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LAVRAS - MG
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Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia, área de concentração em Fitopatologia, para obtenção do título de Mestre.

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RAFAEL COELHO SILVA

O USO ALTERNADO DE PRODUTOS BIOLÓGICOS INTERFERE NAS DOENÇAS DA SOJA E PRODUTIVIDADE ?

DOES BIOLOGICAL PRODUCT'S ALTERNATE USE INTERFERE IN SOYBEAN DISEASES AND YIELD?

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitopatologia, área de concentração em Fitopatologia, para a obtenção do título de Mestre.

Aprovovado em 01 de março de 2024.

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LAVRAS – MG
2024

“Há pessoas que desejam saber só por saber, e isso é curiosidade; outras, para alcançarem fama, e isso é vaidade; outras, para enriquecerem com a sua ciência, e isso é um negócio torpe; outras, para serem edificadas, e isso é prudência; outras, para edificarem os outros, e isso é caridade.”

Santo Agostinho

DEDICATION

First to God, who is above all, and who makes everything in life possible for me;

My family, firstly my father and mother;

To all the people who contributed in some way to me getting here;

Science in Brazil, despite not being valued, deserves its place here.

ACKNOWLEDGMENTS

First and foremost, I thank God, who is almighty and knows what is best for all of us;
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To my postgraduate colleagues, Felipe, Carla, Gabriely, Bárbara, Bruno, José Manoel, Valter, and Victor, who shared many experiences with me;
To the Federal University of Lavras (UFLA), the Department of Phytopathology (DFP), and the Postgraduate Program in Agronomy/Phytopathology, for the opportunity to take the course;
This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Financing Code 001; and also by the Research Support Foundation of the State of Minas Gerais (FAPEMIG);
To everyone who somehow contributed to my journey and whose names were not mentioned above, who, nonetheless, are in my thoughts;
And finally, to everyone who doubted me.

RESUMO

A soja (Glycine max L.) é uma das culturas mais importantes para a economia mundial, com o Brasil sendo líder global em produção. No entanto, a cultura sofre com vários problemas que causam perdas de produtividade, como problemas climáticos, nutrição, adaptabilidade genética e doenças, sendo esta última o fator essencial após o plantio. Existem muitas doenças que afetam a cultura, como mofo branco causado por *Sclerotinia sclerotiorum*, mancha púrpura da semente (*Cercospora kikuchii*) e ferrugem-asiática (*Phakopsora pachyrhizi*), todas, doenças fúngicas que causam grandes danos. O desafio relativo a elas é a seleção de patógenos resistentes causadas pelo uso recorrente de produtos químicos com mesmo mecanismo de ação, diminuindo sua eficácia. Desse modo, conduzimos dois experimentos usando rotação de produtos biológicos: o primeiro usando *Bacillus* spp. para controlar a ferrugem (*Phakopsora pachyrhizi*) na soja; e o segundo usando *Trichoderma* spp. para controlar o mofo branco (*Sclerotinia sclerotiorum*). No primeiro experimento, verificamos que na primeira época de semeadura a combinação dos produtos Twixx seguido do Bioimune, embora não apresentem diferenças no controle da doença aumentaram a produtividade e concequentemente um maior lucro, por outro lado, na segunda época, não ouve diferença estatística no quesito produtividade para os tratamentos Bioimune; Twixx_Bioimune; e Bioimune_Twixx, embora a primeira combinação tenha apresentado maior valor absoluto para produtividade e tenha proporcionado um lucro maior. No segundo experimento, embora tenha havido um efeito da aplicação de *Trichoderma* sobre o rendimento, em comparação com o controle, a rotação de *Trichoderma* não teve diferença entre si; a viabilidade dos escleródios foi reduzida pelo uso do controle biológico, mas o parasitismo foi baixo, indicando a presença de outros organismos que podem ser recrutados pela aplicação de *Trichoderma*. Os experimentos nos mostram que cada patossistema sofre diferentes tipos de interferência do manejo com produtos biológicos e em alguns casos o posicionamento de diferentes produtos biológicos podem ser benéficos ou neutros.

Palavras-chave: manejo biológico; manejo da resistência; recrutamento microbiano.

ABSTRACT

Soybean (Glycine max L.) is one of the most important crops for the global economy, with Brazil being the global leader in production. However, the crop suffers from various problems that cause productivity losses, such as weather-related issues, nutrition, genetic adaptability, and diseases, the latter being the essential factor post-planting. There are many diseases that affect the crop, such as white mold caused by Sclerotinia sclerotiorum, purple seed stain (Cercospora kikuchii), and Asian rust (Phakopsora pachyrhizi), all fungal diseases that cause significant damage. The challenge related to them is the selection of resistant pathogens caused by the recurrent use of chemicals with the same mechanism of action, decreasing their effectiveness. Thus, we conducted two experiments using biological product rotation: the first using Bacillus spp. to control rust (Phakopsora pachyrhizi) in soybean; and the second using Trichoderma spp. to control white mold (Sclerotinia sclerotiorum). In the first experiment, we found that in the first planting season, the combination of Twixx followed by Bioimune, although not showing differences in disease control, increased productivity and consequently higher profits. On the other hand, in the second season, there was no statistical difference in productivity for the Bioimune treatments; Twixx_Bioimune; and Bioimune_Twixx, although the first combination showed a higher absolute value for productivity and provided higher profits. In the second experiment, although there was an effect of Trichoderma application on yield compared to the control, Trichoderma rotation showed no difference among treatments; scleroid viability was reduced by biological control use, but parasitism was low, indicating the presence of other organisms that can be recruited by Trichoderma application. The experiments show us that each pathosystem suffers different types of interference from biological product management and in some cases the positioning of different biological products can be beneficial or neutral.

Keywords: biological management; resistence management; microbial recruitment.

IMPACT INDICATORS

Soybean is a very important crop world wide, it has many souces of protein, oil and carbohydrates. It is the main source of cooking oil, and protein for animal nutrition; and its production increases every year more and more. The present work was developed because of many questions that the farmers had about the position of the products in disease management. Concerning biological control which is the main subject of this work, helps to bring balance to the environment and fewer chemical applications, bringing more life quality to the farmer.

The results we obtained can help to reduce the application of chemical products, and improve the use of biological ones, by utilizing or not different followed products into the agricultural system. This decrease of use, helps to establish a balance of the environment and build a system more self sustainable.

INDICADORES DE IMPACTO

A soja é uma cultura muito importante mundialmente, possuindo muitas fontes de proteína, óleo e carboidratos. É a principal fonte de óleo de cozinha e proteína para a nutrição animal; e sua produção aumenta a cada ano. O presente trabalho foi desenvolvido devido a muitas dúvidas que os agricultores tinham sobre a posição dos produtos no manejo de doenças. Em relação ao controle biológico, que é o principal assunto deste trabalho, ele ajuda a trazer equilíbrio ao ambiente e reduz a necessidade de aplicações químicas, proporcionando mais qualidade de vida ao agricultor.

Os resultados que obtivemos podem ajudar a reduzir a aplicação de produtos químicos e melhorar o uso dos biológicos, utilizando ou não diferentes produtos em sequência no sistema agrícola. Essa diminuição do uso contribui para estabelecer um equilíbrio ambiental e construir um sistema mais autossustentável.

SUMMARY

INTRODUCTION

Soybean (Glycine max L.) is a commodity of global importance, it is produced in all continents but Brazil and United States as larger producers, accounting for approximately 69% of the global production of soybean in 2022/23; it can be used for various purposes, such as a source of food for humans and animals raised for meat consumption, as well as for the production of oils and biofuels (UNITED STATES DEPARTMENT OF AGRICULTURE FOREIGN AGRICULTURAL SERVICE ARGENTINA RAMPS UP SOYBEAN IMPORTS FROM PARAGUAY AND BRAZIL, 2023). The crop was introduced in Brazil in 1882 by Gustavo Dutra, who brought it from the United States, where the Agronomic Institute of Campinas (IAC) conducted the initial research to select cultivars adapted to the local climate (CAVALCANTI, 1892).

The crop is cultivated throughout Brazil, driven by production technologies such as developing new cultivars, opening new agricultural frontiers, and constant improvements (ANA PAULA OLIVEIRA NOGUEIRA et al. 2015). Grain production has steadily increased in Brazil, for example, in the state of Rio Grande do Sul, where, according to the Instituto Rio Grandense do arroz, 39.2% of areas designated exclusively for rice production are now also used for soybean production, resulting in a 205.8% increase in the soybean cultivated area compared to the 2009/10 to 2020/21 crops (IRGA, 2021).

Although economically viable with a comprehensive global market, this crop still faces various diseases that affect its productivity and diminish its commercial value. Examples of fungal-caused diseases include Asian rust (Phakopsora pachyrhizi), White Mold (Sclerotinia sclerotiorum), Purple Seed Stain (Cercospora kikuchii), Red Root Rot (Fusarium solani species complex, mostly Fusarium brasiliensis, F. tucumaniae, F. crassistipitatum), Charcoal Rot (Macrophominaspp.), among others (L. AMORIM et al., 2016).

