

Rotylenchulus reniformis population conditioned by soybean production systems and soil attributes

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Received: 27/05/2024; Accepted: 05/07/2024.

ABSTRACT

A study was conducted to understand the role of different soybean production systems and soil chemical and physical attributes on phytonematode population density in a physical model of production systems. In an area of 28 hectares, the following production systems were established 26 years ago: a) conventional system (CS) - succession of soybeans in summer and oats in winter, and continuous soil preparation with disc harrow; b) no-till system (NTS) - with rotation of summer crops (soybeans and corn) and winter crops (wheat, oats, and fodder radish); c) integrated crop-livestock system (ICLS) - conducted in no-till system and using *Urochloa brizantha* (cultivar Piatã) pasture, in crop-livestock rotation every two years; d) permanent pasture system (PP) - *U. brizantha* (cultivar Piatã) with beef cattle. Twenty six years after the systems were installed, soil samples were taken for nematological analysis and analysis of the soil's chemical and physical attributes. The results showed an almost absolute prevalence of the reniform nematode (*Rotylenchulus reniformis*, Rr) in the area. The PP system had the lowest population (average of 2 Rr/200cc), followed by the ICLS systems (average of 12 to 25 Rr/200cc). The highest population densities were observed in the CS system (1068 Rr/200cc) and the NTS system when soybeans were grown in the last two years before sampling (1361 Rr/200cc). The highest population densities of *R. reniformis* were associated with higher soil density in the arable layer, higher K and P levels in the soil, and lower organic matter contents.

Keywords: Reniform nematode, Cultural management, Crop rotation, Integrated Crop-livestock.

População de *Rotylenchulus reniformis* condicionada por sistemas de produção de soja e atributos do solo

RESUMO

Realizou-se um trabalho visando conhecer o papel de diferentes sistemas de produção de soja e de atributos químicos e físicos do solo sobre a densidade populacional de fitonematoides em um modelo físico de sistemas de produção. Em área de 28 hectares, foram estabelecidos, há 26 anos, os seguintes sistemas de produção a) convencional - SC: sucessão de soja no verão e aveia no inverno, e preparo contínuo do solo com grades de discos; b) plantio direto - SPD: com rotação de culturas de verão (soja e milho) e inverno (trigo, aveia e nabo); c) integração lavoura-pecuária - ILP: conduzido em plantio direto e utilizando pastagem de *Urochloa brizantha* cv Piatã, em rotação lavoura-pastagem a cada dois anos; d) pastagem permanente (PP) de *U. brizantha* cv Piatã com pecuária de corte. Vinte e seis anos após a instalação dos sistemas, foram realizadas amostragens de solo para análises nematológicas e de atributos químicos e físicos do solo. Os resultados apontaram prevalência, quase absoluta, do nematoide reniforme (*Rotylenchulus reniformis*, Rr) na área. A menor população foi encontrada no sistema PP (média de 2 Rr/200cc), seguida dos sistemas de ILP (média de 12 a 25 Rr/200cc). As maiores densidades populacionais foram observadas no SC (1068 Rr/200cc), e no SPD, quando houve cultivo de soja nos dois últimos anos antecedendo a amostragem (1361 Rr/200cc). As maiores densidades populacionais de *R. reniformis* foram associadas a maior densidade do solo na camada arável, teores mais elevados de K e P no solo, e menores teores de matéria orgânica.

Palavras-chave: Nematoide reniforme, Manejo cultural, Rotação de culturas, Integração lavoura-pecuária.



1. Introduction

Phytonematodes are among the main phytosanitary problems in agricultural production. The damage caused to plants is usually associated with the presence of nematodes in high population densities, resulting from the sequence of cultivation of susceptible plant species or varieties and favorable soil and climate conditions (McSorley, 1998). The fact that they are organisms that, at least in some phase of their lives, inhabit the edaphic environment implies that changes in the chemical, physical, and biological conditions of soils can substantially alter their population density, plant tolerance, and, consequently, the damage caused to crops of susceptible species (Galbieri et al., 2016).

In several soybean and cotton production regions, the incidence of high population densities of the reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira 1940) stands out as one of the main causes of plant non-uniformity and reduced crop yield, which can be aggravated in production systems involving the cultivation of cotton in succession to soybean (Sikora et al., 2018; Silva et al., 2021).

