

# Crop succession and rotation with surface liming on nematode management and soybean yield







**Abstract** – The objective of this work was to evaluate the effects of crop production systems under no-tillage and with surface liming, after 10 to 11 years, on nematode populations and soybean (*Glycine max*) grain yield. Twelve treatments were established in a randomized complete block design, with four replicates. The plots consisted of three production systems (monoculture, soybean followed by fallow in the off-season; crop succession, soybean followed by millet in the off-season; and crop rotation, soybean followed by rattlebox, *Urochloa ruziziensis*, and corn, each one in an off-season), and the subplots, of four rates of surface dolomitic limestone (0.0, 2.0, 4.0, and 8.0 Mg ha<sup>-1</sup>). Crop rotation and succession favors a higher soybean grain yield, reducing the population of *Heterodera glycines* in the soil and roots and increasing the populations of *Helicotylenchus* spp. The increment in surface limestone rates reduces soybean grain yield, with an increase in the population of *H. glycines* in the soil and roots and a decrease in the populations of *Pratylenchus brachyurus* and *Helicotylenchus* spp.

**Index terms:** *Glycine max*, *Heterodera glycines*, *Pratylenchus brachyurus*, cover crops, no-tillage, surface liming.

## Sucessão e rotação de culturas com calagem superficial sobre o manejo de nematoides e a produtividade de soja

**Resumo** – O objetivo deste trabalho foi avaliar os efeitos de sistemas de produção em plantio direto e com uso de calagem superficial, após 10 a 11 anos, sobre populações de nematoides e produtividade de grãos de soja (*Glycine max*). Foram estabelecidos 12 tratamentos em delineamento de blocos ao acaso, com quatro repetições. Os fatores avaliados foram dispostos em arranjo de parcelas subdivididas. As parcelas foram constituídas por três sistemas de produção (monocultivo, soja seguida de pousio na entressafra; sucessão de culturas, soja seguida de milho na entressafra; e rotação de culturas, soja seguida de crotalaria, *Urochloa ruziziensis* e milho, cada uma em uma entressafra), por quatro doses de calcário dolomítico em superfície (0,0, 2,0, 4,0 e 8,0 Mg ha<sup>-1</sup>). A rotação e a sucessão de culturas favorece maior produtividade de soja, com redução da população de *Heterodera glycines* no solo e nas raízes e aumento das populações de *Helicotylenchus* spp. O incremento nas doses de calcário em superfície reduz a produtividade de soja, com aumento da população de *H. glycines* no solo e nas raízes e diminuição das populações de *Pratylenchus brachyurus* e *Helicotylenchus* spp.


**Termos para indexação:** *Glycine max*, *Heterodera glycines*, *Pratylenchus brachyurus*, plantas de cobertura, plantio direto, calcário em superfície.

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## Introduction

Worldwide, approximately 100 species of nematodes from 50 different genera are reported to parasitize soybean [*Glycine max* (L.) Merr.] crops (Brida et al., 2016; Bellé et al., 2017). In Brazil, five species are considered the most important for soybean: *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941, a root nematode; *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and *Meloidogyne incognita* (Kofoid & White 1919) Chitwood, 1949, both root-knot nematodes; *Rotylenchulus reniformis* (Linford & Oliveira, 1940), a reniform nematode; and *Heterodera glycines* (Ichinohe, 1952), a cyst nematode (Meyer et al., 2017). In the state of Mato Grosso, the occurrence of these species in soybean, in descending order, is as follows: *P. brachyurus* > *H. glycines* > *M. javanica* > *M. incognita* > *R. reniformis* (Silva et al., 2019).

The dynamics of some species of nematodes is directly affected by crop diversification systems with soybean and cover crops. In the study of Silva et al. (2018), rattlebox (*Crotalaria ochroleuca* G.Don) stood out in the management of *P. brachyurus*. Leandro & Asmus (2015) found that, compared with soybean monoculture, crop rotation with *C. ochroleuca* led to a reduction in the population of *R. reniformis*. Silva et al. (2019) concluded that the sustainability of soybean cultivation in areas infested with *H. glycines* should be based on crop rotation, as soil organic matter (SOM) favors the development of microorganisms capable of parasitizing the eggs and cysts of the nematode.

However, it is important that the producer be aware that these management tactics should not be used in isolation and that the inclusion of nonhost cultures does not eliminate, but only reduce the population of nematodes in the soil. This is confirmed by the researches carried out by Costa et al. (2014) and Dias-Arieira et al. (2021), who observed that the actions used to manage *P. brachyurus* only reduced the population levels of the nematode. According to the authors, no-tillage associated with cover crop rotation increased SOM contents and reduced nematode multiplication. This result may be related to the stimulation of the growth of communities of nematode-suppressive microorganisms, as already reported by Hussain et al. (2018), who detected nematode-suppressing bacteria in the soybean rhizosphere.