Among the options to control soybean diseases, chemical fungicide is the predominant control strategy. Despite its effectiveness, the successive use of these products can lead to the selection of a population of microorganisms resistant to the main fungicide groups(CLÁUDIA VIEIRA GODOY et al., 2020); Another form of control is genetic resistance; as an alternative to these methods of control, there is the use of multisite fungicides: mancozeb, chlorothalonil, and copper-based (KOEFENDER et al., 2023). Biological control of soybean diseasse has improved as an alternative to be applied in the field (). This method is unlike to exerta selection pressure due to thevarious mechanisms of action through which they act (CATELLI et al., 2009; MEYER et al., 2022).

Indeed, the biocontrol products act through competition for space and nutrients, parasitism, production of active or toxic compounds acting as bio-stimulants or antibiotics, and biofilm production, among others, and with no significant effects againts humans (those used in agriculture), this tool is increasingly gaining ground and establishing itself in the market(FLAUSINO DE FARIA et al., 2022; KASPAR et al., 2019; MARINÊS PEREIRA BOMFIM et al., 2010; WANG et al., 2018; YOU et al., 2016).

Considering that the multiple mechanisms of biological products are already established, and their individual use has been proven effective, the objective of this work is to verify whether the alternate use of different biological products influences two important diseases in soybean cultivation (Glycine max): Asian rust (Phakopsora pachyrhizi) and White Mold (Sclerotinia sclerotiorum).

LITERATURE REVIEW

SOYBEAN (*Glycine max* L.)

Most of the plants cultivated today have undergone both artificial and natural selection over the millennia since the sedentarization of humans. The soybean crop (Glycine max L.) is no exception. Even today, we have "perennial" soybeans, considered a spontaneous invasive plant in many locations, with a creeping growth habit, developing near lakes and rivers (EULA MARIA SIQUEIRA SANTOS MOZZAQUATRO et al., 2017). Its place of origin is central-eastern China, and its domestication occurred in the north-central part of China around the 11th century B.C. (EMIDIO RIZZO BONATO AND ANA LIDIA VARIANI BONATO, 1987).

Soybeans undergo development stages categorized into vegetative and reproductive stages. The vegetative stages are marked with the letter "V," ranging from "V1" to "Vn," where "n" is the number of nodes above the cotyledonary node. There are also two earlier stages: "VE," indicating cotyledon emergence, and "VC," indicating complete cotyledon opening. The reproductive stage is marked by the letter "R," ranging from "R1" to "R8," where "R1" indicates the beginning of flowering, and "R8" signifies complete soybean maturation for harvesting (NEUMAIER et al., 2020). Understanding the plant's developmental stages is crucial for recognizing the crop's needs and, consequently, directing proper management, especially for fertilization and applications of products for pest and disease control.

Among the various climatic factors that can affect the commodity, and more detrimentally its productivity, are temperature, water availability, and photoperiod. Temperatures below 20ºC are not suitable for seeding as they impede seed germination rates.

The ideal temperature range for soybean development is 20–30ºC, with temperatures below 10ºC unsuitable for cultivation and temperatures above 40ºC affecting productivity. A critical factor related to temperature is flowering, which is induced only at temperatures above 13ºC. Photoperiod is directly related to cultivar development. Despite being considered a "short-day plant," cultivars produced in locations with a smaller photoperiod amplitude can still produce normally, only delaying flowering. These cultivars have the characteristic of a long juvenile period and are used for cultivation in locations closer to the Equator. Water availability for soybean cultivation is critical during two main periods: germination-emergence and flowering-filling. Therefore, the concentration of rain during these moments in a homogeneous manner is essential for final productivity (JOSÉ RENATO B. FARIAS et al. (2007) e NEUMAIER et al. (2020)).

The expansion of soybean cultivation began in the 1970s, mainly in southern states, with cultivars and techniques coming from foreign countries where cultivation was established. Subsequently, due to the work of breeders, cultivars adapted to tropical regions were developed, making it possible to cultivate soybeans throughout the national territory (LOPES et al., 2009). The development of genetic improvement work allowed an expansion in the soybean cultivation area, leading to increased crop stability and improvements in the production chain (CORREA et al., 2017).

Today, Brazil is the world's largest soybean producer, along with the United States (USDA, 2023), which necessitates extensive studies on how to better work with the crop and address the problems associated with large-scale cultivation.

There are three different types of growth habits in soybeans: determinate, semi-determinate, and indeterminate. Indeterminate varieties can compensate for damage caused by various factors that may influence the crop's final productivity and cause losses, such as water stress, defoliation, or pests (ANDRÉ LUIS THOMAS, 2018). Among these factors are plant diseases, potential agents causing plant damage that will impact the harvest. These diseases can be caused by fungi, bacteria, nematodes, viruses (biotic), or by stress generated by nutrient deficiencies, environmental factors (abiotic) (L. AMORIM et al., 2018).

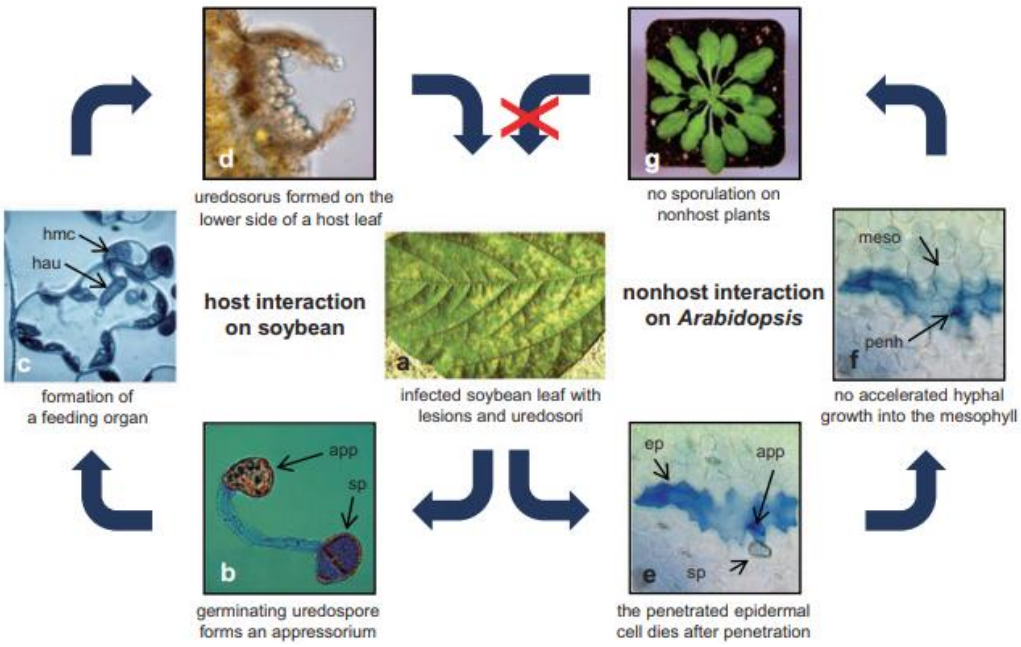
Within these biotic diseases, those caused by fungi in soybean cultivation have the potential to cause significant damage. Notable diseases include Purple Seed Stain (Cercospora kikuchii), Frogeye Leaf Spot (Cercospora sojina), Rust (Phakopsora pachyrhizi), and White Mold (Sclerotinia sclerotiorum), the latter causing damage in higher-altitude locations (Amorim et al., 2016).

In this study, the focus will be on the control of Rust (Phakopsora pachyrhizi) and White Mold (Sclerotinia sclerotiorum).

ASIAN RUST (*Phakopsora pachyrhizi)*

Originally identified in Taiwan, the fungus causing Soybean Asian Rust, Phakopsora pachyrhizi, was first discovered in South America, specifically in Brazil, in 1979 (AMORIM et al., 2016); The first epidemic of the disease in Brazil occurred in 2001, causing significant damage to both the Brazilian and Paraguayan soybean crops (YORINORI et al., 2005). This obligate plant parasitic Basidiomycete fungus is aggressive and can result in yield reductions of up to 90%, affecting soybean grain production (GODOY et al., 2009).

Although the fungus has the potential to infect more than 31 species of 17 legume genera, in Brazil, it is only capable of infecting soybean crops. Belonging to the class Urediniomycetes, the fungus presents teliospores with thin, pale-colored walls, irregular shapes, and multiple layers. The epidemic, however, is caused by urediniospores, anamorphic spores that can vary from 2-10, with 4-8 being the most common. These spores originate epidermally, may appear superficial, surrounded by paraphyses, and can have a cup or bowl-shaped to spherical form. At opportune moments, they develop an opening through which the spores are released. They exhibit a yellowish to pale brown color (ONO et al., 1992).



Source: (Goellner et al., 2010).

Infection by this fungus begins with direct penetration through the cuticle. Once established, the fungus produces its spores, which are wind-disseminated. A period of 6 hours

of leaf wetness is required for infection to occur (under optimal temperatures of 15-25ºC), and this can increase with temperature variations. Symptoms can appear at any phenological stage of the crop, and their severity depends on the initial inoculum present in the field. However, symptoms typically begin to appear when canopy closure occurs, establishing a more favorable microclimate (AMORIM et al., 2016; ANDRADE and ANDRADE, 2002; CARVALHO and FIGUEIREDO, 2000).

Symptoms initiate as tiny darker spots than healthy tissue on the adaxial leaf surface, ranging from grayish to greenish and evolving into a brown color. Tan-colored lesions indicate susceptibility, while reddish-brown (RB) lesions indicate resistance. Lesions on the adaxial part usually have an angular shape delimited by the vein, and uredinia on the corresponding abaxial part of the lesion (ANDRADE and ANDRADE, 2002).