Rotylenchulus reniformis is a polyphagous species that commonly occurs in regions with a tropical or subtropical climate (Robinson et al., 1997). The characteristic of entering a state of anhydrobiosis under conditions of low soil humidity (Tsai and Apt, 1979) allows the nematode to survive for long periods without favorable host plants, which can complicate management programs for this pest. The lack of a clear symptomatological picture means that this nematode is sometimes overlooked, and the unevenness of crops is attributed to other causes of a physical or chemical nature in the soil.

Cultivar resistance can be an important management tool for *R. reniformis* in soybean cultivation, given the existence of good sources of resistance (Jiao et al., 2015; Klepadlo et al., 2018). However, few commercially available cultivars combine resistance to *R. reniformis* with other desirable agronomic characteristics. In Brazil, soybean breeding programs have not considered the introgression of resistance to this nematode in their elite cultivars. Exceptions are soybean cultivars, which, during the introgression of resistance genes to *Heterodera glycines*, bring resistance to *R. reniformis* (Davis et al., 1996).

However, these cultivars have only been developed for regions where *H. glycines* is prevalent. Therefore, soybean rotation and succession programs with non-host crops become an important management strategy for *R. reniformis*.

Despite the polyphagous nature of *R. reniformis*, there are economically important crops that behave as non-hosts, such as rice, corn, sorghum, oats, wheat, and sugarcane, which have proven to be very useful in the management of *R. reniformis* (Robinson et al., 1997; Sikora et al., 2018). In addition, growing forage grasses is an excellent alternative for reducing the nematode population density in the soil (Gardiano et al., 2012). Thus, crop livestock integration systems can be alternatives for the cultural management of this nematode.

Unlike other phytonematodes in soybean crops, *R. reniformis* occurs in higher population densities and causes more significant damage in clay textured soils (Robinson et al., 1997; Star et al., 1993). The role of other attributes of the soil environment on the population density and damage caused by *R. reniformis* is still poorly studied. Thus, understanding the relationship between the changes in the soil environment caused by different land use and management systems and the population density of *R. reniformis* is fundamental to formulating strategies for coexistence with this phytonematode, giving sustainability to agricultural activity. Based on this, this study aimed to determine the impact of integrated, diversified production systems established 26 years ago, as well as variations in the physical and chemical attributes of the soil, on the population density of *R. reniformis*.

2. Material and Methods

In 1995, four production systems were established in an area of 28 hectares of the Experimental Station of the Embrapa Western-Region Agriculture, in Dourados, MS, Brazil (22°16'55.0"S, 54°48'18.1" W, and an altitude of 400 meters), on a Latossolo Vermelho Distroférico típico, caulínítico, with 630, 215, and 155 g kg⁻¹ of clay, silt, and sand, respectively. The chemical attributes of the soil where the study was conducted are shown in Table 1.

Table 1. Average values of the main chemical attributes in the soil layers at the time the experiment was set up.

Soil layer cm	pH H ₂ O	Al	Ca	Mg	K	P
			-----cmol _c dm ⁻³ -----			mg dm ⁻³
0-5	5.5	0.45	4.3	2.5	0.87	19
5-15	5.3	0.87	4.0	1.8	0.37	14
15-30	5.3	0.79	3.8	1.8	0.21	8

P and K: Mehlich extractor.

The following describes the systems: The Conventional System - CS (a) represents the traditional form of agriculture in the region when the experiment was set up, occupying a 2 ha plot and being conducted with soybeans in the summer and oats in the winter. The soil was prepared conventionally, using disk harrows (heavy + light), without crop rotation. The soybean crop was fertilized according to current technical recommendations, and the oat crop was grown to harvest the grain. Varieties recommended for the region and integrated pest management were used, among other cultural practices (Seixas et al., 2020).

The No-Till System – NTS (b) comprises no-till farming with crop rotation, with soybeans and corn as summer crops and wheat, oats, and fodder radish as winter crops. The area occupied by the system was 6 ha divided into three plots of 2 ha each (NTSa, NTSb, and NTSc), depending on the crop sequence. According to current technical recommendations, the soybean, corn, and wheat crops were fertilized each time.

