

Perceptions on regenerative practices in suppressing soybean root diseases

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ABSTRACT: The main hypothesis of this study is that a conservationist system combined with soil regenerative practices can reduce the incidence of *Macrophomina phaseolina* in soybean crops and consequently increase productivity. The study was conducted in the experimental area of the Federal University of Santa Maria, Frederico Westphalen Campus, RS, Brazil, during the 2023/2024 harvest. The experimental design consisted of main blocks in which three production systems were allocated: 1) no-tillage black oats system with physical intervention; 2) no-tillage black oats system; and 3) no-tillage polyculture system. The results showed that the no-tillage polyculture system presented 36.5% and 31.7% lower incidence of *Macrophomina phaseolina* and consequently higher grain productivity with an increase of 23% (675 kg ha⁻¹) and 15.08% (473 kg ha⁻¹) in relation to the no-tillage black oats system with physical intervention and no-tillage black oats system. The weight of a thousand grains was the variable which best explained the increase in productivity, with a significant increase of 7.9% and 7.01% in the no-tillage polyculture system compared to the no-tillage black oat system with physical intervention and no-tillage black oat system. The no-tillage system combined with regenerative practices is the best way to minimize losses due to root diseases in soybean crops.

Key words: cover crops; *Macrophomina phaseolina*; no-tillage; soil health.

Percepções sobre práticas regenerativas na supressão de doenças radiculares da soja

RESUMO: A hipótese principal desta pesquisa é que, sistema conservacionista aliado a práticas regenerativas de solo possa reduzir a incidência de *Macrophomina phaseolina* na cultura da soja, e consequentemente aumentar a produtividade. O estudo foi conduzido na área experimental da Universidade Federal de Santa Maria, Campus Frederico Westphalen - RS, durante a safra 2023/2024. O delineamento experimental foi constituído por blocos principais, onde foram alocados três sistemas de produção: 1) sistema plantio direto aveia preta com intervenção física, 2) sistema plantio direto aveia preta e 3) sistema plantio direto policultivo. Os resultados demonstram que o sistema plantio direto policultivo apresentou 36,5% e 31,7% menor incidência de *Macrophomina phaseolina* e consequentemente maior produtividade de grãos com aumento de 23% (675 kg ha⁻¹) e 15,08% (473 kg ha⁻¹) em relação ao sistema plantio direto aveia preta com intervenção física e sistema plantio direto aveia preta. O peso de mil grãos foi a variável que melhor explicou o incremento de produtividade, com aumento significativo de 7,9% e 7,01% no sistema plantio direto policultivo em comparação ao sistema plantio direto aveia preta com intervenção física e plantio direto aveia preta. O sistema plantio direto aliado a práticas regenerativas é o caminho para minimizar as perdas por doenças radiculares na cultura da soja.

Palavras-chave: culturas de cobertura, *Macrophomina phaseolina*, plantio direto, saúde do solo



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Introduction

New challenges are proposed with each harvest by the climate, management and genetics in Brazilian crops in search of increased productivity. In addition, the adverse climatic conditions occurring around the world (for example, increased temperatures and periods without rainfall during the summer) have made food production increasingly difficult (Zullo Jr. et al., 2008; Kassam et al., 2009; Crusciol et al., 2022; Amado, 2023), especially in specific regions such as southern Brazil where extreme weather events are occurring more frequently. Due to such uncertainty, high crop yields, as well as the maintenance of high productivity levels over time, are threatened by extreme weather events.

High yields, as already evidenced by studies through the Brazilian Soybean Strategic Committee (CESB), present a clear example that soybeans have a very high productivity potential proposed by genetics to be efficiently capable of converting all production of photoassimilated into grains (Sako et al., 2015; Battisti et al., 2017). A second path is production stability, in which the productive environment has the ability to withstand stress situations through the interaction between soil conditions and the plant in such a way that productivity is maintained or losses are minimal, regardless of the climatic condition proposed during the harvest (Bossolani et al., 2021; Bossolani et al., 2022).