In regions where winter is mild, such as Brazil and the southern United States, there is no inoculum breakdown or host death. Therefore, disease development on different hosts increases the disease inoculum, resulting in higher field infection pressure (KELLY et al., 2015).

Controlling the fungus in Brazil, where it survives only on soybean plants, primarily involves crop rotation, spontaneous soybean plant control, implementation of a sanitary break (ranging from 60 to 90 days, depending on the region), and the use of early-cycle cultivars combined with timely planting. Monitoring the disease through spore collection and field evaluations is indispensable, although fungicides are used preventively (AMORIM et al., 2016).

Another critical and highly sought-after form of control is chemical control, which can be achieved using site-specific fungicides such as triazoles (demethylation inhibitors - DMI), strobilurins (quinone outside inhibitors - QoI), and carboxamides (succinate dehydrogenase inhibitors - SDHI); or multisite fungicides such as Mancozeb, chlorothalonil, and copper oxychloride. Additionally, a mechanism used for resistance management is the rotation of active ingredients in different fungicides (GODOY et al., 2020).

According to (EMBRAPA, 2019) , 90% of the DNA sequences of Phakopsora pachyrhizi consist of non-coding repetitive DNA sequences, ensuring adaptability to the pathogen, particularly the development of populations resistant to control measures currently on the market and varietal resistance breakdown. As noted by Juliatti et al. (2019) , many products once recommended by the Ministry of Agriculture, Livestock and Supply for disease control are no longer effective due to reduced efficacy of commonly used active ingredients.

Finally, other types of controls used for disease management include the use of alternative controls such as essential oils, plant extracts, homeopathic medicines, resistance inducers, and biological control. However, only products based on microorganisms (biological control) are registered for controlling this disease, specifically products formulated with bacteria of the genus Bacillus sp. (MEYER et al., 2022).

LATE SEASON DISEASES (*Cercospora kikuchii* and *Septoria glycine*)

The fungi Cercospora kikuchii (Matsu. & Tomoyasu) and Septoria glycine (Hemmi) are constantly associated to each other in soybean fields, causing the late season diseases complex (MARTINS et al., 2004); the first usually cause more damage under 23-27°C and high moisture levels (HENNING et al., 2014). The first pathogen provokes symptoms of dark to brownish spots that gather and form bigger spots; the main characteristic of this fungus is to promote a purple spot in soy grains (ENCISO-MALDONADO and FERNÁNDEZ-GAMARRA, 2021), and usually can be visualized only after R6 to R7 stages, after causing the real damage, where this disease causes around 30% of yield losses in the crop due to defoliation (AMORIM et al., 2016).

Although the main species related to late season disease in soybean from the Genus Cercospora is Cercospora kikuchii, other species as C. cf. flagellaris and C. cf. sigesbeckiae are also related to this disease complex symptoms (SOARES et al., 2015)

Septoria glycine is another widespread pathogen that causes losses in soybean, causing a disease named brown spot (ALLEN et al., 2017). It infects the plant in early stages, making a brown spot on the leaf surface, the spot starts smalls, and then grow according to the development of the disease. In advanced stages it causes defoliation on the crop. The minimum leaf wetting time needed is 6 hours, under a temperature of 15 – 30°C, where its pycnidia is the primary and secondary inoculum. The spore germinates and develop one or more germinating tubes (HENNING et al., 2014). The losses it causes in the crop are also around 27% (LIN et al., 2021).

The management concerns about crop rotation and healthy seeds manly. The chemical products can be used in seed treatment and aerial application; the principal products applied in the field are: benzimidazoles, triazoles, strobilurins, carboxamides and multisite (PRICE et al., 2015); additionally biological products can be used to control these diseases, where the main microorganism commercially used are bacteria from the genus Bacillus sp. (MEYER et al., 2022).

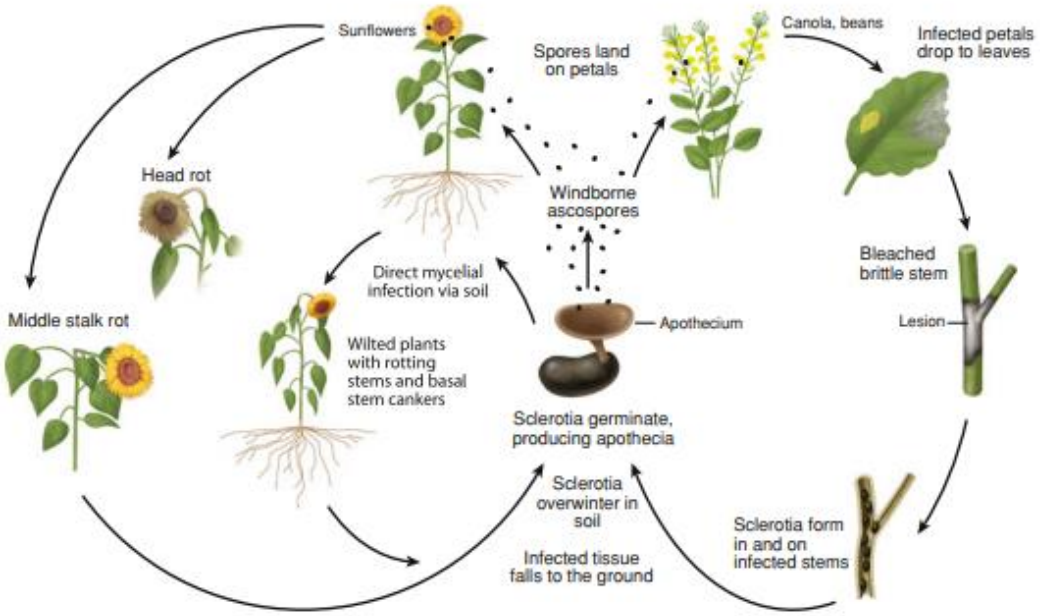
WHITE MOLD (*Sclerotinia sclerotiorum)*

The fungus causing White Mold, Sclerotinia sclerotiorum (Lib.) de Bary, is an Ascomycete belonging to the Sclerotiniaceae family, with the ability to infect over 400 species of wild and cultivated plants. Among the most affected crops are soybeans, sunflowers, and canola (BOLAND AND HALL, 1994). The fungus can form sclerotia, clusters of melanized hyphae, which remain viable in the soil for several years until favorable conditions arise. These sclerotia are typically located in the top 3 cm of the soil layer and can germinate to form hyphae or apothecia when conditions are favorable. The fungus can reproduce sexually or asexually, with asexual reproduction occurring when it emits hyphae and infects only one plant and sexual reproduction occurring when it forms the apothecium and releases ascospores, resulting in field epidemics causing nearly 100% damage (WEBSTER and WEBER, 2007).

Under conditions of high humidity and temperatures between 10-21ºC, sclerotia germinate and give rise to apothecia. In apothecia, asci are produced, and a single sclerotium can produce 15 to 20 apothecia. These apothecia, in turn, release ascospores daily for 2 to 17 days, with each apothecium capable of releasing up to 2 million ascospores. Ascospores can be wind-dispersed (Figure 1) and reach distances of up to 100 m, serving as primary inoculum. Alternatively, under temperatures between 20-25ºC, sclerotia germinate and emit hyphae that locally infect the host plant (JACCOUD FILHO et al., 2017).

A crucial factor in the pathogenicity of this fungus is the production of oxalic acid. This molecule is a significant pathogenicity factor as it chelates calcium ions in the plant cell wall, suppresses the plant's hypersensitivity response, and acidifies the infected tissue. Mutants lacking this gene have significantly attenuated virulence (CESSNA et al., 2000; GODOY et al., 1990; KABBAGE et al., 2013).

Figure1. Life cycle of *Sclerotinia sclerotiorum*.



Source: (ROLLINS et al., 2014).

The symptoms of the disease begin with edema spots that progress to light brown, accompanied by the formation of a white cottony mycelium. Infection typically originates in the plant's flowers, the most common entry point for ascospores, but it can occur on any part of the plant. The most critical phase for the crop is from the onset of flowering (R2) to the beginning of pod formation (AMORIM et al., 2016).

Disease management involves proper irrigation practices, crop rotation, the use of non-host plant residues (generally grasses), the use of healthy seeds, seed treatment, chemical control, biological control, resistance induction, and integrated crop-livestock systems. The main chemical fungicides are based on fluazinam, procymidone, thiophanate-methyl, and carbendazim. Another factor influencing sclerotia germination is the use of herbicides; the herbicide with the active ingredient lactofen induces sclerotia germination at an inopportune time, making pathogen infection more challenging (JACCOUD FILHO et al., 2017).

There are two main microorganisms used in biological control of white mold in tropical climates: Trichoderma spp., which has high parasitic capacity and antagonistic activity against the fungus (FERNANDO C. JULIATTI et al., 2019); and Bacillus sp., which exhibits antagonistic activity, competition, and resistance induction (CAWOY et al., 2011).