Although it has been shown in the literature that the use of cover crops in the no-tillage system can reduce nematode populations, more information is necessary regarding the management of limestone in these crop systems. In their researches, Hua et al. (2020) found that changes in soil pH can interfere in the development of the cyst nematode (*H. glycines*) and of root-knot nematodes (*Meloidogyne* spp.). However, there are no known studies involving the effects of changes in soil pH in long-term agricultural systems with different annual and cover crops.

The objective of this work was to evaluate the effects of crop systems under no-tillage and with surface liming, after 10 to 11 years, on nematode populations and soybean grain yield.

## Materials and Methods

The experiment was installed in the 2008/2009 crop season at the experimental station of Fundação MT, located in the municipality of Itiquira, in the state of Mato Grosso, Brazil, in the Cerrado biome (17°09'33"S, 54°45'11"W, at an altitude of 490 m). The used data were collected in the 2017/2018 and 2018/2019 crop seasons. The predominant climate in the region, according to the Köppen-Geiger classification, is of the Aw type, with an average annual precipitation between 1,200 and 1,800 mm and an average annual temperature between 18 and 25°C.

The soil of the area is classified as an Oxisol, with flat relief and a very clayey texture (Teixeira et al.), corresponding to a Latossolo Vermelho distrófico (Santos et al., 2018). Before the beginning of the experiment, the chemical-physical attributes in the 0.0–0.1 m layer were: pH (CaCl<sub>2</sub>) 5.2; 22, 90, 7.4, 2.3, 108, 22.9, and 0.33 mg dm<sup>-3</sup> P (Mehlich-1), K, Zn, Cu, Fe, Mn, and B, respectively; 3.4, 1.2, 5.7, and 9.6 cmol<sub>c</sub> dm<sup>-3</sup> Ca, Mg, exchangeable acidity (H+Al), and cation exchange capacity (CEC), respectively; base saturation (V) and aluminum saturation (m) of 51 and 0.4%, respectively; 42.4 g dm<sup>-3</sup> soil organic matter (SOM); and 658, 192, and 150 g kg<sup>-1</sup> clay, sand, and silt, respectively. In the 0.1–0.2 m layer, the following values were obtained: pH (CaCl<sub>2</sub>) 5.0, 16 and 43 mg dm<sup>-3</sup> P (Mehlich-1) and K, respectively; 3.1, 1.0, 5.03, and 9.2 cmol<sub>c</sub> dm<sup>-3</sup> Ca, Mg, H+Al, and CEC, respectively; V and m of 45 and 1.1%; respectively; and 39.7 g dm<sup>-3</sup> SOM. The experimental area has been under soybean

monoculture for at least 25 years – from the 1983/1984 to the 2007/2008 crop season.

In the 2008/2009 crop season, 12 treatments were established in a randomized complete block design, which resulted from the combination of two factors: crop system and limestone rates. The evaluated factors were arranged in split-plots, with four replicates. The plots consisted of the three following production systems: monoculture, soybean followed by fallow, with the application of herbicide in the off-season; crop succession, soybean followed by millet (*Pennisetum glaucum* R.Br.) in the off-season; and crop rotation, soybean followed by rattlebox – in 2009, 2012, 2015, and 2018 –, by *Urochloa ruziziensis* (R.Germ. & C.M.Evrard) Morrone & Zuloaga – in 2010, 2013, and 2016 –, and by corn (*Zea mays* L.) – in 2011, 2014, and 2017 –, all in the off-season. In the subplots, four rates of dolomitic limestone (0.0, 2.0, 4.0, and 8.0 Mg ha<sup>-1</sup>) were applied to soil surface, without incorporation, in the 2008/2009, 2012/2014, and 2016/2017 crop seasons. The used rates of dolomitic limestone (31.2% CaO, 21.3% MgO, and 104% neutralizer power) were defined according to Sousa & Lobato (2004). The dimensions of the plots were 10x20 m and of the subplots, 5.0x10 m.

In all crop seasons at soybean pre-sowing, 90 kg ha<sup>-1</sup> K<sub>2</sub>O were applied via potassium chloride in haul and in the sowing furrow, as well as 54, 48, and 24 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, Ca, and S-SO<sub>4</sub><sup>-2</sup>, respectively, via simple superphosphate (00-18-00 + 16% Ca and 8% S-SO<sub>4</sub><sup>-2</sup>) at 0.08 to 0.1 m depth.

The used soybean cultivar was BMX Desafio RR, which is from maturation group 7.4 and has an undetermined growth. The seed were treated with insecticide, fungicide, cobalt, molybdenum, and inoculant containing strains of *Bradyrhizobium elkanii* and *Bradyrhizobium japonicum*. Sowing was carried out on 10/31/2017 and 10/13/2018, with 24 seed distributed per meter in the furrow, at a depth of 0.04 m, with a spacing of 0.45 m between lines. Soybean was harvested on 3/6/2018 and 2/13/2019.