The no-tillage system is based on soil conservation and follows basic principles such as minimum soil disturbance, permanent cover, and crop rotation (Pires et al., 2020). These principles enabled advancement in agriculture in tropical and subtropical climates, and have been and will be the guiding axis for producers to achieve stability and high productivity in their crops. In this sense, crops managed in low complexity systems (i.e. without crop rotation), negative carbon balance (i.e. biomass input lower than the decomposition rate of soil organic matter), and with low species diversity can result in threats to productivity with increased pressure from soil pathogens. In the case of soybeans, the occurrence of *Macrophomina phaseolina* constitutes such a threat, which is a necrotrophic fungus occurring in production systems with low complexity (Basandrai et al., 2021; Marquez et al., 2021; Rangel-Montoya et al., 2022). This fungus causes the root disease known as root rot and its incidence has compromised the plant performance of soybean crops globally (Hartman et al., 1999). This disease causes plants to end their life cycle before the senescence stage, directly affecting productivity, interrupting the transport of water, nutrients and photoassimilates by the roots and are therefore not relocated to the plant area (Short et al., 1978; Rangel-Montoya et al., 2022).

The agronomic performance of these crops is extremely dependent on soil health. The diversity of beneficial microorganisms present in the soil is sought to the detriment of the ability of pathogenic organisms to cause plant diseases so that crops can express their maximum potential (Pires et al., 2020). Mendes et al. (2011) showed that there are soils

where fungi that cause root diseases are suppressed by the presence of other organisms, reducing the incidence of root diseases from 69 to 3%. In this sense, investigations proposing management practices which restore the populations of beneficial/suppressive microorganisms and reduce the incidence of root diseases, such as that caused by *Macrophomina phaseolina*, will be extremely necessary for better understanding of production systems that can promote soil health and consequently plant health for the coming years.

The hypothesis of this study is that conservation systems combined with other regenerative practices which result in high dry matter production by cover crops, species biodiversity and sowing of the main crop on cover crops which are still green/physiologically active, can improve soil health and consequently reduce the presence of pathogens such as *Macrophomina phaseolina* in soybean crops. The main objective of this study was to quantify the incidence of *Macrophomina phaseolina* in soybean crops, in addition to the yield and final grain yield components in three production systems.

Materials and Methods

The study was developed in the southern region of Brazil, in the municipality of Frederico Westphalen, RS, with coordinates 27° 21' 32" S, 53° 23' 38" W, and an average altitude of 566 m. The experimental field is located in the Department of Agronomic and Environmental Sciences of the Federal University of Santa Maria, *Campus* Frederico Westphalen.

The soil is classified as a dystrophic red latosol, deep and well-drained (Santos et al., 2018). The chemical characteristics of the soil before installation of the experiment at the depth of 0-10 cm identified a pH in H₂O of 5.7, organic matter content of 36.7 g dm⁻³, P mehlisch⁻¹ of 8.8 mg dm⁻³, K⁺ of 0.68 cmol_c dm⁻³, Ca⁺² of 5.51 cmol_c dm⁻³, Mg⁺² of 2.49 cmol_c dm⁻³, S of 6.21 mg dm⁻³, and while Al⁺³ was not detected.

The experiment began in April 2022 when three models of production systems were implemented, allocated in main blocks with dimensions of 45 m x 18 m, in a useful area of 810 m² for each production system. The systems studied were: 1) no-tillage system with physical intervention using black oats (*Avena strigosa* Schreb.) as winter cover crop; 2) no-tillage system using black oats as winter cover crop; and 3) no-tillage system using polyculture composed of different species as winter cover crops. The species used in polyculture were black oats, white oats (*Avena sativa* (L.)), rye (*Secale cereale* (L.)), common vetch (*Vicia sativa*), hairy vetch (*Vicia villosa*), white lupine (*Lupinus albus*) and fodder turnip (*Raphanus sativus* (L.)).