BIOLOGICAL CONTROL OF PLANT DISEASES

According to the definition by Cook and Baker, (1983), biological control is "the reduction of inoculum or disease-determining activities caused by a pathogen, carried out by

one or more organisms other than humans". Agrios, (2005), defines it as the total or partial destruction of a pathogen population by organisms found in nature. In the case of biological control of diseases, a microorganism that does not cause disease in the plant exerts an antagonistic effect against another pathogenic microorganism (AMORIM et al., 2018).

Biological control can be divided agriculturally into two different forms: classical biological control, where organisms are introduced once and reach equilibrium with the harmful organism, requiring no reintroduction of the beneficial organism, and applied biological control, which involves the mass introduction of beneficial organisms into the crop, showing significant results in the short term (PARRA, 2014).

This approach has been used as an alternative to chemical control in managing Asian soybean rust (Phakopsora pachyrhizi), as chemical control, though commonly employed, can have environmental consequences by contaminating soil and water systems, leading to environmental degradation (VEIGA et al., 2006); Another influencing factor is the selection of pathogen populations resistant to key disease control molecules, such as the fungicides tebuconazole, cyproconazole, and azoxystrobin, caused by their repeated and non-rotational use (GODOY et al., (2018) e XAVIER et al., (2015)).

Microorganisms used in the biological control of diseases have various mechanisms of action that can intervene against plant pathogens, including hyperparasitism, induction of resistance, competition for substrate, production of antagonistic volatile molecules, production of toxic metabolites to microorganisms, production of biofilms, among others (BENNETT and INAMDAR, 2015; MEYER et al., 2022; PEREIRA DA SILVA, 2016); The genus Bacillus is the only one with registered products up to 2022 (Meyer et al., 2022); Bacteria of this genus can produce various types of secondary metabolites, making it an efficient biological control agent (Kaspar et al., 2019) , addressing the issue of developing resistant populations of the fungus, as each microorganism is an individual capable of producing different substances, even within the same species.

Soil-associated fungi causing diseases are among the most challenging to control (ALABOUVETTE et al., 2005) , including white mold (BOLAND and HALL, 1994). Biological control of this disease is achieved by different types of microorganisms, such as Bacillus spp., Trichoderma spp., Clonostachys spp., Pseudomonas spp., Streptomyces spp., and Coniothyrium minitans spp. (Baharlouei et al., 2011; Faria et al., 2022; Nobre et al., 2005; Webster and Weber, 2007; Zhang, 2004). In Brazil, currently, biological products for white mold control are limited to Bacillus spp. and Trichoderma spp.. While bacteria of the genus

Bacillus produce antagonistic compounds against S. sclerotiorum, Trichoderma primarily parasitize sclerotia, resulting in a reduction of up to 70% in carpogenic germination (FARIA et al., 2022; MEYER et al., 2022).

GENUS *BACILLUS*

Bacteria of the genus Bacillus, such as Bacillus subtilis, are found in various places ubiquitously, demonstrating unusual genetics and adaptability, allowing them to colonize different types of niches (EARL et al., 2008). Some of these, however, are human pathogens, such as B. cereus and B. anthracis, while others, like B. subtilis, pose no risks as food-borne (MAUGHAN and VAN DER AUWERA, 2011). Among the species most used for biological control in Brazil are B. subtilis, B. amyloliquefaciens, B. velezensis, and B. thuringiensis (MEYER et al., 2022).

Bacteria of this genus are gram-positive, meaning they have thick peptidoglycan walls; they have aerobic or facultative anaerobic metabolism capable of producing the catalase enzyme; their shape is rod-like, and they can form endospores, ensuring their survival in adverse environments (DE VOS et al., 2009).

Various mechanisms make these microorganisms effective biological control agents, including the production of compounds from secondary metabolism, direct competition, production of volatile compounds, antibiotics, biofilm formation, siderophore production, resistance induction, and the production of phytohormones (WANG et al., 2018; YOU et al., 2016).

Stein, (2005), identified that the genome of Bacillus subtilis individuals dedicates 4 to 5% to the production of secondary metabolites, with over 24 of these substances having antimicrobial capacity.

There are various types of peptides produced by different Bacillus species, including cyclic and linear lipopeptides, dihydroisocoumarins, bacylisins, and many others, of ribosomal or non-ribosomal origin (KASPAR et al., 2019).

Among the lipopeptides, the cyclic ones are the most studied; their composition, formed by a polar cyclic peptide linked to a branched or non-branched apolar chain, gives these molecules surfactant activity. The majority, belong to three different families: Iturins, Fengycins, and Surfactins (CAULIER et al., 2019; LUO et al., 2015).

The Surfactin family is the most studied of the three, with surfactant capabilities extending to cancer treatments. Its high potential for causing cell lysis and easy production,

extraction, and isolation contribute to it being the most popular family in studies. However, it is less selective than the others, as its high surfactant capacity acts on various cell types, including viruses (KASPAR et al., 2019).

On the other hand, Iturins have both antibacterial and antifungal abilities; they form pores through self-assembly and are inserted into the cytoplasm. Their efficiency depends on concentration, with an effect on a broad range of fungi starting from 10 µg mL-1, while their efficiency against bacteria is limited to some gram-positive bacteria (MAGET-DANA et al., 1985). There are several types of Iturins, differing greatly in composition and organisms affected (KASPAR et al., 2019).

Compared to the other two, Fengycins are less toxic to plants, showing selectivity for filamentous fungi like Rhizoctonia solani (LOEFFLER et al., 1986); This selectivity may be due to an "all-or-nothing" mechanism proposed by (PATEL et al., 2011) , which has a greater effect on the membrane permeability of filamentous fungi, possibly due to specific lipid composition. The linear chain part of these molecules influences the types of microorganisms they affect, their effects, and some in biofilm formation (KASPAR et al., 2019). Other peptides, such as geopeptins, were able to inhibit the mobility of zoospores of Phytophthora capsici, showing the broad variety of these compounds and their efficiency in controlling organisms associated with plant diseases (TAREQ et al., 2015).

The production of enzymes is also an important mechanism of action for these bacteria, such as the production of glucanases, proteases, and chitinases. These enzymes directly act on the pathogen, hindering fungal penetration by acting on their cell walls and using the released components as a carbon source for their metabolism (ARORA et al., 2007; EL-BENDARY et al., 2016).

The activation of plant resistance is also an efficient mechanism exerted by these Firmicutes. They can produce secondary metabolites that stimulate the expression of pathogen-related proteins and others associated with plant defense, such as chitinase, phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase (JIAO et al., 2020) Systemic resistance can also be activated through the jasmonic acid and salicylic acid pathways, preventing disease development (NGUVO and GAO, 2019).

It is also worth noting other control mechanisms, such as biofilm formation and the production of volatile organic compounds, which can act directly on the pathogen or induce plant resistance, can also be deployed by those organisms. Biofilm, in turn, makes the bacterium more competitive for space and nutrients, such feature assures the the buffered environmental

to the buildup of the biocontrol population and the expression of quorum-sensing regulated genes related to the bacterial survival and plant protection, there hampering the plant from pathogen attack (BAIS et al., 2004; ZHANG et al., 2020).

GENUS *TRICHODERMA*

Trichoderma spp. is a genus of the Ascomycota phylum and the order Hypocreales. They are characterized by forming perithecia with pale to vivid colors, although some members do not have a known teleomorphic phase. In their anamorphic phase, they have conidiogenic cells in the form of terminal or lateral phialides, either grouped or not. Fungi of this class have a high parasitic capacity and produce antimicrobial compounds (WEBSTER and WEBER, 2007). The species most used in biological control in Brazil are *Trichoderma asperellum, T. harzianum T. koningopsis e T. stromaticum*.

Another capability that makes this fungus interesting for use in biological control is its ability to solubilize nutrients, such as phosphate, as well as enhance its acquisition by plants and promote plant growth (BONONI et al., 2020).

Due to its high competitiveness, this fungus stands out for rapid growth, colonization of various substrates, tolerance to toxic substances, and abundant sporulation. Its high parasitic capacity occurs because the fungus can produce various types of substances antagonistic to other microorganisms, induce resistance, and inactivate some enzymes in plants (GERALDINE et al., 2013; HARMAN et al., 2004; SHORESH et al., 2010).

Species of *Trichoderma*, as mentioned earlier, are widely used in biological control in Brazil. One of the main targets of biological control is the use of this fungus to control *Sclerotinia sclerotiorum* (FARIA et al., 2022). although they show excellent growth at temperatures of 25-28ºC, some species, despite having reduced mycelial growth, are still capable of significant growth under humidity between 55-70% (JACCOUD FILHO et al., 2017; BOMFIM et al., 2010). Another factor that contributes to their effectiveness against this pathogen is the secretion of lytic enzymes that cause the rupture of the fungal sclerotia cell wall after penetrating them (GERALDINE et al., 2013).

OBJECTIVES

General objectives

To check if there is an influence of the alternating use of different biological products on Asian rust *(Phakopsora pachyrhizi*), and white mold (*Sclerotinia sclerotiorum*) on soybean.

Specific objectives

CHARPTER 1: " Does the alternate Bacillus-based product sapplication has the same performance on the disease management and plant yield as the single product applicationin soybean?"

Analyze the interaction among *Bacillus* based products to control foliar diseases in soybean, introducing them alone and in different sequences, to verify the effectiveness or ineffectiveness of it.

CHARPTER 2: "Does alternating different Trichoderma-formulated products control Sclerotinia sclerotiorum?"