Soon after soybean harvest, rattlebox, millet (cultivar ADR 300), and *U. ruziziensis* were sown at 0.17 m spacing between lines. The used seed were certified and did not receive any chemical treatment. The sowing rate of millet and rattlebox was 20 kg ha<sup>-1</sup>, and that of *U. ruziziensis* was 11 kg ha<sup>-1</sup>, equivalent to 400 points of crop value per hectare. The cover crops did

not receive any type of mineral fertilization. *Urochloa ruziziensis* was desiccated with 1,080 g ha<sup>-1</sup> glyphosate in June, when the amount of dry phytomass of the aerial part considered beneficial to the crop system was defined visually. Millet and rattlebox, however, were not desiccated, being allowed to complete their biological cycle.

The corn hybrid used was P3707 VYH, which was sown with three seed distributed per meter at 0.45 m spacing between lines. At pre-sowing, 60 kg ha<sup>-1</sup> K<sub>2</sub>O were applied via potassium chloride in haul and in the sowing furrow, as well as 11 and 52 kg ha<sup>-1</sup> N and P<sub>2</sub>O<sub>5</sub>, respectively, via monoammoniumphosphate (11-52-00). When the plants were in stage V4, 100 kg ha<sup>-1</sup> N were applied via urea in haul.

After soybean harvest and before the sowing of the cover crops in February, soil sampling was carried out in the 0.0–0.1 and 0.1–0.2 m layers. Twelve simple samples were collected per subplot in between lines, to make up composite samples in their respective layers and treatments, in which pH in CaCl<sub>2</sub> was determined.

Nematode populations were counted in the 2017/2018 and 2018/2019 crop seasons. Sampling was carried out in the 0.0–0.1 m layer during the full flowering of soybean. For this, five simple samples containing soil and roots were collected per subplot, being homogenized to obtain the composite sample. To determine the populations of *P. brachyurus*, *R. reniformis*, *Helicotylenchus* spp., and *H. glycines*, samples of 200 cm<sup>3</sup> soil per subplot were processed by flotation, sieving, and centrifugation. For the extraction of juvenile and adult nematodes of *P. brachyurus*, *R. reniformis*, *Helicotylenchus* spp., and *H. glycines* in the soil, the methodology proposed by Jenkins was used (1969). Plant roots were washed, weighed on a high precision scale, standardized to 5.0 g roots per subplot, and crushed in a blender for 1.0 min with hypochlorite solution. Then, the juvenile nematodes present in the roots were extracted using the method of processing, sifting, and fluctuation in centrifugation with kaolin and sucrose according to Coolen & D'Herde (1972). The cysts of *H. glycines* were extracted by the methodology proposed by Abrantes et al. (2000). Juvenile and adult nematodes were counted with the aid of Peter's chamber under an optical microscope. The nematode population was estimated from the density obtained in 5.0 g roots.

At soybean harvest, two sampling points were delimited in each subplot, each consisting of four adjacent lines measuring 2.0 m in length. The plants were collected and processed mechanically in a stationary track. Yields were obtained by weighing the grains in each subplot, with values converted to kg ha<sup>-1</sup> and corrected to 13% humidity.

Nematode populations were transformed into  $\sqrt{x + 1}$  square root. Subsequently, the results were subjected to the F-test of the analysis of variance, and the averages of the crop systems were compared by Tukey's test, at 5% probability. The means of limestone rates were analyzed by regression, fitting models of significant equations by the F-test ( $p < 0.01$  and  $p < 0.05$ ). The obtained results were also subjected to the multivariate analysis of variance, highlighting the absolute values of the eigenvectors greater than 0.5, regardless of whether positive or negative. The studied variables were processed in the Statistica, version 7.0, software (TIBCO Software Inc., Palo Alto, CA, USA), allowing the identification of main components and the performance of the cluster analysis.

## Results and Discussion

The populations of *P. brachyurus*, *R. reniformis*, and *Helicotylenchus* spp. in the soil and soybean roots, in both crop seasons, were influenced by the interaction among crop systems and surface limestone rates (Table 1). The systems with floristic diversity (crop succession and rotation) stood out in the reduction of the population of *P. brachyurus* in the soil and roots (Tables 2 and 3). Crop rotation proved to be an important tool for the management of the studied nematodes, except of *Helicotylenchus* spp., due to the inclusion of species antagonistic to the development of these nematodes, especially of rattlebox in crop rotation and millet in crop succession. According to Silva et al. (2019), both of these crops show soil nematode-suppressive properties in agricultural areas.

For the management of *P. brachyurus* in systems with crop rotation, the use of limestone does not seem to be an adequate technique, particularly since, when higher rates are applied, there may be a slight increase in the population of this nematode in the roots of soybean grown in succession. This shows that systems with crop rotation are weakly dependent on surface liming for the control of *P. brachyurus*, to the point

that high rates of lime can increase the population levels of this nematode in soybean roots. However, in the monoculture system, limestone can be used for the management of *P. brachyurus*. In the 2018/2019 crop season, the increase in limestone rates linearly

**Table 1.** F-values and coefficient of variation (CV) of populations of *Pratylenchus brachyurus*, *Rotylenchulus reniformis*, *Helicotylenchus* spp., and *Heterodera glycines* in samples of 200 cm<sup>3</sup> soil and 5.0 g roots collected at full flowering of soybean (*Glycine max*), as well as soybean grain yield, in three crop production systems under different rates of surface dolomitic limestone in two crop seasons.