The Integrated Crop-Livestock System – ICLS (c) is a no-till system that alternates crops (soybeans/oats) with perennial pasture grass (*Urochloa brizantha* - cultivar Piatã). Crop-pasture rotation was conducted every two years. The system occupied 8 ha of area divided into two plots of 4 ha each (ICLSa and ICLSc), depending on the crop sequence. Soybeans were always sown after grazing, and the pasture was desiccated with herbicides. Grazing was conducted with Nelore heifers or heifers from crossbreeding Nelore with European breeds, with stocking rates adjusted to keep the forage supply constant at around 7% (7 kg of dry mass of forage per 100 kg of live weight per day). Fertilization was only conducted for the soybeans (300 kg ha⁻¹ of the 0-20-20 NPK formulation), and no fertilizers or correctives were used in the establishment and maintenance of the pasture.

The Permanent Pasture System (d) PP comprises permanent pasture of brizantha grass (*U. brizantha*, cultivar Piatã) with beef cattle grazed continuously,

using Nelore animals or animals from crossbreeding Nelore with European breeds, and managed according to the available technical recommendations. The area for this system was 8 ha. The cattle were managed in the same way as in the ICLS system. Table 2 shows the crop sequences in the last four years of each production system, which were repeated over 26 years (1995 to 2021).

During the period from July to September 2021, when the area had been under different production systems for 26 years, soil was collected in a grid of points with a spacing of 30 m x 30 m (Figure 1) for chemical, physical, and nematological analysis, in the different production systems, totaling 26 points in the CS, 82 points in the NTS, 87 points in the ICLS, and 47 points in the PP. The samples for nematological analysis were obtained with a mechanical auger at a depth of 0.0 to 0.2m and stored in plastic bags inside thermal boxes (expanded polystyrene) until they were processed for the extraction of nematodes from the soil (Jenkins, 1964).

After extraction, the nematodes were inactivated at 55 °C for 5 min and fixed in 2% formalin. The genera and species of phytonematodes present in the samples were identified based on morphometric characteristics (Robinson et al., 1997; Cares and Huang, 2000). To quantify the phytonematode populations, 1 mL aliquots of the nematode suspension were used on a Peter's counting slide using a light microscope (40x). Soil samples for chemical analysis were extracted with a mechanical drill at a depth between 0.05-0.15 m and sent to the soil analysis laboratory, where pH, Al⁺³, Ca⁺², Mg⁺², H⁺ + Al³⁺, K⁺, P, and total C were determined, and SB, CEC, effective CEC, m%, V (%), and OM were calculated.

Undisturbed samples were collected from the soil layers using 100 cm³ metal rings, following the procedure described by Teixeira (2017) to determine soil density. Soil density was assessed in the 0-0.10 m and 0.10-0.20 m layers. Soybean yields were determined for all cultivated systems in the 2020/2021 crop season.

Table 2. Crop sequence in the last four years of production systems conducted from 1995 to 2021 at the Experimental Station of the Embrapa Western-Region Agriculture, Dourados, MS.

System	Crop season							
	2017	2017/18	2018	2018/19	2019	2019/20	2020	2020/21
CS	O	S	O	S	O	S	O	S
NTSa	W	S	N	C	O	S	W	S
NTSb	O	S	W	S	N	C	O	S
NTSc	N	C	O	S	W	S	N	C
ICLSa	O	B	B	B	B	S	O	S
ICLSb	B	S	O	S	O	B	B	B
PP	B	B	B	B	B	B	B	B

CS = Conventional System, with soybean/oat succession; NTS = No-Till System, with crop rotation; ICLS = Integrated Crop-Livestock System; PP = Permanent Pasture System; S = soybean; O = oats; W = wheat; N = turnip rape; C = corn; P = brachiaria grass.