Verify if the pattern of applications, of two different products based on *Trichoderma* have a benefic, malefic, or neutral outcome, for controlling white mold in soybean, and how the interaction of them can impact the development of the crop.

REFERENCES

Ademir Assis Henning, Álvaro Manuel Rodrigues Almeida, Cláudia Vieira Godoy, Claudine Dinali Santos Seixas, José Tadashi Yorinori, Leila Maria Costamilan, Léo Pires Ferreira, Maurício Conrado Meyer, Rafael Moreira Soares, Waldir Pereira Dias, 2014. **Manual de identificação de doenças de soja**. Empresa Brasileira de Pesquisa Agropecuária Embrapa Soja 5, 76.

Alabouvette, C., Olivain, C., Haridon, L.', Aimé, S., Steinberg, C., 2005. 8. **USING STRAINS OF FUSARIUM OXYSPORUM TO CONTROL FUSARIUM WILTS: DREAM OR REALITY?**

Allen, T.W., Bradley, C.A., Sisson, A.J., Byamukama, E., Chilvers, M.I., Coker, C.M., Collins, A.A., Damicone, J.P., Dorrance, A.E., Dufault, N.S., Esker, P.D., Faske, T.R., Giesler, L.J., Grybauskas, A.P., Hershman, D.E., Hollier, C.A., Isakeit, T., Jardine, D.J., Kelly, H.M., Kemerait, R.C., Kleczewski, N.M., Koenning, S.R., Kurle, J.E., Malvick, D.K., Markell, S.G., Mehl, H.L., Mueller, D.S., Mueller, J.D., Mulrooney, R.P., Nelson, B.D., Newman, M.A., Osborne, L., Overstreet, C., Padgett, G.B., Phipps, P.M., Price, P.P., Sikora, E.J., Smith, D.L., Spurlock, T.N., Tande, C.A., Tenuta, A.U., A.Wise, K., Allen Wrather, J., 2017. **Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2010 to 2014**. Plant Health Prog 18, 19–27. https://doi.org/10.1094/PHP-RS-16-0066

Ana Paula Oliveira Nogueira, Tuneo Sediyama, Gomes, J.D., 2015. **MELHORAMENTO GENÉTICO DA SOJA**, Millenium. ed. Campinas.

André Luis Thomas, 2018. Soja: **Tipos de crescimento da planta**. Porto Alegre-RS.

Arora, N.K., Min, J.K., Sun, C.K., Maheshwari, D.K., 2007. **Role of chitinase and β-1,3-glucanase activities produced by a fluorescent pseudomonad and in vitro inhibition of Phytophthora capsici and Rhizoctonia solani.** Can J Microbiol 53, 207–212. https://doi.org/10.1139/W06-119

Baharlouei, A., Sharifi-Sirchi, G.R., Shahidi Bonjar, G.H., 2011. **Biological control of Sclerotinia sclerotiorum (oilseed rape isolate) by an effective antagonist Streptomyces**. Afr J Biotechnol 10, 5785–5794. https://doi.org/10.5897/AJB10.2017

Bais, H.P., Fall, R., Vivanco, J.M., 2004. **Biocontrol of Bacillus subtilis against Infection of Arabidopsis Roots by Pseudomonas syringae Is Facilitated by Biofilm Formation and Surfactin Production**. Plant Physiol 134, 307–319. https://doi.org/10.1104/pp.103.028712

Bennett, J.W., Inamdar, A.A., 2015. **Are some fungal volatile organic compounds (VOCs) mycotoxins?** Toxins (Basel). https://doi.org/10.3390/toxins7093785

Boland, G.J., Hall, R., 1994**. Index of plant hosts of sclerotinia sclerotiorum.** Canadian Journal of Plant Pathology 16, 93–108. https://doi.org/10.1080/07060669409500766

Bononi, L., Chiaramonte, J.B., Pansa, C.C., Moitinho, M.A., Melo, I.S., 2020. **Phosphorus-solubilizing Trichoderma spp. from Amazon soils improve soybean plant growth**. Sci Rep 10. https://doi.org/10.1038/s41598-020-59793-8

C. Juliatti, F., A. Rezende, A., Cezar Marinho Juliatti, B., P. Morais, T., 2019. **Trichoderma as a Biocontrol Agent against Sclerotinia Stem Rot or White Mold on Soybeans in Brazil: Usage and Technology** , in: Trichoderma - The Most Widely Used Fungicide. IntechOpen. https://doi.org/10.5772/intechopen.84544

CARVALHO JÚNIOR, A.A., FIGUEIREDO, M.B., 2000. **A verdadeira identidade da ferrugem da soja no Brasil**. Summa Phytopathol 26, 197–200.

Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., Mahillon, J., 2019. **Overview of the antimicrobial compounds produced by members of the Bacillus subtilis group**. Front Microbiol. https://doi.org/10.3389/fmicb.2019.00302

Cavalcanti, A.B.U., 1892. **Relatório do campo de ensaio**. Boletim da Estação Agronômica de Campinas. Campinas.

Cawoy, H., Bettiol, W., Fickers, P., Ongena, M., 2011. **Bacillus-based biological control of plant diseases**, in: Pesticides in the Modern World-Pesticides Use and Management. pp. 273–302.

Cessna, S.G., Sears, V.E., Dickman, M.B., Low, P.S., 2000. **Oxalic Acid, a Pathogenicity Factor for Sclerotinia sclerotiorum , Suppresses the Oxidative Burst of the Host Plant, The Plant Cell**.

Cláudia V. Godoy, Carlos M. Utiamada, Maurício C. Meyer, Hercules D. Campos, Ivani de O. N. Lopes, Alfredo R. Dias, C.C.D., Cláudia B. Pimenta, David S. Jaccoud Filho, Eder N.

Moreira, Edson P. Borges, Edson R. de Andrade Junior, Fabiano V. Siqueri, Fernanda C. Juliatti, Fernando C. Juliatti, Fernando Favero, Heraldo R. Feksa, Ivan Pedro Araújo Júnior, José Fernando J. Grigolli, José Nunes Junior, Luana M. de R. Belufi, Luciana C. Carneiro, Luís Henrique C. P. da Silva, Luiz Nobuo Sato, Marcelo G. Canteri, Marcelo R. Volf, Marcio Goussain, Moab D. Dias, Mônica Paula Debortoli, Mônica C. Martins, Ricardo S. Balardin, Silvânia H. Furlan, Tiago Madalosso, Valtemir J. Carlin, Wilson S. Venancio, 2018. **Eficiência de fungicidas para o controle da ferrugem-asiática da soja, Phakopsora pachyrhizi, na safra 2017/2018**: Resultados sumarizados dos ensaios cooperativos. Londrina-PR.

Cláudia Vieira Godoy, Claudine Dinali Santos Seixas, Maurício Conrado Meyer, Rafael Moreira Soares, 2020. **Ferrugem-asiática da soja: bases para o manejo da doença e estratégias antirresistência**. Londrina-PR.

Cook, R.J., Baker, K.F., 1983. **The Nature and Practice of Biological Control of Plant Pathogens**. APS Press, Saint Paul.

Correa, F., Domingos Júnior, F., Mazetto Júnior, J., Costa, D., Torres, J., 2017. **PRODUTIVIDADE DE CULTIVARES DE SOJA EM SEQUEIRO NO MUNICÍPIO DE PERDIZES, MG**. Enciclopédia Biosfera 14, 1064–4071. https://doi.org/10.18677/encibio_2017a85

David de S. Jaccoud Filho, Luciane Henneberg, Edilaine M.G. Grabicoski, 2017. **Mofo Branco**. TODAPALAVRA, Ponta Grossa.

Earl, A.M., Losick, R., Kolter, R., 2008. **Ecology and genomics of Bacillus subtilis**. Trends Microbiol. https://doi.org/10.1016/j.tim.2008.03.004

El-Bendary, M.A., Hamed, H.A., Moharam, M.E., 2016. **Potential of Bacillus isolates as bio-control agents against some fungal phytopathogens**. Biocatal Agric Biotechnol 5, 173–178. https://doi.org/10.1016/j.bcab.2016.02.001

Embrapa, 2019. **O genoma altamente complexo do fungo P. pachyrhizi causador da mais devastadora doença da soja foi decifrado por um consórcio único de empresas públicas e privadas**. Londrina.

Emidio Rizzo Bonato, Ana Lidia Variani Bonato, 1987. **A SOJA NO BRASIL**: História e Estatística. Londrina.

Eula Maria Siqueira Santos Mozzaquatro, De Oliveira Almirão, D., Righi, A.P., João Cleber de Sousa Lopes, 2017. **VIABILIDADE ECONÔMICA DA CULTURA DA SOJA EM UMA PROPRIEDADE RURAL**.

Faria, A.F. de, Schulman, P., Conrado Meyer, M., Diniz Campos, H., Cruz-Magalhães, V., Vieira Godoy, C., Araújo Guimarães, R., Henrique Carregal Pereira da Silva, L., Marcos Goussain, M., Cagnin Martins, M., Nunes Junior, J., Story Venancio, W., Fantin, L., Brustolin, R., de Souza Jaccoud Filho, D., Celeste Carneiro, L., 2022**. Seven years of white mold biocontrol product's performance efficacy on Sclerotinia sclerotiorum carpogenic**

germination in Brazil: a meta-analysis.
https://doi.org/https://dx.doi.org/10.2139/ssrn.4120095

GEORGE N. AGRIOS, 2005. **PLANT PATHOLOGY**, 5th ed. Elsevier, Burlington.