Source of variation	2017/2018		2018/2019	
	Soil	Root	Soil	Root
<i>P. brachyurus</i> <sup>(1)</sup>				
Production system (P)	12.18**	6.34*	20.76**	23.25**
Limestone rate (L)	5.73**	6.70**	34.72**	41.29**
P x L	2.66*	10.40**	10.83**	9.09**
CV of plot (%)	48.40	32.15	47.62	22.35
CV of subplot (%)	36.78	31.16	35.75	19.79
<i>R. reniformis</i> <sup>(1)</sup>				
Production system (P)	22.93**	279.08**	1.18 <sup>ns</sup>	4.43 <sup>ns</sup>
Limestone rate (L)	4.54*	8.61**	1.91 <sup>ns</sup>	0.31 <sup>ns</sup>
P x L	7.80**	16.28**	9.16**	12.15**
CV of plot (%)	10.62	13.71	22.08	24.48
CV of subplot (%)	10.80	18.37	17.98	27.01
<i>Helicotylenchus</i> spp. <sup>(1)</sup>				
Production system (P)	29.13**	156.49**	37.08**	181.36**
Limestone rate (L)	86.34**	56.22**	44.39**	34.66**
P x L	30.81**	29.01**	44.39**	34.66**
CV of plot (%)	74.84	23.13	78.27	24.87
CV of subplot (%)	30.06	26.23	37.10	20.25
<i>H. glycines</i> <sup>(1)</sup>				
Production system (P)	21.54**	209.18**	17.52**	137.89**
Limestone rate (L)	59.96**	57.28**	19.54**	45.64**
P x L	1.34 <sup>ns</sup>	9.17**	0.97 <sup>ns</sup>	1.51 <sup>ns</sup>
CV of plot (%)	26.77	20.12	40.34	15.61
CV of subplot (%)	27.86	34.53	35.40	25.11
Grain yield				
Production system (P)	50.57**		309.8**	
Limestone rate (L)	1.17 <sup>ns</sup>		3.30*	
P x L	3.84**		0.67 <sup>ns</sup>	
CV of plot (%)	5.80		4.60	
CV of subplot (%)	4.79		9.17	

<sup>(1)</sup>Results transformed into  $(x+1)^{0.5}$ . \*\* and \*Significant at 1 and 5% probability, respectively. <sup>ns</sup>Nonsignificant.

**Table 2.** Populations of *Pratylenchus brachyurus*, *Rotylenchulus reniformis*, *Helicotylenchus* spp., and *Heterodera glycines* in samples of 200 cm<sup>3</sup> soil and 5.0 g roots collected at full flowering of soybean (*Glycine max*), as well as soybean grain yield, in three crop production systems under different rates of surface dolomitic limestone in the 2017/2018 crop season<sup>(1)</sup>.

Production system <sup>(2)</sup>	Limestone rate (Mg ha <sup>-1</sup> )				Regression equation <sup>(3)</sup>	R <sup>2</sup>
	0.0	2.0	4.0	8.0		
<i>P. brachyurus</i> in the soil <sup>(4)</sup>						
Monoculture	20.0a	15.0a	15.0a	10.0a	ns	-
Crop succession	15.0a	10.5a	0.0b	0.0b	$\hat{y} = 13.0 - 1.93x$	0.77
Crop rotation	2.5b	5.0b	2.5b	2.5b	ns	-
<i>P. brachyurus</i> in the roots <sup>(4)</sup>						
Monoculture	612.5a	212.5a	82.5a	137.5a	$\hat{y} = 599.0 - 214.6x + 19.7x^2$	0.99
Crop succession	332.5b	325.0a	105.0a	25.0b	$\hat{y} = 345.5 - 42.46x$	0.87
Crop rotation	37.5c	45.0b	185.0a	187.5a	$\hat{y} = 40.01 + 21.07x$	0.74
<i>R. reniformis</i> in the soil <sup>(4)</sup>						
Monoculture	1,990a	2,640a	2,017a	2,437a	ns	-
Crop succession	1,882a	1,860b	1,277b	1,170b	$\hat{y} = 1,895 - 99.29x$	0.81
Crop rotation	672b	1,537b	1,830ab	1,902a	$\hat{y} = 1,004 + 137.61x$	0.69
<i>R. reniformis</i> in the roots <sup>(4)</sup>						
Monoculture	72.5a	30.0a	85.0a	102.5a	$\hat{y} = 52.0 + 5.86x$	0.42
Crop succession	22.5b	17.5ab	2.5c	17.5b	$\hat{y} = 24.5 - 8.0x + 0.88x^2$	0.77
Crop rotation	0.0c	7.5b	22.5b	12.5b	$\hat{y} = 1.7 + 8.5x - 0.84x^2$	0.87
<i>Helicotylenchus</i> spp. in the soil <sup>(4)</sup>						
Monoculture	0.0c	0.0c	0.0b	0.0b	ns	-
Crop succession	142.5b	20.0b	2.5b	0.0b	$\hat{y} = 94.0 - 15.07x$	0.57
Crop rotation	892.5a	137.5a	82.5a	67.5a	$\hat{y} = 593.5 - 85.29x$	0.53
<i>Helicotylenchus</i> spp. in the roots <sup>(4)</sup>						
Monoculture	0.0c	0.0b	0.0b	0.0b	ns	-
Crop succession	12.5b	12.5a	0.0b	0.0b	$\hat{y} = 12.5 - 1.79x$	0.71
Crop rotation	102.5a	12.5a	7.5a	10.0a	$\hat{y} = 66.0 - 9.39x$	0.48
<i>H. glycines</i> in the soil <sup>(4)</sup>						
Monoculture	430a	557a	1,412a	2,120a		
Crop succession	122ab	160ab	1,640a	2,107a	$\hat{y} = 74.3 + 233x$	0.93
Crop rotation	7b	47b	637b	2,550a		
<i>H. glycines</i> in the roots <sup>(4)</sup>						
Monoculture	57.5a	35.0a	532.5a	997.5a	$\hat{y} = 44.5 + 128.61x$	0.93
Crop succession	7.5b	12.5a	67.5b	202.5b	$\hat{y} = 17.5 + 25.71x$	0.94
Crop rotation	0.0b	0.0a	42.5b	55.0c	$\hat{y} = 2.5 + 7.68x$	0.84
Grain yield (kg ha <sup>-1</sup> )						
Monoculture	4,090b	4,300b	4,180c	4,330b	ns	-
Crop succession	5,050a	4,940a	4,630b	4,440b	$\hat{y} = 5,040 - 0.08x$	0.94
Crop rotation	5,070a	5,190a	5,410a	5,140a	ns	-