The soil in the no-tillage system with physical intervention was only prepared at the beginning of the experiment in 2022 with chiseling, turning with disc plowing, followed by harrowing. The sowing rate used for the cover crops was: 80 kg ha⁻¹ in both systems with black oats, and 120 kg ha⁻¹ for polyculture. The cover crops were desiccated 24 hours before

sowing of the soybean crop with herbicide application: active ingredient (Saflufenacil) concentration (700 g kg^{-1}), Glyphosate (620 g L^{-1}) and Setoxidim (184 g L^{-1}). The soybean cultivar used in the first year and second year was Monsoy 5947, and the results of the *Macrophomina phaseolina* incidence, yield components and final grain yield were quantified in the second year, so that the results are better expressed as a function of the cumulative effect of the managements established in the different production systems during two consecutive harvests. Soybean sowing in the second year took place on December 21, 2023, at a density of 12 seeds m^{-2} , while the base fertilization was 250 kg ha^{-1} conducted on February 23 2023 with a formulation of N, P_2O_5 and K_2O .

The dry matter production of the cover crops was estimated when they reached full flowering stage. The collection was performed in an area of 0.250 m^2 , collecting nine replicates in each production system. The samples were kept in a drying oven under constant air circulation at a temperature of 65°C .

Next, 3 replicates of 10 random plants were collected in each production system during the R7.3 phenological stage to evaluate the *Macrophomina phaseolina* incidence in the soybean crop, close to the harvest stage. The plants were identified in the field and transported to the laboratory to perform the evaluations. A cross-section was initially made in each plant individually at the base of the root identifying the presence or absence of the disease. Then, the grains were separated individually from these same plants to compute the weight of one thousand grains, finding the potential for loss caused by the disease.

For the yield components of each system, 3 replicates were collected containing 10 plants in each replication obtained randomly to estimate the number of grains per plant and weight of one thousand grains. Yield was calculated over a useful area of 2.7 m^2 in each replication, where 3 replicates were collected in each production system, the harvest was carried out manually and then the grains were weighed and the moisture corrected to 13%.

The data were submitted to analysis of variance (ANOVA) by Tukey's at 5% probability of error through the SISVAR statistical program (Ferreira, 2011).

Results

We observed variability in the *Macrophomina phaseolina* incidence in soybean cultivation between the different production systems (Figure 1A). The no-tillage black oats with physical intervention and no-tillage black oats production systems were statistically superior to polyculture, with an average difference of more than 36.5% and 31.7%, respectively, on the *Macrophomina phaseolina* incidence. The weight of a thousand grains over the absence or presence of *Macrophomina phaseolina* (Figure 1B) was statistically higher when there was absence of the disease, representing a mean increase of 14.2% in relation to the presence of *Macrophomina phaseolina* in soybean crops. The dry matter production in the different production

systems (Figure 1C) showed that the dry matter production was very similar (6342 and 6755 kg ha^{-1}) when the same black oat cover crop plant was used, regardless of the production system. When the no-tillage production system with polyculture as cover crop was chosen, there was an increase in dry matter production of up to 103 and 91.4% in relation to the two production systems.

