatments were sown: (i) buckwheat (*Fagopyrum esculentum*) IPR-92 Altar (ii) pigeon pea (*Cajanus cajan*) IAPAR 43, (iii) *Stylosanthes* 'Campo Grande', and (iv)

TABLE 1. Chemical characteristics of soil samples collected in Umuarama, Paraná, Brazil, for use in the experiments.

pH $CaCl_2$	P	OM	Ca	K	Mg	Al	CEC	BS
	mg dm^{-3}	---%---	------------------cmolc dm^{-3}----------------					--%--
5.04	2.46	0.27	1.50	0.09	0.25	0.18	3.59	51.27

OM - organic matter, CEC - cation-exchange capacity, BS - base saturation.

Crotalaria spectabilis. Cover crop plants were grown for 60 d under the same conditions as the soybeans. After this period, the following herbicide treatments were applied: (i) fomesafen (Flex®, Syngenta, 250 g active ingredient (a.i.) L^{-1}, rate of 1 L ha^{-1}, spray volume of 300 L ha^{-1}), (ii) chlorimuron-ethyl (Classic®, FMC, 250 g a.i. L^{-1}, rate of 80 g ha^{-1}, spray volume of 300 L ha^{-1}), and (iii) bentazone (Basagran® 600, BASF, 600 g a.i. L^{-1}, rate of 1.2 L ha^{-1}, spray volume of 250 L ha^{-1}). The herbicides were applied using a pressurized backpack sprayer with CO_2, equipped with flat fan nozzles 110.015, providing a spray volume of 120 L ha^{-1}. Plants without herbicide treatment were subjected to manual weeding and used as control.

After 15 d without irrigation, plant residues were weighed and deposited in the same pots from which they were cut. Pots were sown with soybean, and plants were cultivated for 60 d under the same conditions mentioned before. After this period, soybean plants were harvested and the root system was carefully separated from shoots, washed, and placed on absorbent paper to remove excess water. Root fresh weight was determined; and root samples were subjected to nematode extraction, as described above. Total nematode numbers were counted using a Peters chamber under an optical microscope. The number was divided by root weight to determine the nematode population density (nematodes g^{-1} root).

Shoots were evaluated with a measuring tape for shoot height and fresh and dry weights were determined using a semi-analytical scale (Gehaka, 0.001 g, BG 440). For dry weight determination, shoots were dried in a forced-air oven at 65°C for 72 h until constant weight was achieved. Figure 1 depicts a timeline schedule of experiments 1 and 2.

Effect of herbicides on *Meloigogyne javanica* hatching

The experiment was conducted in duplicate, following a completely randomized design with five treatments: 1) control - distilled water; 2) bentazone; 3) chlorimuron; 4) fomesafen, and 5) abamectin. The experimental unit consisted of a polypropylene Falcon tube to which 4 ml of herbicide (at the rate used under greenhouse conditions) and 1 ml of a suspension containing 100 eggs of *M. javanica* were added. Six replicates were used, meaning that each treatment was applied to six experimental units. Egg masses of *M. javanica* were collected from soybean roots, placed in a plastic tube containing 0.10% sodium hypochlorite, and shaken manually for 1 min to detach the eggs. The mixture was sieved through a 60-mesh sieve placed on top of a 500-mesh sieve under running water. The eggs were collected from the bottom sieve and the suspension was calibrated using an optical microscope. The control consisted of distilled water and an abamectin-based nematicide (Avicta® Completo, Syngenta, 500 g a.i. L^{-1}, rate of 60 ml 100 kg^{-1} seeds and a spray volume of 200 L ha^{-1}). Tubes were incubated at 27°C in the dark for 10 d with manual shaking for 2 min three times a day. The hatching percentage was determined by counting the number of hatched juveniles and remaining eggs using a Peters chamber under an optical microscope.

Statistical analysis

The data were subjected to analysis of variance, and when significant, means were compared by the Scott–Knott test at $P<0.05$ using SISVAR software (Ferreira, 2011).