<sup>(1)</sup>Means followed by equal letters, in the same columns, do not differ by Tukey's test, at 5% probability. <sup>(2)</sup>Monoculture, soybean followed by fallow, with the application of herbicide in the off-season; Crop succession, soybean followed by millet (*Pennisetum glaucum*) in the off-season; and Crop rotation, soybean followed by rattlebox (*Crotalaria ochroleuca*), *Urochloa ruziziensis*, and corn (*Zea mays*), each one in an off-season. <sup>(3)</sup>Regression equation significant at 1% probability. <sup>(4)</sup>Nonsignificant. <sup>(5)</sup>Means were compared by transforming the results into  $(x+1)^{0.5}$ .

**Table 3.** Populations of *Pratylenchus brachyurus*, *Rotylenchulus reniformis*, *Helicotylenchus* spp., and *Heterodera glycines* in samples of 200 cm<sup>3</sup> soil and 5.5 g roots collected at full flowering of soybean (*Glycine max*) in the 0.0–0.1 m soil layer, as well as soybean grain yield, in three crop production systems under different rates of surface dolomitic limestone in the 2018/2019 crop season<sup>(1)</sup>.

Production system <sup>(2)</sup>	Limestone rate (Mg ha <sup>-1</sup> )				Regression equation <sup>(3)</sup>	R <sup>2</sup>
	0.0	2.0	4.0	8.0		
<i>P. brachyurus</i> in the soil <sup>(4)</sup>						
Monoculture	60.0a	10.0a	10.0a	0.0a	$\hat{y} = 42.0 - 6.29x$	0.63
Crop succession	0.0c	0.0b	2.5b	0.0a	ns	-
Crop rotation	25.0b	5.0ab	5.0ab	0.0a	$\hat{y} = 18.0 - 2.64x$	0.66
<i>P. brachyurus</i> in the roots <sup>(4)</sup>						
Monoculture	780.0a	277.5b	240.0b	100.0a	$\hat{y} = 607.0 - 73.61x$	0.72
Crop succession	340.0b	165.0c	80.0c	0.0b	$\hat{y} = 286.0 - 39.93x$	0.88
Crop rotation	240.0b	440.0a	400.0a	145.0a	$\hat{y} = 254.9 + 101.8x - 14.5x^2$	0.95
<i>R. reniformis</i> in the soil <sup>(4)</sup>						
Monoculture	1,930a	2,000a	1,410a	640b	$\hat{y} = 2,112 - 176.29x$	0.92
Crop succession	1,090b	1,400ab	1,340a	1,780a	ns	-
Crop rotation	470b	880b	1,720a	1,932a	$\hat{y} = 591.5 + 188.32x$	0.87
<i>R. reniformis</i> in the roots <sup>(4)</sup>						
Monoculture	120.0a	120.0a	70.0a	20.0b	$\hat{y} = 130 - 13.57x$	0.94
Crop succession	30.0b	20.0b	40.0a	130.0a	$\hat{y} = 8.0 + 13.43x$	0.82
Crop rotation	45.0b	40.0b	42.5a	40.0b	ns	-
<i>Helicotylenchus</i> spp. in the soil <sup>(4)</sup>						
Monoculture	0.0b	0.0b	0.0a	0.0a	ns	-
Crop succession	0.0b	0.0b	0.0a	0.0a	ns	-
Crop rotation	292.5a	80.0a	10.0a	10.0a	$\hat{y} = 207.5 - 31.25x$	0.64
<i>Helicotylenchus</i> spp. in the roots <sup>(4)</sup>						
Monoculture	0.0b	0.0b	0.0b	0.0a	ns	-
Crop succession	0.0b	0.0b	0.0b	0.0a	ns	-
Crop rotation	30.0a	23.7a	13.7a	0.0a	$\hat{y} = 30.2 - 3.82x$	0.99
<i>H. glycines</i> in the soil <sup>(4)</sup>						
Monoculture	480a	2,470a	2,750a	3,280a		
Crop succession	252a	845a	2,505a	3,040a	$\hat{y} = 505 + 294x$	0.92
Crop rotation	20a	180b	1,000b	1,600b		
<i>H. glycines</i> in the roots <sup>(4)</sup>						
Monoculture	65a	285a	400a	567a		
Crop succession	10b	90b	140b	205b	$\hat{y} = 50.2 + 35.0x$	0.96
Crop rotation	10b	20c	110b	170b		
Grain yield (kg ha <sup>-1</sup> )						
Monoculture	3,964b	3,882b	4,035b	3,660c		
Crop succession	5,556a	5,568a	5,224a	4,674b	$\hat{y} = 5,230 - 0.07x$	0.85
Crop rotation	5,848a	6,035a	5,948a	5,608a		