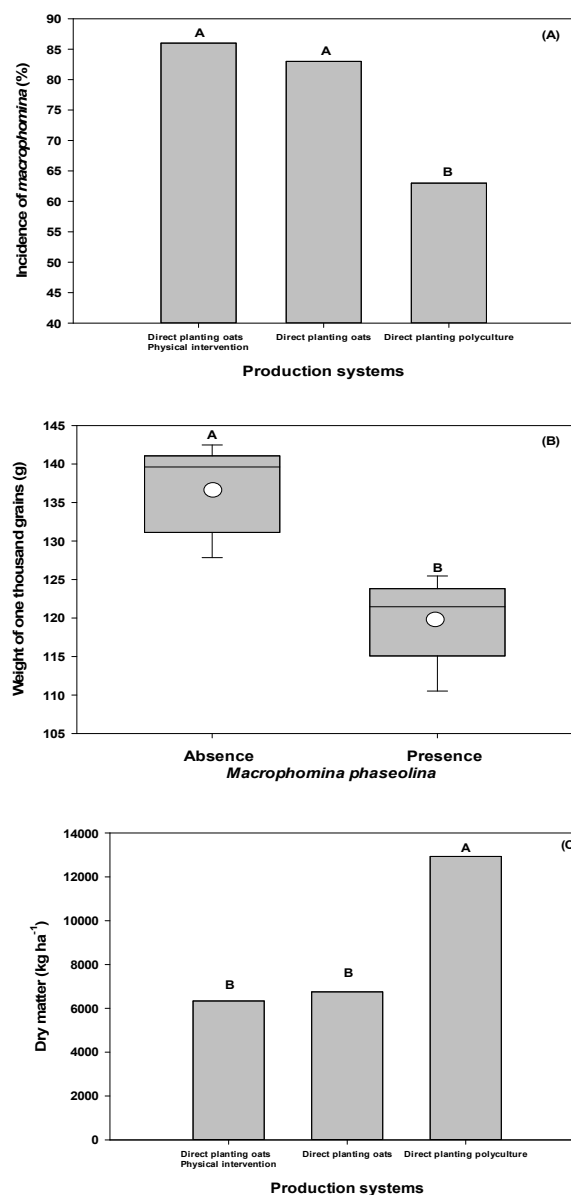


Figure 1. (A) Incidence of *macrophomine*, (B) weight of one thousand grains on the absence and presence of *macrophomine* and (C) Dry matter of cover crops in different production systems. Frederico Westphalen, RS, Brazil, 2024. Coefficient of variation CV, (A) CV 53.6%, (B) CV 5.57% and (C) CV 17.86%.

It was identified that there was no difference between the production systems for the yield component number of grains per plant (Figure 2A), with an upward trend of approximately 5.7% and 4.2% in the no-tillage black oats with physical intervention in relation to the no-tillage black oats system and the polyculture no-tillage system. For the weight of one thousand grains (Figure 2B), the polyculture no-tillage system

was statistically superior to the other systems, with increases in the order of 7.9% and 7.01% in relation to the no-tillage black oats with physical intervention and no-tillage black oats. Regarding the final grain yield for the soybean crop (Figure 2C), the results showed that the polyculture no-tillage system was superior to the others, with a significant increase of 23% (675 kg ha^{-1}) and 15.08% (473 kg ha^{-1}) in relation to no-tillage black oats with physical intervention and no-tillage black oats.