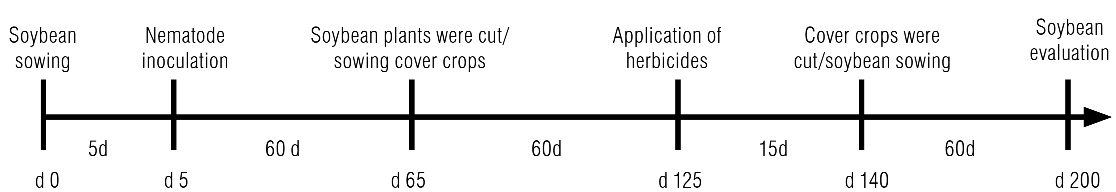

FIGURE 1. Timeline schedule of 2 experimental trials, conducted in a greenhouse.

Results and discussion

Meloidogyne javanica reproduction and soybean development in succession to cover crops subjected to herbicide treatment

There was significant plant cover × herbicide treatment effects on total nematode number and nematode population density in both trials (Tabs. 2-3). Soybean grown after buckwheat had higher nematode populations in both trials.

TABLE 2. Total numbers of *Meloidogyne javanica* in roots of soybean plants grown in succession to crops cover subjected to herbicide treatment after nematode inoculation.

Crop Cover	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	51,774 aA	28,533 aB	3,473 aC	19,109 aB
Pigeon pea	3,772 bA	522 bA	1,000 bA	1,898 bA
Stylosanthes	957 bA	271 bA	221 cA	284 bA
Crotalaria	1,310 bA	758 bA	121 cA	104 bA
CV (%)	71.72			
	Trial 2			
Buckwheat	15,750 aA	5,400 aB	325 aC	937 aC
Pigeon pea	450 bA	25 bA	225 aA	175 aA
Stylosanthes	180 bA	75 bA	175 aA	300 aA
Crotalaria	154 bA	125 bA	262 aA	175 aA
CV (%)	80.67			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P<0.05$ by the Scott–Knott test. CV – coefficient of variation. Original data were transformed to $\sqrt{x}+0.5$ before analysis. The species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

In assessing differences between plant cover within each herbicide treatment, we observed that total nematode number was higher in soybeans grown after buckwheat in Trial 1, regardless of herbicide application (Tab. 2). In Trial 2, we observed no differences between the plant cover in treatments receiving either chlorimuron or fomesafen. Herbicide application led to a decrease in total nematode number in soybeans grown after buckwheat, compared with the control without herbicide, in Trials 1 and 2. The other treatments did not differ from the control. Therefore, in general, variations in nematode populations were only observed in soybeans grown after buckwheat, which allowed for higher populations of *M. javanica* in soybean.

The results of nematode population density (Tab. 3) were similar to those of total nematode number. We found that nematode population density was higher in soybeans grown in succession to buckwheat in Trial 1, regardless of herbicide application. In Trial 2, there were no differences between plant cover treated with chlorimuron or fomesafen.

TABLE 3. Number of *Meloidogyne javanica* per gram of roots of soybean grown in succession to crop cover subjected to herbicide treatment after nematode inoculation.

Crop Cover	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	8,481 aA	9,549 aA	1,728.8 aB	9,419.4 aA
Pigeon pea	907.8 bA	466.2 bA	966 bA	1,125.8 bA
Stylosanthes	235.7 bA	161 bA	84.1 cA	98.2 cA
Crotalaria	291 bA	342.8 bA	54.2 cA	36.2 cA
CV (%)	66.85			
	Trial 2			
Buckwheat	6,064.1 aA	6,777.5 aA	754.4 aB	600.1 aB
Pigeon pea	387 bA	41.7 bA	750.2 aA	193 aA
Stylosanthes	96.8 bA	135.5 bA	388 aA	252.8 aA
Crotalaria	113.3 bA	273.7 bA	508.5 aA	219.5 aA
CV (%)	71.67			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P<0.05$ by the Scott–Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x}+0.5$ before analysis. The species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

Regarding the effects of herbicides within each plant cover treatment, differences were observed only in treatments with buckwheat. Chlorimuron led to a reduction in nematode population density in Trial 1 compared with the other treatments and chlorimuron and fomesafen led to reductions in the variable in Trial 2.