<sup>(1)</sup>Means followed by equal letters, in the same columns, do not differ by Tukey's test, at 5% probability. <sup>(2)</sup>Monoculture, soybean followed by fallow, with the application of herbicide in the off-season; Crop succession, soybean followed by millet (*Pennisetum glaucum*) in the off-season; and Crop rotation, soybean followed by rattlebox (*Crotalaria ochroleuca*), *Urochloa ruziziensis*, and corn (*Zea mays*), each one in an off-season. <sup>(3)</sup>Significant at 1% probability. <sup>(4)</sup>Nonsignificant. <sup>(5)</sup>Means were compared by transforming the results into  $(x+1)^{0.5}$ .

decreased the population of *P. brachyurus* in the soil, indicating that the correction of soil acidity could help to reduce the damage of this nematode to soybean (Table 3). One hypothesis to justify this result is that the lower acidity of the soil increases the resistance of root cell walls (Allan et al., 1990), as it favors the deposition of pectins (calcium pectates) between cellulose microfibrils during cell wall synthesis (Taiz & Zeiger, 2013), making it difficult for the nematode to penetrate, move, and feed within the roots. The reduction in soil acidity can also favor groups of microorganisms antagonistic to the nematode or even cause harm to *P. brachyurus* (Hussain et al., 2018).

For crop rotation in 2017/2018, there was a linear increase in the population of *P. brachyurus* in soybean roots with the increase of limestone rates, but the infestation levels of the nematode were lower than those in the monoculture and in crop succession with the lowest limestone rates (Table 2). These results may be explained by the effect of the predecessor crop, leading to a greater root growth of soybean sown in succession to rattlebox and, consequently, to an increase in the population of the nematode.

Crop rotation promoted the reduction of the population of *R. reniformis* in the soil and soybean roots. Leandro & Asmus (2015) also found significant reductions in the populations of *R. reniformis* in the soil of plots cultivated with *U. ruziziensis* and *C. ochroleuca* when compared with those only with soybean. The researchers emphasized that the cultivation of *U. ruziziensis* or rattlebox in the off-season, with soybean sown in succession, can be a strategy for the management of *R. reniformis* in infested areas.