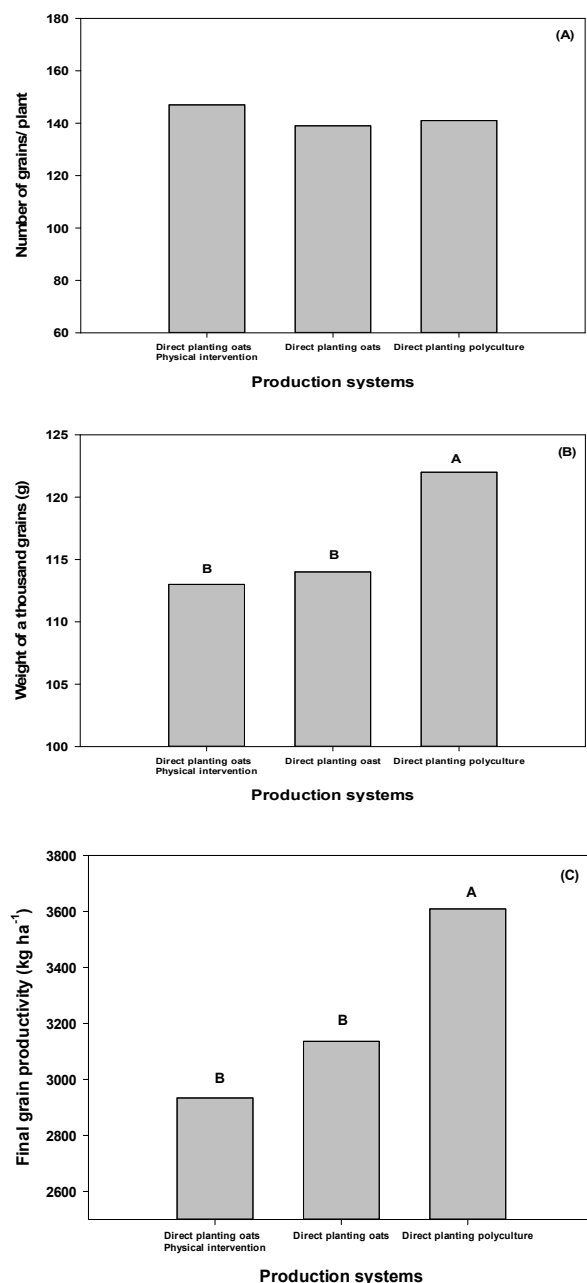


Figure 2. Yield components, (A) number of grains per plant, (B) weight of one thousand grains and (C) final grain yield of soybean crop in different production systems. Frederico Westphalen, RS, Brazil, 2024. Coefficient of variation CV, (A) CV 11.08%, (B) CV 2.34% and (C) CV 9.36%.

Discussion

The no-tillage system with the use of polyculture as a cover crop proved to be efficient in mitigating the *Macrophomina phaseolina* incidence in the soybean crop after two consecutive harvests. This information is extremely relevant due to the fact that *Macrophomina phaseolina* is a pathogenic fungus which is difficult to control (Basandrai et al., 2021). These organisms have survival structures (microsclerotia) which remain in the soil and crop residues throughout seasons (Gupta et al., 2012; Vibha, 2016), making it difficult to control them in soybean crops globally. The impact of this fungus can result in significant economic losses (Almeida et al., 2005; Kaur et al., 2012; Vibha, 2016; Rangel-Montoya et al., 2022).

In this sense, straw production (dry matter from the different production systems) helps us to understand the dynamics of the disease. The systems which had black oats as the only cover crop for two consecutive seasons resulted in lower biomass production in relation to polyculture; consequently, there was lower *Macrophomina phaseolina* incidence in soybean cultivation cultivated in areas where there was polyculture of cover crops. These results indicate that dry matter production close to 6 t ha^{-1} by black oats, associated with low plant diversity, is not enough to mitigate the incidence of *Macrophomina phaseolina*. The amount of biomass produced by black oats resulted in little protection on the soil surface, constituting a physical barrier during soybean cultivation, increasing the probability of microsclerotia germination of *Macrophomina phaseolina*, its dissemination and infection in host plants (Linhares et al., 2018).

Studies conducted in Brazil have already shown that the control of *Macrophomina phaseolina* in areas under low quality no-tillage and low crop rotation was not sufficiently able to dilute losses by *Macrophomina phaseolina*, mainly due to the fact that there is little residual straw on the surface, reducing soil moisture and increasing temperature fluctuations that favor the pathogen (Linhares et al., 2018; Almeida et al., 2003). On the other hand, no-tillage areas with constant use of cover crops that consequently contribute more residues and accumulate more carbon in the soil throughout the harvests can increase the abundance of microorganisms beneficial to plant protection and health, drastically reducing the population of pathogens that detract from plant health such as *Macrophomina phaseolina* (Khan et al., 2020; Passinato et al., 2021).

Furthermore, polyculture, which differently produced twice as much dry matter $> 12 \text{ ton ha}^{-1}$ when compared to black oats, allowed a longer time of soil cover during soybean development, helping to break dormancy and germination of *Macrophomina phaseolina* microsclerotia, keeping the temperature more stable and with higher humidity (Basandrai et al., 2021). In addition, polyculture contains different species of legumes, cruciferous and grasses which stimulate the diversification of microbial communities in the soil, potentially favoring the prevalence of beneficial fungal and bacterial microorganisms, antagonists of soil pathogens and

promoters of plant health (Khalili et al., 2016; Khan et al., 2020; Pires et al., 2020; Passinato et al., 2021; Rangel-Montoya et al., 2022).