Overall, differences in *M. javanica* reproduction were only observed when buckwheat was used as a crop cover; nematode reproduction was higher in soybeans grown after buckwheat, although chlorimuron led to a reduction in pathogen multiplication. Buckwheat had varied responses to nematodes and are considered susceptible to *Meloidogyne* spp. (Melo *et al.*, 2022). In a study assessing the response of green manure to *M. javanica* populations, Chidichima *et al.* (2021) observed a reproduction factor (RF) of 2.94 to 53.46 on buckwheat, explaining the large nematode population in soybeans grown in succession to buckwheat.

Given the susceptibility of buckwheat to *M. javanica*, care must be taken when using this crop cover. However, buckwheat should not be disregarded completely, as it is an important component for the maintenance of crop systems, acting in the cycling of macro- and micronutrients (Klein *et al*., 2010; Gonçalves *et al*., 2016), improving soil microbiota (Kautz *et al*., 2004; Tejada *et al*., 2008), and favoring the growth of other plant species with the production of soil organic matter (Yadav *et al*., 2000; Gonçalves *et al*., 2016). Furthermore, buckwheat extract was found to be rich in polyphenols, including rutin and quercetin, tannins, and aldehydes such as salicylaldehyde that may be responsible for the plant nematicidal activity against *Meloidogyne* spp. (Kalinova *et al*., 2011; Aissani *et al*., 2018). Use of this crop coverage on lands affected by root-knot nematodes requires planning and monitoring.

Green manure species may behave as weeds after successive crops, given their high contribution to the soil seed bank, leading to their classification as remnant plants (Soltani *et al*., 2011). As such, cover crops may serve as alternative hosts to nematodes in the field (Braz *et al*., 2016; Matias *et al*., 2018).

The reduction in *M. javanica* reproduction on soybeans succeeding chlorimuron-treated buckwheat might be related to herbicidal effects. Chlorimuron is a systemic sulfonylurea capable of quickly inducing plant death (Oliveira Junior *et al*., 2011). These and previous findings underscore the importance of managing invasive species as a delay. Lack of control of nematode-susceptible plants may promote an increase in the population of remaining nematodes (Nelson *et al*., 2006; Werle *et al*., 2013; Castro-Carvajal *et al*., 2015; Kashiwaki *et al*., 2016; Kashiwaki *et al*., 2020).

Crotalaria spectabilis, *Stylosanthes*, and *Cajanus cajan* had the best nematode reduction potential. Studies have shown that *C. spectabilis* is effective in suppressing *M. javanica* reproduction (Inomoto *et al*., 2008; Miamoto *et al*., 2016; Soares *et al*., 2022). *Crotalaria spectabilis* is known to be antagonistic and serve as a trap to nematodes (Warnke *et al*., 2008; Curto *et al*., 2015; Miamoto *et al*., 2016). The plant accumulates secondary metabolites with nematicidal action in the roots, such as pyrrolizidine alkaloid (monocrotaline) (Colegate *et al*., 2012).

In line with our results, Miamoto *et al.* (2016) observed low *M. javanica* penetration and reproduction in *Stylosanthes capitata* after 60 d of cultivation, indicating that the plant is not very attractive to the pathogen. In other studies, no galls were detected in the roots of *S. capitata*, with a reduction of up to 98.4% of nematode populations compared with the control, and so is classified as immune to nematodes (Lenne, 1981; Sharma, 1984).

Cajanus cajan was shown to reduce *M. javanica* populations (Miamoto *et al.,* 2016) and is used as a nematode-resistant standard, with reproduction factor (RF) values of 0.11 to 0.13 (Araújo Filho *et al*., 2010). Chidichima *et al.* (2021), however, observed RF values of 0.58-2.99 that varyied according to *M. javanica* populations, suggesting a controversial antagonistic effect of *C. cajan* (Miamoto *et al*., 2016) attributed to the different behaviors of cultivars (Araújo Filho *et al*., 2010).