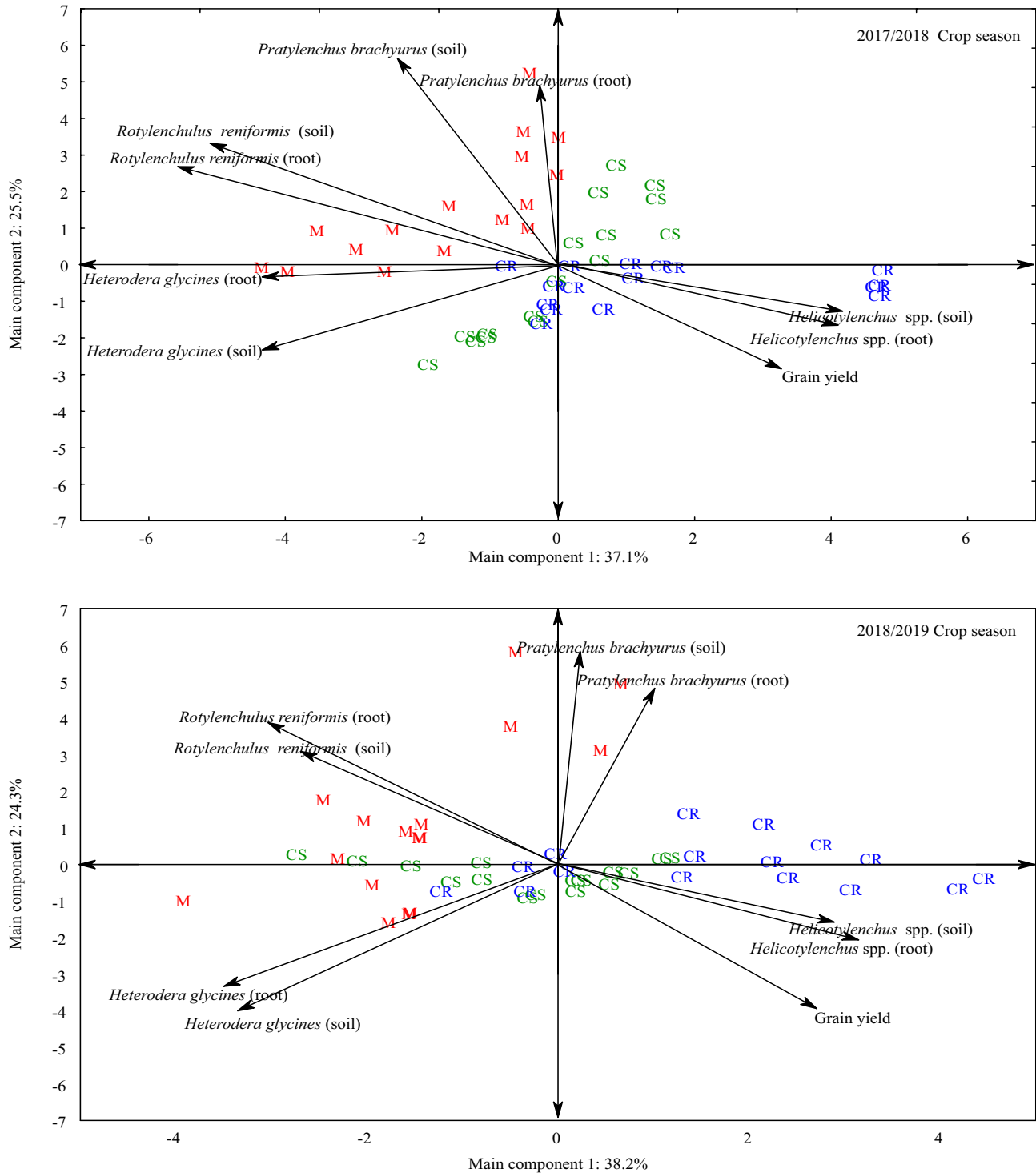
However, crop rotation and succession had a multiplier effect on the population of *R. reniformis* in the soil when increasing limestone rates were applied. Contrarily, in the monoculture, the population of this nematode was reduced in the soil with the application of limestone. This result reinforces the thesis that less diversified systems are more dependent on limestone to remain productive (Claudius-Cole et al., 2015).

The largest populations of *Helicotylenchus* spp. in the soil and soybean roots under crop rotation can be related to nematode multiplication favored by *C. ochroleuca*, a species that was included in the crop production system and that was cultivated in the 2017 off-season before soybean in the 2017/2018 crop

season. Similarly, Claudius-Cole et al. (2015) observed a greater reproduction of *Helicotylenchus* spp. in crop systems with the presence of rattlebox. In the present study, the results obtained in 2017/2018 are indicative that, under crop rotation and succession, increasing rates of limestone were efficient in reducing populations of this nematode in the soil and roots.

There was also a reduction in the population of *H. glycines* in the soil and soybean roots under crop rotation and succession, which reinforces the importance of this system in providing microclimate conditions that are nematode suppressive. In this same line, Silva et al. (2018) observed a reduction in *H. glycines* in plots cultivated with *C. ochroleuca* in the state of Mato Grosso. These results are similar to those of Silva et al. (2019), who found that SOM favors the development of microorganisms capable of parasitizing *H. glycines* eggs and cysts. The populations of *H. glycines* both in the soil and soybean roots, however, were increased with increasing rates of limestone. These results can be attributed to the fact that the lower pH values of the soil without limestone application guarantee a greater activity of the fungi that attack *H. glycines* cysts as mentioned by Silva et al. (2019). In a greenhouse experiment, Rocha et al. (2006) verified that from 3.03 Mg ha<sup>-1</sup> limestone there was an increase in the population of *H. glycines* in plant roots, which is in alignment with the present study. This information is important since the evaluated soybean cultivar – BMX Desafio RR – is susceptible to *H. glycines* as reported by Bellé et al. (2017). Therefore, different results may be obtained when using other cultivars that are tolerant and/or resistant to *H. glycines*.