Geraldine, A.M., Lopes, F.A.C., Carvalho, D.D.C., Barbosa, E.T., Rodrigues, A.R., Brandão, R.S., Ulhoa, C.J., Lobo Junior, M., 2013. **Cell wall-degrading enzymes and parasitism of sclerotia are key factors on field biocontrol of white mold by Trichoderma spp**. Biological Control 67, 308–316. https://doi.org/10.1016/j.biocontrol.2013.09.013

Godoy, C. V, Flausino, A.M., Santos, L.C.M., Ponte, E.M. Del, 2009. **Eficiência do controle da ferrugem asiática da soja em função do momento de aplicação sob condições de epidemia em Londrina, PR**.

Godoytl1, G., Steadmant, J.R., Dickmant, M.B., Dam+, R., 1990. **Use of mutants to demonstrate the role of oxalic acid in pathogenicity of Sclerotinia sclerotiorum on Phaseolus vulgaris**, Physiological and Molecular Plant Pathology.

Goellner, K., Loehrer, M., Langenbach, C., Conrath, U., Koch, E., Schaffrath, U., 2010. **Phakopsora pachyrhizi, the causal agent of Asian soybean rust:** Pathogen profile. Mol Plant Pathol 11, 169–177. https://doi.org/10.1111/j.1364-3703.2009.00589.x

Guillermo Andrés Enciso-Maldonado, Marta Alicia Fernández-Gamarra, 2021**. Cercospora kikuchii ¿Patógeno potencial de la soja?** Revista Impacto en Ciencia y Tecnología 69–72.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. **Trichoderma species - Opportunistic, avirulent plant symbionts**. Nat Rev Microbiol.
https://doi.org/10.1038/nrmicro797

IRGA - Instituto Rio Grandense do Arroz Irrigado, 2021**. SAFRA 2020/2021 chega ao fim com produtividade recorde no RS**.

Jiao, R., Munir, S., He, Pengfei, Yang, H., Wu, Y., Wang, J., He, Pengbo, Cai, Y., Wang, G., He, Y., 2020. **Biocontrol potential of the endophytic Bacillus amyloliquefaciens YN201732 against tobacco powdery mildew and its growth promotion**. Biological Control 143. https://doi.org/10.1016/j.biocontrol.2019.104160

José Renato B. Farias, Alexandre L. Nepomuceno, Norman Neumaier, 2007. **Ecofisiologia soja**. Londrina - PR.

Juliatti, F.C., Mesquita, A.C.O., Teixeira, F.G., Beloti, I.F., Mota, L.C.B.M., Fonseca, L.J., de Sousa, L.A., Souza, M.S.X. de A., Silva, N.S., Zancan, N.L.B., de Morais, T.P., 2019. **Characterization of soybean genotypes showing partial resistance to soybean rust**. Summa Phytopathol 45, 313–319. https://doi.org/10.1590/0100-5405/190552

JULIO CARLOS PEREIRADA SILVA, 2016. **COMPOSTOS ORGÂNICOS VOLÁTEIS DE PLANTAS E O ETANOL NO CONTROLE DE MELOIDOGYNE INCOGNITA**. UFLA, Lavras-MG.

Kabbage, M., Williams, B., Dickman, M.B., 2013. **Cell Death Control: The Interplay of Apoptosis and Autophagy in the Pathogenicity of Sclerotinia sclerotiorum**. PLoS Pathog 9. https://doi.org/10.1371/journal.ppat.1003287

Kaspar, F., Neubauer, P., Gimpel, M., 2019. **Bioactive Secondary Metabolites from Bacillus subtilis: A Comprehensive Review**. J Nat Prod. https://doi.org/10.1021/acs.jnatprod.9b00110

Kelly, H.Y., Dufault, N.S., Walker, D.R., Isard, S.A., Schneider, R.W., Giesler, L.J., Wright, D.L., Marois, J.J., Hartman, G.L., 2015. **From select agent to an established pathogen: The response to Phakopsora pachyrhizi (soybean rust) in North America**. Phytopathology 105, 905–916. https://doi.org/10.1094/PHYTO-02-15-0054-FI

Koefender, J., Camera, J.N., Schoffel, A., Golle, D.P., Mugnol, J.A.B., Bortolotto, R.P., Schneider, T., Flores, E.F., 2023. **Associação de fungicidas no controle da ferrugem asiática da soja**. Peer Review 5, 56–65. https://doi.org/10.53660/666.prw1804c

L. Amorim, J.A.M. Rezende, A. Bergamin Filho, 2018. **Manual de Fitopatologia**, 5th ed. Agronômica Ceres Ltda, Ouro Fino-MG.

L. Amorim, J.A.M. Rezende, A. Bergamin Filho, L.E.A. Camargo, 2016. **Manual de Fitopatologia**, 5th ed. Editora Agronômica Ceres Ltda, Ouro Fino-MG.

Lin, H.A., Villamil, M.B., Mideros, S.X., 2021. **Characterization of Septoria brown spot disease development and yield effects on soybean in Illinois**. Canadian Journal of Plant Pathology 43, 62–72. https://doi.org/10.1080/07060661.2020.1755366

Lizandra L. Catelli, 2009. **RESISTÊNCIA DA SOJA À FERRUGEM ASIÁTICA E AO OÍDIO: HERANÇA DE CARACTERES QUALI-QUANTITATIVOS E MAPEAMENTO GENÉTICO**. Faculdade de Ciências Agrárias e Veterinárias - Unesp, Jaboticabal.

Loeffler, W., Tschen, S.-M., Vanittanakom, N., Kugler, M., Knorpp, E., Hsieh, T.-F., Wu, T.-G., 1986. **Antifungal Effects of Bacilysin and Fengymycin from Bacillus suhtilis F-29-3 A Comparison with Activities of Other Bacillus Antihiotics**. J. Phytopathology 115, 204–213.

Lopes, H., Pontes, J., Barros Telles Do Carmo, B., José, A., Porto, V., 2009. **Problemas logísticos na exportação brasileira da soja em grão**.

Luo, C., Liu, X., Zhou, X., Guo, J., Truong, J., Wang, X., Zhou, H., Li, X., Chen, Z., 2015. **Unusual biosynthesis and structure of locillomycins from Bacillus subtilis 916**. Appl Environ Microbiol 81, 6601–6609. https://doi.org/10.1128/AEM.01639-15

Maget-Dana, R., Ptak, M., Peypoux, F., Michel, G., 1985. **Pore-forming properties of iturin A, a lipopeptide antibiotic, BiochimicaetBiophysicaActa**.

Marcelo Motta Veiga, Dalton Marcondes Silva, Lilian Bechara Elabras Veiga, Mauro Velho de Castro Faria, 2006. **Análise da contaminação dos sistemas hídricos por agrotóxicos numa pequena comunidade rural do Sudeste do Brasil**. Cad. Saúde Pública 2391–2399.

Marinês Pereira Bomfim, Abel Rebouças São José, Tiyoko Nair Hojo Rebouças, Saulo Sousa de Almeida, IvanVilas Boas Souza, Nilma Oliveira Dias, 2010. **Antagonic effect in vitro and in vivo of Trichodermaspp. to Rhizopus stolonifer in yellow passion fruit**. Summa Phytopathol 36, 61–67.

Martins, M.C., Guerzoni, R.A., De, G.M., Câmara, S., Mattiazzi, P., Lourenço, S.A., Amorim, L., 2004. **Escala Diagramática para a Quantificação do Complexo de Doenças Foliares de Final de Ciclo em Soja**.

Maughan, H., Van der Auwera, G., 2011. **Bacillus taxonomy in the genomic era finds phenotypes to be essential though often misleading**. Infection, Genetics and Evolution. https://doi.org/10.1016/j.meegid.2011.02.001

Meyer, M.C., Bueno, A. de F., Mazaro, S.M., Silva, J.C. da, 2022. **BIOINSUMOS NA CULTURA DA SOJA**. Embrapa Soja.

MEYER, M.C., GODOY, C. V., UTIAMADA, C.M., CAMPOS, H.D., MEDEIROS, F.H.V. de, OLIVEIRA, M.C.N. de, MAZARO, S.M., SENGER, M., BONANI, J.C., CARVALHO, A.G. de, BRUSTOLIN, R., NUNES JUNIOR, J., DIAS, A.R.; F.L.H., GOUSSAIN JUNIOR, M.M., GOMES, L.M.M.R., FARIAS, A. de, CHAVES, I.C.P. de V., MARTINS, M.C., BELUFI, L.M. de R., KONAGESKI, F.T., KONAGESKI, T.F., STEFANELO, M.S., GRIGOLLI, J.F.J., 2023. **Avaliação da associação de fungicidas químicos e biológicos no controle de doenças foliares da soja, safra 2022/2023**: resultados sumarizados da rede de experimentos cooperativos 198, 1–15.

Neumaier, N., Renato, J., Farias, B., Lima Nepomuceno, A., Mertz-Henning, L.M., Salvador, J., Foloni, S., Alexandra, L., Moraes, C., Luiz, S., Capítulo, G., 2020. **Estádios fenológicos da soja Ecofisiologia da soja, in:** Sistemas de Produção. pp. 33–54.