These beneficial microorganisms can be stimulated to increase their populations, mainly by root exudates (high carbon lability). They also include low molecular weight compounds, such as amino acids, organic acids, sugars and other secondary metabolites, as well as high molecular weight compounds such as mucilage (polysaccharides) and proteins (El Zahar Haichar et al., 2014) which are released by the roots into the soil rhizosphere in greater quantities, quality and time when using different plant species (Jones et al., 2004). The increase in the diversity of microorganisms and the guarantee of their survival is enhanced when we associate the diversity of plant species with a broader cycle, high dry matter volumes (i.e. straw) on the soil surface and active roots for as long as possible in the productive environment (Vargas Gil et al., 2009; Chavarría et al., 2016).

Allied to this, we investigated the potential to reduce the weight of a thousand grains (a yield component which defines productivity) in the soybean crop, for which it was clear that the soybean plants presented a significant reduction in grain weight when *Macrophomina phaseolina* was identified and present in the plants which. Different studies have already demonstrated the potential for soybean yield losses due to this soil pathogen which initially proliferates in the roots, dehydrating the vascular system tissues of the plant stem responsible for transporting water, nutrients, and photoassimilates (Lodha & Mawar, 2020).

The number of grains per plant did not differ between the production systems, even with a higher incidence of *Macrophomina phaseolina* in the no-tillage black oats with physical intervention and no-tillage black oats systems. For the conditions of this study, it was possible to verify that *Macrophomina phaseolina* shows greater presence and consequently potential for losses during the end of the crop cycle after defining the number of pods and beginning of grain filling by the plant (stage R5.1). Some studies around the world conducted in other intensive systems have already reported *Macrophomina phaseolina* incidence in an initial phenological stage, compromising production with irreversible losses when associated with a low-quality production environment, dry climate with water restriction and the presence of the pathogen in high populations in the soil (Machado, 1987; Wrather et al., 1997; Crusciol & Costa, 2018).

The relationship for the weight of a thousand grains and final grain yield was very similar to the effect of the production systems on the *Macrophomina phaseolina* incidence. Polyculture differed from the other systems, as it presented lower *Macrophomina phaseolina* incidence and consequently higher weight of a thousand grains and productivity. In this sense, our study reinforces that root disease caused by the fungus *Macrophomina phaseolina* has high potential to reduce productivity, and it has been shown that practices aimed at promoting soil health are important to promote plant health, ensure that plants of economic interest can perform their functions with minimal stress

during their cycle, and that root diseases are suppressed by the end of the plant cycle.

Conclusions

The no-tillage conservation system associated with the diversity of plant species using a polyculture of cover crops is a great opportunity to restore soil health and consequently alleviate *Macrophomina phaseolina* incidence in soybean crops.

The weight of one thousand grains was the yield component which best explained the yield loss in the soybean crop due to *Macrophomina phaseolina* incidence in the roots, which had a potential reduction of up to 12.5% of grain weight.

Compliance with Ethical Standards

Author contributions: Conceptualization: UB, MJRS, CJB, MRC, LCP, TJCA; Data curation: UB, MJRS, CJB, MRC, LCP, TJCA; Investigation: UB, MJRS, CJB, MRC, LCP, TJCA; Methodology: UB, MJRS, CJB, MRC, LCP, TJCA; Software: UB, MJRS, CJB, MRC, LCP, TJCA; Supervision: UB, MJRS, CJB, MRC, LCP, TJCA; Validation: UB, MJRS, CJB, MRC, LCP, TJCA; Visualization: UB, MJRS, CJB, MRC, LCP, TJCA; Writing – original draft: UB, MJRS, CJB, MRC, LCP, TJCA; Writing – review & editing: UB, MJRS, CJB, MRC, LCP, TJCA.

Conflict of interest: There is no conflict of interest.

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