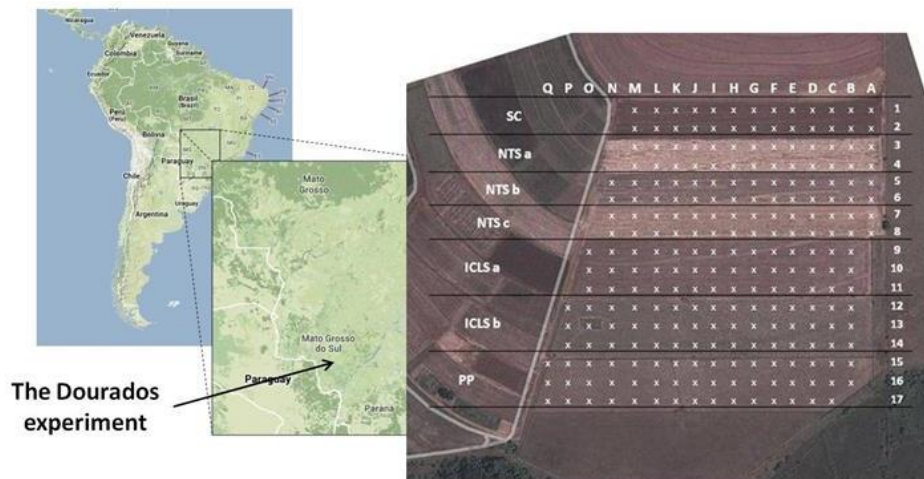


Figure 1. Location of the experiment and grid of points used for soil sampling conducted between July and September 2021.

A multivariate principal component analysis was conducted to understand the role of the soil chemical and physical variables on the variability in the occurrence of *R. reniformis*. To do this, we used data on pH in water, Al^{+3} , Ca^{+2} , Mg^{+2} , $H^+ + Al^{+3}$, K^+ , P, OM, and soil density at the 0-0.10 m and 0.10-0.20 m soil layers from all the points where these variables were collected, regardless of the system. The analysis was conducted using R software (R Core Team, 2023) with the aid of the FactoMineR package (Le et al., 2008). Analysis of variance and mean comparison tests were conducted using AgroEstat software (Barbosa and Maldonado Júnior, 2015).

3. Results and Discussion

The results of the nematological assessments showed the almost absolute prevalence of the reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) in the experimental area, with population densities ranging from zero to 4,370 nematodes/200 cc of soil. Phytonematodes of the genera *Helicotylenchus*, *Scutellonema*, *Meloidogyne*, and *Pratylenchus* were also found, but at very low population densities and in very sporadic and erratic frequency. Thus, considering its importance as a cause of severe damage to soybeans, *R. reniformis* was used to analyze horizontal distribution and population density according to production systems.

The average population densities (nematodes/200 cc of soil) of *R. reniformis* in the different production systems are shown in Figure 2. There was significant variation in the nematode population density depending on the production system. The lowest average population density (2 nematodes/200cc of soil, close to the detection limit) was found in the PP system (permanent pasture). The nematode population was also very low in the integrated crop-livestock systems, being lowest in the ICLSB system (12 nematodes/200cc of soil), which in the last two years was in the livestock cycle. The highest

population densities of the nematode were observed in the conventional system, with soybean succession in spring/summer and oats in fall/winter, and in the no-till system (NTSa), in which soybean (a species susceptible to the nematode) had been grown in the last two years before sampling (Table 2). The effect of rotation with the corn crop used in no-till systems (NTSb and NTS c) in reducing the nematode population density is evident. In these systems, the nematode population was higher than in the ICLS and PP systems.

The presence of the reniform nematode in the system with permanent pasture (PP), albeit in very low quantities, suggests that the species was already present in the experimental area when the experiment was set up 26 years ago. The successive cultivation of a favorable host crop (in this case, soybeans) led to a significant increase in its population density in the soil, both in the CS and in the NTSa systems, where soybeans had been cultivated in the last two crop seasons. However, in the ICLS systems, alternating soybeans with two years of livestock farming on *U. brizantha* pasture prevented an increase in the nematode population in the soil. In the NTS, rotation with corn after two years of soybeans did not increase the nematode population in the same way as in the CS system, but it was less effective than in the ICLS systems.

The reniform nematode has the peculiar characteristic of surviving long periods without favorable hosts (Starr, 1998). Depending on the population density, one year of rotation with non-host crops may be insufficient to reduce the population (Lawrence, 2022). This is evident when comparing ICL systems, in which there is a two year rotation with brachiaria grass, and NTS, in which there is a one year rotation of corn for every two years of soybean cultivation. The permanent pasture of brachiaria grass kept the nematode population extremely low, almost at the detection limit. These data confirm the need to use crop rotation systems with a greater number of crop cycles of non-host crops for cultural management in highly infested areas.