In the current study, there were significant interactive effects of crop coverage and herbicide treatment on soybean height in both trials (Tab. 4). In general, when there were significant differences between treatments, only chlorimuron negatively influenced soybean height compared with the other herbicides.

TABLE 4. Height (cm) of soybean plants grown in succession to cover crops subjected to herbicide treatment after nematode inoculation.

Cover crop	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	34.1 ^{aA}	42.5 ^{aA}	21.5 ^{aB}	23.7 ^{bB}
Pigeon pea	34.7 ^{aA}	36.1 ^{aA}	25.1 ^{aB}	34.6 ^{aA}
Stylosanthes	39.4 ^{aA}	32.1 ^{aB}	27.1 ^{aB}	36.7 ^{aA}
Crotalaria	35.6 ^{aA}	35.2 ^{aA}	23.7 ^{aB}	40 ^{aA}
CV (%)	10.26			
	Trial 2			
Buckwheat	33.8 ^{bB}	45.2 ^{aA}	26.7 ^{bB}	40.6 ^{aA}
Pigeon pea	45.7 ^{aA}	44.1 ^{aA}	27.1 ^{bB}	42.6 ^{aA}
Stylosanthes	46.8 ^{aA}	42.5 ^{aA}	34.7 ^{aB}	42.1 ^{aA}
Crotalaria	40.3 ^{bA}	41.7 ^{aA}	33.1 ^{aA}	35.5 ^{aA}
CV (%)	9.51			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P<0.05$ by the Scott–Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x} + 0.5$ before analysis. Species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

Interaction effects were also exerted on root fresh weight in both trials (Tab. 5). In Trials 1 and 2, soybean plants grown after untreated buckwheat had higher root weight. In comparing herbicide treatments, we found that root weight was higher in soybean grown in succession to herbicide-free cover crops in both trials.

TABLE 5. Root fresh weight (g) of soybean plants grown in succession to crop covers subjected to herbicide treatment after nematode inoculation.

Cover crop	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	8.4 aA	2.7 aB	2.0 aB	2.0 aB
Pigeon pea	4.5 bA	1.7 aB	1.4 aB	2.1 aB
Stylosanthes	4.1 bA	1.5 aA	2.3 aA	2.4 aA
Crotalaria	5.1 bA	2.0 aB	1.8 aB	1.9 aB
CV (%)	23.27			
	Trial 2			
Buckwheat	2.8 aA	0.7 aC	0.4 aC	1.6 aB
Pigeon pea	1.3 bA	0.7 aB	0.3 aC	1.0 bA
Stylosanthes	1.7 bA	0.6 aC	0.5 aC	1.2 aB
Crotalaria	1.4 bA	0.4 aC	0.4 aC	0.8 bB
CV (%)	18.65			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P<0.05$ by the Scott–Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x}+0.5$ before analysis. Species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

Shoot fresh weight was influenced by interaction effects in Trial 1 (Tab. 6), wherein lower means were observed in soybeans grown after chlorimuron-treated buckwheat and pigeon pea and fomesafen-treated buckwheat. In Trial 2, only the main effects of herbicide treatment were significant, with higher means in soybeans grown after untreated plants.

TABLE 6. Shoot fresh weight (g) of soybean plants grown in succession to crop coverages subjected to herbicide treatment after nematode inoculation.

Cover crop	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	9.4 aA	7 aA	2.4 bB	2.3 bB
Pigeon pea	7.6 aA	5.3 aA	2.7 bB	5.8 aA
Stylosanthes	9.4 aA	5.8 aB	4.7 aB	6.8 aB
Crotalaria	10.1 aA	6.1 aB	3.7 aB	6.4 aB
CV (%)	21.09			
	Trial 2			
Mean	4.9 A	2.6 C	1.8 D	4.1 B
CV (%)	16.81			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P<0.05$ by the Scott–Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x}+0.5$ before analysis. Species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

Finally, for shoot dry weight, only the main effects of herbicide treatment were significant (Tab. 7). Shoot dry weight was higher in groups without herbicide application in both trials.