Nguvo, K.J., Gao, X., 2019. **Weapons hidden underneath: bio-control agents and their potentials to activate plant induced systemic resistance in controlling crop Fusarium diseases**. Journal of Plant Diseases and Protection. https://doi.org/10.1007/s41348-019-00222-y

Nobre, S.A.M., Maffia, L.A., Mizubuti, E.S.G., Cota, L. V., Dias, A.P.S., 2005. **Selection of Clonostachys rosea isolates from Brazilian ecosystems effective in controlling Botrytis cinerea**. Biological Control 34, 132–143. https://doi.org/10.1016/j.biocontrol.2005.04.011

NONGNUCH VANITTANAKO, WOLFGANG LOEFFLER, 1986. **FENGYCIN - A NOVEL ANTIFUNGAL PRODUCED BY BACILLUS LIPOPEPTIDE SUBTILIS ANTIBIOTIC F-29-3**.

Ono, Y., Buriticá, P., Hennen, J.F., 1992. **Delimitation of Phakopsora, Physopella and Cerotelium and their species on Leguminosae**. Mycol Res 96, 825–850. https://doi.org/10.1016/S0953-7562(09)81029-0

Parra, J.R.P., 2014. **Biological control in Brazil:** An overview. Sci Agric. https://doi.org/10.1590/0103-9016-2014-0167

Patel, H., Tscheka, C., Edwards, K., Karlsson, G., Heerklotz, H., 2011. **All-or-none membrane permeabilization by fengycin-type lipopeptides from Bacillus subtilis QST713**. Biochim Biophys Acta Biomembr 1808, 2000–2008. https://doi.org/10.1016/j.bbamem.2011.04.008

Paul De Vos, George M. Garrity, Dorothy Jones, Noel R. Krieg, Wolfgang Ludwig, Fred A. Rainey, Karl-Heinz Schleifer, William B. Whitman, 2009. **Bergey's Manual of Systematic Bacteriology**, 2nd ed. Springer, London. https://doi.org/10.1007/b92997

Paulino José Melo Andrade, Donita Figueiredo de Alencar Araripe Andrade, 2002. **Ferrugem Asiática: uma Ameaça à Sojicultura Brasileira**. Dourados-MS .

Price, P.P., Purvis, M.A., Cai, G., Padgett, G.B., Robertson, C.L., Schneider, R.W., Albu, S., 2015. **Fungicide resistance in cercospora kikuchii, a soybean pathogen**. Plant Dis 99, 1596–1603. https://doi.org/10.1094/PDIS-07-14-0782-RE

Rollins, J.A., Cuomo, C.A., Dickman, M.B., Kohn, L.M., 2014. **Genomics of Sclerotinia sclerotiorum, in: Genomics of Plant-Associated Fungi and Oomycetes: Dicot Pathogens**. Springer-Verlag Berlin Heidelberg, pp. 1–17. https://doi.org/10.1007/978-3-662-44056-8_1

Shoresh, M., Harman, G.E., Mastouri, F., 2010. **Induced systemic resistance and plant responses to fungal biocontrol agents**. Annu Rev Phytopathol 48, 21–43. https://doi.org/10.1146/annurev-phyto-073009-114450

Soares, A.P.G., Guillin, E.A., Borges, L.L., Da Silva, A.C.T., De Almeida, Á.M.R., Grijalba, P.E., Gottlieb, A.M., Bluhm, B.H., De Oliveira, L.O., 2015. **More Cercospora species infect soybeans across the Americas than meets the eye.** PLoS One 10. https://doi.org/10.1371/journal.pone.0133495

Stein, T., 2005. **Bacillus subtilis antibiotics: Structures, syntheses and specific functions**. Mol Microbiol. https://doi.org/10.1111/j.1365-2958.2005.04587.x

Tareq, F.S., Hasan, C.M., Lee, H.S., Lee, Y.J., Lee, J.S., Surovy, M.Z., Islam, M.T., Shin, H.J., 2015. **Gageopeptins A and B, new inhibitors of zoospore motility of the phytopathogen Phytophthora capsici from a marine-derived bacterium Bacillus sp. 109GGC020**. Bioorg Med Chem Lett 25, 3325–3329. https://doi.org/10.1016/j.bmcl.2015.05.070

United States Department of Agriculture Foreign Agricultural Service **Argentina Ramps Up Soybean Imports from Paraguay and Brazil, 2023**.

Wang, X.Q., Zhao, D.L., Shen, L.L., Jing, C.L., Zhang, C.S., 2018. **Application and mechanisms of Bacillus subtilis in biological control of plant disease, in:** Role of Rhizospheric Microbes in Soil: Stress Management and Agricultural Sustainability. Springer Singapore, pp. 225–250. https://doi.org/10.1007/978-981-10-8402-7_9

Webster, J., Weber, R., 2007. **Introduction to Fungi**, 3rd ed. Cambridge university press.

Xavier, S.A., Koga, L.J., Barros, D.C.M., Canteri, M.G., De Lopes, I.O.N., Godoy, C.V., 2015. **Sensitivity variation of phakopsora pachyrhizi populations to demethylation inhibitor fungicides in Brazil**. Summa Phytopathol 41, 191–196. https://doi.org/10.1590/0100-5405/2068

Yorinori, J.T., Paiva, W.M., Frederick, R.D., Costamilan, L.M., Bertagnolli, P.F., Hartman, G.E., Godoy, C. V., Nunes, J., 2005. **Epidemics of soybean rust (Phakopsora pachyrhizi) in Brazil and Paraguay from 2001 to 2003, in:** Plant Disease. pp. 675–677. https://doi.org/10.1094/PD-89-0675

You, C., Zhang, C., Kong, F., Feng, C., Wang, J., 2016. **Comparison of the effects of biocontrol agent Bacillus subtilis and fungicide metalaxyl-mancozeb on bacterial communities in tobacco rhizospheric soil.** Ecol Eng 91, 119–125. https://doi.org/10.1016/j.ecoleng.2016.02.011

Zhang, D., Yu, S., Yang, Y., Zhang, J., Zhao, D., Pan, Y., Fan, S., Yang, Z., Zhu, J., 2020. **Antifungal Effects of Volatiles Produced by Bacillus subtilis Against Alternaria solani in Potato**. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.01196

Zhang, Y., 2004. **BIOCONTROL OF SCLEROTINIA STEM ROT OF CANOLA BY BACTERIAL ANTAGONISTS AND STUDY OF BIOCONTROL MECHANISMS INVOLVED**.

CHARPTER 1 – SOYBEAN RUST AND FINAL CYCLE DISEASES

Does the alternate Bacillus-based product application has the same performance on the disease management and plant yield as the single product application in soybean?

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Abstract

Biological control is has proven to reduce foliar diseases in soybeans - Phakopsora pachyrhizi (Asian rust), Cercospora kikuchii (purple spot) and Septoria glycines (brown spot) however little is know about the intereaction between different products. Two field assays were conducted where severity and yield parameters were evaluated. The combination of two strains of Bacillus amyloliquefaciens, rotated with Bacillus subtilis, showed the highest yield in the early sowing season (Tuckey 5%), and an increase of U$98.24 per hectare in the late sowing season, compared to the second most productive treatment. The results indicate that biological control rotation works, but it depends on the biological products being rotated and their position in the rotation, as it determines their efficacy.

Introduction

Soybean (Glycine max L.), one of the fourth most widely cultivated crops globally, with a planting area of 102.77 million hectares and an annual yield of 239.36 million tons, has Brazil and the United States as the largest world producers, responsible for approximately 69% of the

global productivity of soybean in 2022/23 (United States Department of Agriculture Foreign Agricultural Service Argentina Ramps Up Soybean Imports from Paraguay and Brazil, 2023; FAO, 2020).

Many diseases affect soybean in Brazil, among them, we have Cercospora kikuchii, Septoria glycines (both together named late season diseases) and Phakopsora pachyrhizi (rust); these foliar diseases cause many losses in the crop every year, that can be avoided by the application of fungicides (Brown et al., 2024; Hossain et al., 2023). The disease caused by the fungus Cercospora sp. has many species related to the disease complex symptoms, such as: Cercospora kikuchii, C. cf. flagellaris C. cf. sigesbeckiae (Soares et al., 2015).

Soybean rust on the other hand causes defoliation in the plant, that can be not compensated at high levels of the disease, especially high inoculum at early stages (Primiano and Amorim, 2023). The main alternative to avoid these losses, is the use of resistant cultivars, saving millions of dollars per year (Ishiwata and Furuya, 2020); additionally, the disease management is made with the use of chemical and biological control products (Meyer et al., 2023).

Due to repeated used of the same product, chemical fungicides are losing their efficacy, by selecting resistant individuals (Fernando Cezar Juliatti et al., 2019); the use of biological products is an alternative to prevent this selection if used correctly in disease management, for these tools are multisite fungicides (Meyer et al., 2022). Most biological products registered in Brazil, belong to the genus Bacillus and Trichoderma, where Bacillus is the main one for foliar diseases (“AGROFIT-Sistema de agrotóxicos fitossanitários,” 2024). These Bacillus have a variety of modes of action, caused by the presence of lipopeptides, biofilm compounds, and volatile compounds, involved in disease management (Tran et al., 2022).