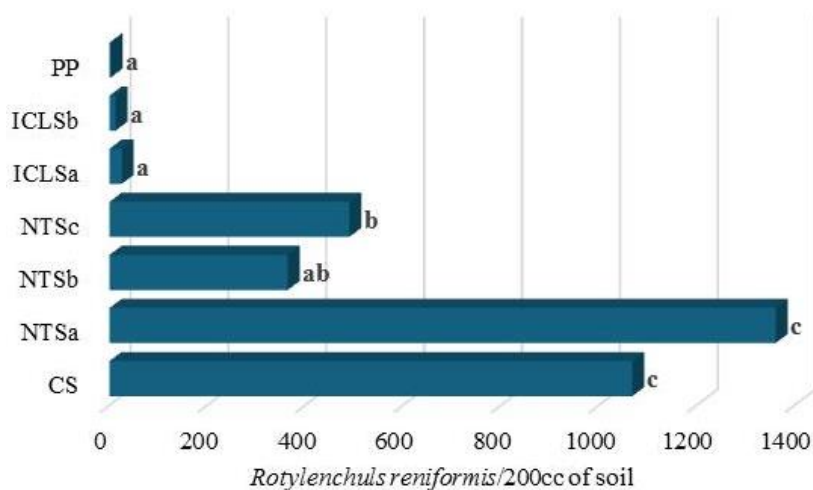


Figure 2. Population density (nematodes/200cc of soil) of *Rotylenchulus reniformis* in soil under different production systems, implemented 26 years ago at the Experimental Station of the Embrapa Western-Region Agriculture, Dourados, MS. (CS = Conventional System, in monoculture; NTS = No-Till System, with crop rotation; ICLS = Integrated Crop-Livestock System; PP = Permanent Pasture System). Columns followed by the same letter do not differ ($p < 0.05$).

When these same production systems were in their eighth year of operation, Sereia et al. (2007) analyzed the frequency and abundance of phytonematodes, showing that *R. reniformis* was the main species present in the area. The results obtained by Sereia et al. (2007) already pointed to the great difference between the population density of the reniform nematode in the different production systems, with 2,282, 26, 3, and zero nematodes/200cc of soil, respectively, in CS, NTS, ICLS, and PP.

The biggest change observed since then has been the increase in the nematode population in no-till areas (NTSa), especially when the last two crop seasons were cultivated with soybeans (a susceptible host) in the summer. This implies that the return of the host crop, in this case, soybeans, after a period of rotation with non-host crops, leads to a resumption of the increase in the nematode population density. Therefore, crop rotation programs in infested areas to manage *R. reniformis* should be permanent, periodically growing a non-host species.

Soybean grain yields in the different systems in which the crop was grown in the 2020/2021 crop season are shown in Figure 3. It can be seen that the NTSa, NTSb, and ICLSa systems had higher grain yields than the CS system, with an increase of 1,079, 645, and 639 kg/ha, respectively, compared to the CS system. These increases, however, should not be attributed solely to differences in *R. reniformis* population densities. Improvements in other soil attributes associated with the more diversified and integrated systems (NTS and ICLS), such as higher carbon contents (Salton et al., 2013), may have played a more important role than the nematode in the observed differences in grain yield.

A Pearson correlation analysis considering all

sampling points indicated no significant correlation ($r = -0.1078$; $p = 0.2496$) between nematode population density and soybean grain yield. However, when considering the variability within the NTSa system, which showed the widest range of values for the population density of *R. reniformis* in the soil (from zero to 4,370 nematodes/200cc of soil), the correlation between the nematode population and grain yield was highly significant (Figure 4), indicating that, in the same system, the nematode plays an important role in the expression of soybean grain yield.

The principal component analysis (Figure 5) shows that the highest nematode population densities are associated with higher soil densities, especially in the 0.10-0.20 m soil layer, and with higher phosphorus and potassium contents. On the other hand, lower nematode population densities are associated with higher organic matter contents. The availability of roots from susceptible plants as a food source is one of the main factors responsible for the colonization of soil horizons by *R. reniformis* (Moore et al. 2011). The nematode can inhabit soil extracts below the arable layer (Robinson et al., 2000), accompanying root growth at depth.