Treatments under crop succession and rotation favored soybean grain yield (Figure 1 and Table 4). These results are consistent with those obtained during two consecutive crop seasons by Veronese et al. (2012), who concluded that millet and *U. ruziziensis* in the off-season increased soybean grain yield when compared with soybean monoculture. In crop rotation, the highest yields were obtained at the highest limestone rates; however, in the monoculture, increasing rates of limestone resulted in a decrease in soybean grain yield. The supremacy of crop rotation over other crop systems is attributed to the reduction in the populations of *H. glycines* in the soil and roots due to the inclusion of diversified crops – such as *C. ochroleuca*, *U. ruziziensis*, and corn – in the



**Figure 1.** Main component analysis explaining 62.6 and 62.5% of the total variation of the results obtained in the 2017/2018 and 2018/2019 crop seasons, respectively, for the nematode populations in the soil and root samples collected at full flowering of soybean (*Glycine max*), as well as soybean grain yield, under the following three production systems: monoculture (M), soybean followed by fallow, in the off-season; crop succession (CS), soybean followed by millet (*Pennisetum glaucum*) in the off-season; and crop rotation (CR), soybean followed by rattlebox (*Crotalaria ochroleuca*), *Urochloa ruziziensis*, and corn (*Zea mays*), each one in an off-season.



**Table 4.** Multivariate analysis of variance showing the correlation coefficient between each major component and nematode populations in the soil and root samples collected at full flowering of soybean (*Glycine max*), as well as soybean grain yield, in the 2017/2018 and 2018/2019 crop seasons<sup>(1)</sup>.

Variable	2017/2018 crop season		2018/2019 crop season	
	Main component 1	Main component 2	Main component 1	Main component 2
<i>Pratylenchus brachyurus</i> in the soil	-0.1679	0.8237 <sup>(1)</sup>	0.1871	0.8663 <sup>(1)</sup>
<i>Pratylenchus brachyurus</i> in the roots	-0.0089	0.7328 <sup>(1)</sup>	0.3626	0.7751 <sup>(1)</sup>
<i>Rotylenchulus reniformis</i> in the soil	-0.5306 <sup>(1)</sup>	0.5034 <sup>(1)</sup>	-0.4370	0.4334
<i>Rotylenchulus reniformis</i> in the roots	-0.6872 <sup>(1)</sup>	0.4408	-0.3724	0.6339 <sup>(1)</sup>
<i>Helicotylenchus</i> spp. in the soil	0.7932 <sup>(1)</sup>	-0.1469	0.7029 <sup>(1)</sup>	-0.1172
<i>Helicotylenchus</i> spp. in the roots	0.7869 <sup>(1)</sup>	-0.1337	0.7849 <sup>(1)</sup>	-0.1121
<i>Heterodera glycines</i> in the soil	-0.7192 <sup>(1)</sup>	-0.4432	-0.8243 <sup>(1)</sup>	-0.2404
<i>Heterodera glycines</i> in the roots	-0.7119 <sup>(1)</sup>	-0.0691	-0.7787 <sup>(1)</sup>	-0.2429
Soybean grain yield	0.5489 <sup>(1)</sup>	-0.3155	0.7403 <sup>(1)</sup>	-0.3166
Proportion of total variance explained (PTV, %)	37.1	25.5	38.2	24.3
Proportion of accumulated variance explained (PAV, %)	37.1	62.6	38.2	62.5

<sup>(1)</sup>More discriminatory values.

off-season over time. Silva et al. (2018) also observed higher soybean grain yields in crop rotation systems with *C. ochroleuca*.

The multivariate analysis was responsible for explaining 62% of the total variance of the original results (Figure 1), which confirm the importance of crop rotation and succession for the management of *P. brachyurus*, *R. reniformis*, and *H. glycines* in the soil and soybean roots (Table 4). The greater diversity of crops and greater input of phytomass and SOM in the crop succession and rotation systems may have favored the multiplication of bacteria and nematophagous fungi that attack *H. glycines* cysts, decreasing their survival, as shown by Silva et al. (2019). Conversely, in another study, Sereia et al. (2007) found that soybean monoculture increased the population of *R. reniformis*.

Crop rotation was also characterized by a strong correlation of *Helicotylenchus* spp. in the soil and roots with grain yield. The presence of *C. ochroleuca* seems to be a determining factor for these results. Another hypothesis is that the insertion of off-season corn in this crop system may also have favored the multiplication of *Helicotylenchus* spp., which, according to Inomoto (2009), is a nematode that has been showing high populations when associated with the corn crop. However, it is still necessary to evaluate whether low populations of *Helicotylenchus* spp. can be considered pathogenic in diversified systems (crop

rotation) with the inclusion of soybean, which had high average grain yields.

## Conclusions

1. Crop rotation favors higher soybean (*Glycine max*) grain yields and is efficient in reducing the populations of the *Heterodera glycines*, *Pratylenchus brachyurus*, and *Rotylenchulus reniformis* nematodes in the soil and plant roots, despite increasing the populations of *Helicotylenchus* spp.

2. The increase in surface limestone rates reduces soybean grain yield, increasing *H. glycines* in the soil and plant roots and decreasing the populations of *P. brachyurus* and *Helicotylenchus* spp.

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