TABLE 7. Shoot dry weight (g) of soybean plants grown in succession to cover crops subjected to herbicide treatment after nematode inoculation.

Herbicide	Trial 1	Trial 2
Control	2.07 a	1.08 a
Bentazone	1.32 b	0.67 c
Chlorimuron	0.86 c	0.41 d
Fomesafen	1.25 b	0.81 b
CV (%)	21.29	14.50

Means within columns followed by the same letter are not significantly different at $P<0.05$ by the Scott–Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x}+0.5$ before analysis. The control was distilled water.

Chlorimuron application reduced soybean plant height, shoot dry weight, and shoot fresh weight. Alencar *et al.* (2022), investigating the selectivity of different post-emergence herbicides on soybean, found that chlorimuron-ethyl at 17.5 g a.i. ha^{-1} affected early plant development with a phytotoxicity of more than 30%. Alonso *et al.* (2013) reported that when combined glyphosate, chlorimuron-ethyl and fomesafen caused phytotoxicities of 65% and 40%. Chlorimuron is an inhibitor of acetolactate synthase with rapid absorption, high soil activity, and residual effects. Thus, it is suggested that the use of herbicides and their mixtures can affect phenological characteristics of soybean, such as plant height, pod number, and, consequently, yield (Oliveira Junior *et al.*, 2011; Alonso *et al.*, 2013).

Effect of herbicides on *Meloidogyne javanica* hatching

The nematicide abamectin was the most effective in reducing *M. javanica* hatching in both trials with hatching percentages of 0% and 3.15% (Tab. 8). Among the herbicides, bentazone did not affect *M. javanica* hatching in either trial, showing similar results to the control with distilled water. In Trial 1, fomesafen also had no effect on hatching compared with the control and did not differ from chlorimuron. In water, hatching reached 82.63% and 83.00% in Trials 1 and 2, respectively.

TABLE 8. *In vitro* hatching percentage of *Meloidogyne javanica* juveniles after 10 d of exposure of eggs to herbicide treatments.

Treatment	Trial 1	Trial 2
Control	82.63 a	83.00 a
Bentazone	66.55 a	73.38 a
Chlorimuron	24.57 b	52.32 b
Fomesafen	61.02 a	52.58 b
Abamectin	0.00 b	3.15 d
CV (%)	23.02	18.21

Means within columns followed by the same letter are not significantly different at $P<0.05$ by the Scott–Knott test, CV – coefficient of variation. The control was distilled water.

Despite the direct effect of some herbicides on nematodes, it is possible that in agricultural lands their activity is related to the mode of absorption and translocation of the active ingredients in plants. Systemic herbicides are transported via the phloem and xylem (Oliveira Junior *et al.*, 2011), possibly reaching the feeding sites of sedentary endoparasites such as *Meloidogyne* in the plant roots. There are few data on the direct effects of herbicides on nematode hatching. Studies are limited to the soybean cyst nematode (*Heterodera glycines* Ichinohe) (Wong *et al.*, 1993; Levene *et al.*, 1998; Barbosa *et al.*, 2014).

There is little information on the interaction of cover crops and herbicides on the control of *M. javanica* in soybeans. Nevertheless, it is evident that there are specific relationships between these factors; and further research is needed to elucidate these topics, particularly under field conditions, including testing other active ingredients less harmful to the crop. Herbicides should not be used to control nematodes, but they can assist in the integrated management of pathogens by controlling host plants.

Acknowledgments

The authors thank the Brazilian National Council for Scientific and Technological Development (CNPq) for the research and productivity grant awarded to CRDA (grant no. 303269/2020-0) and the doctoral fellowship awarded to AC (grant no. 157130/2021-5).

Conflict of interest statement

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

Author's contributions

CYF and CRDA designed the experiments, CYF carried out the field and laboratory experiments, AC contributed to the data analysis, CYF, AC, and CRDA wrote the article. All authors reviewed the final version of the manuscript.

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