However, physical impediments, such as soil densification, limit root growth in depth, causing roots to concentrate in surface layers and, consequently, the possibility of an increase in nematode population density in horizons where conditions are more conducive to biological activity and population growth. The association of high populations of *R. reniformis* with higher K levels had already been observed in a study conducted by Pettigrew et al. (2005) on cotton and, at first glance, could suggest that this nematode occurs in higher population densities in soils with good chemical fertility.

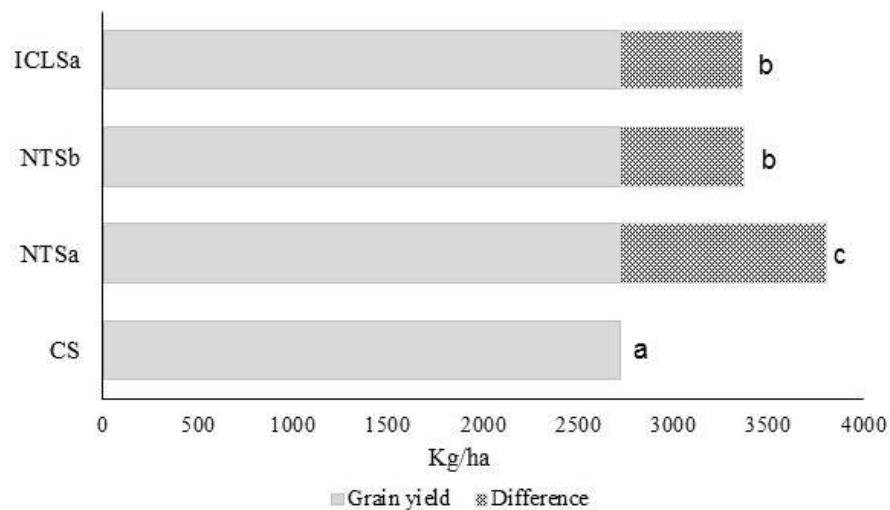


Figure 3. Grain yield (kg/ha) of soybeans in systems implemented 26 years ago, 2020/2021 crop season. Dourados, MS. Columns followed by the same letter do not differ ($p < 0.05$).

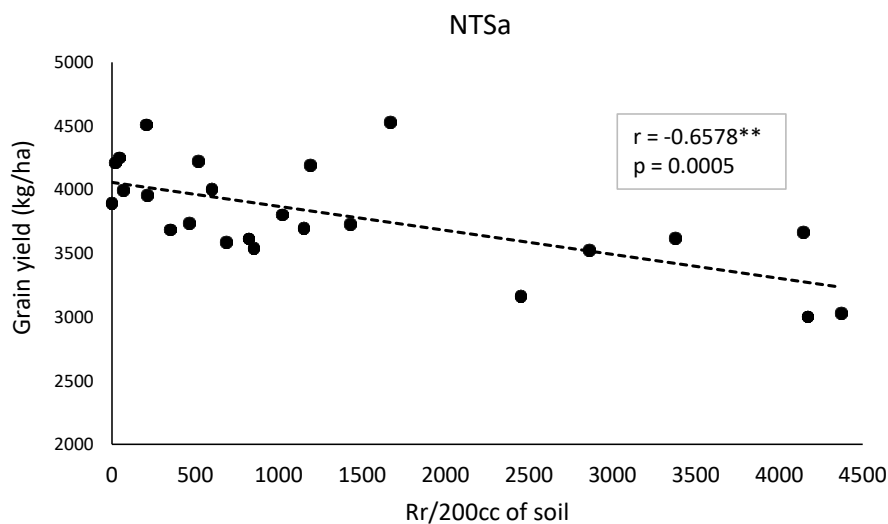


Figure 4. Correlation between soybean yield (kg/ha) and population density of *Rotylenchulus reniformis* in the soil (nematodes/200cc of soil) in the NTSa system. Dourados, MS.

One of the possibilities for the similar results obtained in this study is that soybeans grown in soil with higher nutrient contents would have a larger root volume, offering more food for the nematode to reproduce and increasing its population density. However, another possibility is that plants with roots heavily parasitized by the nematode would not be able to extract and translocate these nutrients to the shoot, and, over time, they would accumulate in the soil. Reinforcing this hypothesis, a study conducted by Kahn and Hussain (1990) with cowpea (*Vigna unguiculata*) showed that plants inoculated with *R. reniformis* showed a decrease in P and K content in the shoot and accumulation in the roots, when compared to non-inoculated plants.

Organic matter can improve the soil's chemical, physical, and biological aspects. Organic carbon is a

source of energy for the edaphic microbiota, which includes various species of fungi, bacteria, and even predatory nematodes, which act in the natural control of phytonematodes, either by antagonism, parasitism or competition (Rodríguez Kábana, 1986). In addition, organic matter decomposition releases toxic compounds to phytonematodes (Oka, 2010)

Evaluating the effect of soil attributes on the phytonematode population in a field study conducted in the municipality of Ceres, GO, Oliveira et al. (2023) observed that organic matter inhibited the presence of phytonematodes, a fact similar to that observed in the present study. Thus, production systems that provide a greater supply of organic matter contribute to maintaining the biological balance of the soil and limit the population growth of phytonematodes, as verified in the current study.

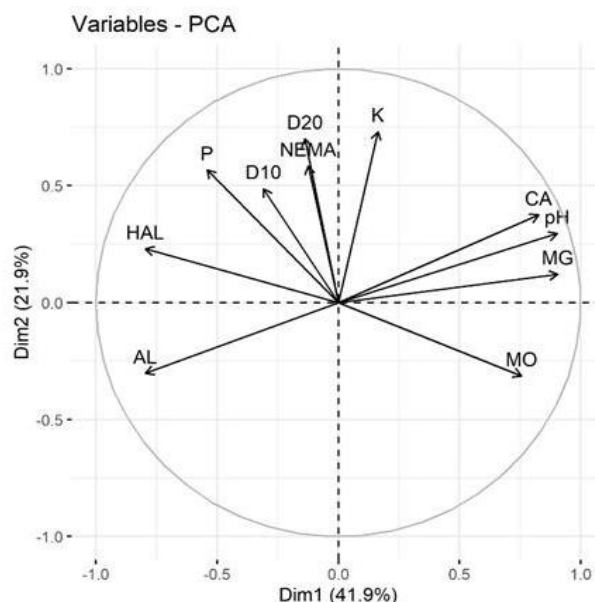


Figure 5. Principal component analysis of soil chemical and physical attributes and the population density of *Rotylenchulus reniformis*. pH = pH in water; AL = Al^{3+} ; CA = Ca^{2+} ; MG = Mg^{2+} ; HAL = $H^+ + Al^{3+}$; K = K^+ ; MO = Organic matter; D10 = soil density in 0.0-0.10 m layer; D20 = soil density 0.10-0.20 m layer; NEMA = population density of *R. reniformis* in the soil.

4. Conclusions

In integrated crop-livestock systems, no-till systems, and permanent pastures, the reniform nematode (*R. reniformis*) population remains low, even after almost three decades of farming. Conventional systems, with monocultures of favorable host species, allow the population density of the nematode to grow over time. Regardless of the production system, the population density of *R. reniformis* is greater in soil with higher densities in the subsurface layers, with higher P and K contents, and low organic matter content.

Authors' Contribution

The authors contributed to this scientific article as follows: Guilherme Lafourcade Asmus conceived the experiment and was responsible for planning the nematological evaluations, organizing the data, and writing the manuscript. Júlio Cesar Salton outlined the experimental design in the field and was responsible for conducting the systems during the experimental period and for the organic matter analysis of the soil. Michely Tomazi and Fabrícia da Silva Ramos analyzed and interpreted the soil's physical and chemical attributes. Rafael Silva Ferreira helped with the physical and chemical analysis of the soil, and was responsible for the statistical analysis and revision of the manuscript.

Acknowledgments

To the AGRISUS Foundation, to the AISA/ITAIPU BINACIONAL Project, and to FUNDECT 18/2021 MS-Carbono Neutro TO 017/2022 for their financial support.

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