Therefore, two commercial Bacillus sp. based products were tested to control Phakopsora pachyrhizi, Cercospora kikuchii, and Septoria glycines in soybean fields in Brazil.

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Materials and Methods

Field Assay

The experiment was conducted on a rural property in Nepomuceno-MG, at coordinates 21°19'10.8"S 45°06'48.1"W (Early sowing 1), and the soybeans sown in the second period at 21°18'53.6"S 45°06'14.1"W (Late sowing 2), during the 2023-24 summer season. The cultivar used was Brasmax Zeus IPRO, sown at two different moments on October 11, 2022, and November 22, 2022. Planting was carried out with 14 seeds per meter, with a row spacing of 0.5 meters row distance. The plots consisted of 6 treatments and 4 blocks, with each plot having an effective area of 18 square meters. Four foliar sprays of biological fungicide were made to the soybeans sown on the first planting V4, 45 days after emergence (R1), 18 days after the last application (R3), 28 days after the last application (R5). Bacillus subtilis (Bioimune) 2L/ha and Bacillus amyloliquefaciens; Bacillus amyloliquefaciens (Twixx) were applied alone and alternate to the crop. Positive control with chemicals was performed using the following applications: 1st Application (45 days after emergence) strobirulin + pyrazole carboxamide (Vessarya) (0.6 L/ha) + Adjuvant (Quid oil) (0.2 L/ha); 2nd application (18 days after the first application) strobilurins + dithiocarbamate + triazole (Tridium) 2.0 L/ha + adjuvant (Strides) 0.25% v/v; and 3rd Application (36 days after the first application) triazole (Cypress) 0.3 L/ha + isophthalonitrile (Bravonil) 1.5 L/ha. In the late sowing, 5 applications of biological fungicides were performed: V4, 45 days after emergence (R1), 18 days after the last application (R3), 28 days after the last application (R5) and 42 days after the last application (R6); Bacillus

subtilis (Bioimune) 2L/ha and Bacillus amyloliquefaciens ; Bacillus amyloliquefaciens (Twixx) were applied alone and alternate to the crop. The positive control was performed with the following applications: 1st (45 days after emergence) bixafem + proticonazole + Trifloxistrobin (FoxXpro) 0.5 L/ha + Adjuvant (Aureo) 0.25% v/v; 2nd (14 days after the first application) epoxiconazole + fluxapyroxad + pyraclostrobin (Ativum) 0,8L/ha + Adjuvant (Mees) 0.25% v/v; 3rd (28 days after the first application) strobilurins + dithiocarbamate + triazole (Tridium) 2.0 Kg/ha + adjuvant (Strides) 0.25% v/v; 4th (42 days after the first application) triazole (Cypress) 0.3 L/ha + isophthalonitrile (Bravonil) 1.5 L/ha, the last one only in the second season. Procimidone (ParrudoBR) was applied at a dose of 1L/ha to control of Sclerotinia sclerotiorum in Area 1,

glyphosate (Roundup WG), at the dose of 2Kg/ha was applied to control weed, and thiamethoxam + lambda-c-yalothrine (Engeo Pleno S), at the dose of 200ml/ha to control insects when necessary. The volume of spray used was 150L/ha.

The severity of Asian rust (Phakopsora pachyrhizi) was measured (disease, according to the scale proposed by Godoy et al., 2006, end-of-season diseases, according to the scale developed by Martins et al., (2004)). Three assessments for disease severity were conducted with a 15-day interval between them between R5 (beginning of seed) and R6 (full-seed) growth stages; it was used to establish the critical point of the disease, as shown by (Barro et al., 2021). Disease severity is expressed as mean percentage value for the plot and considers the percentage area affected by remaining leaves and 100% for a defoliated canopy. The parameters: yield (kg/ha), was measured by the weight of the grains collected from the main 4 lines (useful plot) of the center of the plot with the discount of the moisture; thousand-grain weight, measured by weighting one hundred grains 8 times and transforming it into one thousand grains weight; number of pods, measured by counting the number of pods of the main stem; and number of

grains per pod, measured by dividing the number of grains by the number of pods of the main stem.

Statistical analysis

The data were submitted to Shapiro-Wilk normality test (p-value > 0.05), Bartlett's homogeneity test (p-value > 0.05), and Dixon's test (p-value ≤ 0.05), with the removal of outliers if necessary. For the mean comparison, the Tukey test was conducted for the mentioned analyses (p-value ≤ 0.05). The software used was the program R.

RESULTS

Rust severity index

Four assessments were made to evaluate the severity of the disease. In the Figure 1, are presented the results for Asian rust in soybean. The image A, B and C that show the first experiment, the point that the severity rises mainly between the 3 and the 4 evaluations for the bottom and the middle third of the canopy. We can also see a similar pattern for the late sowing.

The severity of the 4th evaluation (between R5 and R6) of each third of the canopy was submitted to statistical analysis (Figure 2). The image A indicates that only BCA 1 differs from BCA 2 ($p = 0.014$); On image B and C (Middle ($p = 0.11$) and superior ($p = 0.51$)) third of the canopy respectively), there is no significant difference between the treatments. On the other hand, in the late sowing, we have in the three thirds of the canopy a pattern where all treatments differ from the negative control, where D indicates the bottom ($p < 0.001$), E the middle ($p < 0.001$) and F the superior third ($p < 0.001$).

Additionally, the average of the disease index from the bottom, middle and superior third of the canopy was submitted by statistical analysis (Figure 3). For the early sowing BCA 1 suffered from less severity compared the negative control ($p = 0.01$), but on the late sowing,

the results show similar result to the separated thirds, were all treatments present less disease severity compared to the negative control ($p < 0.001$).

Final cycle diseases severity index

As the rust severity was measured for rust, the same was applied for the DFC's (Figure 4). Analogous results to the rust, a sudden increase of the disease severity occurred in the 3rd (R4) to the 4th (R6) evaluation, in this case for all the thirds of the canopy and both sowing times.

The severity from the material collected in the interval R5 to R6 is presented in the Figure 5. The severity of the bottom third (image A), expresses that the treatments did not reduce the percentage set side by side to negative control ($p = 0.0016$). Images B and C, middle and superior thirds respectively did not show significant difference, for the early sowing. On the late sowing thought, the bottom third, Image D, the treatments BCA 2, BCA 1_BCA 2 and BCA 2_BCA 1, were better than BCA 1 and positive control, witch, in turn are superior to the negative control ($p < 0.001$). The middle part of the canopy had the smallest severity when treated with the biologicals, then whit the chemicals (positive control), which was lower than the negative control ($p <0.001$). Last, in image F, the superior part of the canopy was affected by all the treatments, except negative control.

Lastly the average of the severity taken in R5-R6 (Figure 6), in the first experiment there was no significant difference between the treatments and the negative control ($p = 0.02$); in the second one, BCA 2, BCA 1_BCA 2 and BCA 2_BCA 1, was better than BCA 1, that was better than the positive control, which was better than the negative control ($p < 0.001$).

Productivity parameters

The treatment BCA_1_BCA_2 resulted in the highest yield in both experiments (Figure 7) 21.2% and 8.02% yield increase respectively against the second best, while in the late sowing moment, it was not different from the other treatments, except the negative control. In the first experiment treatments Positive control, BCA 2, and BCA_2_BCA_1, also resulted in 26.7%, 17.92% and 19.7% increase compared to the negative control ($p < 0.001$). On the other hand, in the late sowing all treatments were higher than the negative control (Positive control 26.98%, BCA 2 27.59, BCA 1_BCA 2 36.53 and 28.43), except for BCA 1 ($p = 0.0018$), that in both experiments did not differ from the negative control.

The variables bellow, in the Figure 8, present the data from both experiments, from the two sowing seasons. In the first season there was no significant difference between the treatments (Thousand grains weight ($p = 0.08$); pod number ($p = 0.33$); and grains per pod ($p = 0.6$)). In the late sowing, the variable pod number, did not show a significant difference between the treatments ($p = 0.027$), while for thousand grains all the treatments had a significant effect ($p < 0.001$) over negative control increasing its values. Furthermore, in grains per pod the treatments BCA 2, BCA 1_BCA2 and BCA 2_BCA 1 ($p = 0.084$), did differ from the negative control, simultaneously the treatments positive control and BCA 1, resulted ambiguity, not differing from the following treatments.

Economic analysis

Table 1 – Economic analysis made by comparing the application values and the yield, considering that all other management actions were the same for the treatments.

		Amount (U$)	
Treatments	Yield (Bags)	Application	Profit

Negative Control	34,12[(1)]	33.61[(2)]	0[(1)]	0[(2)]	796,42[(1)]	784,43[(2)]
Positive Control	51,63[(1)]	47.90[(2)]	86,17[(1)]	115,56[(2)]	1118,98[(1)]	1002,38[(2)]
BCA 1	43,73[(1)]	44.78[(2)]	48,38[(1)]	60,48[(2)]	972,19[(1)]	984,59[(2)]
BCA 2	45,86[(1)]	48.22[(2)]	105,80[(1)]	132,25[(2)]	964,68[(1)]	993,18[(2)]
BCA 1_BCA 2	65,50[(1)]	52.95[(2)]	77,09[(1)]	89,19[(2)]	1451,59[(1)]	1146,71[(2)]
BCA 2_BCA 1	47,03[(1)]	48.70[(2)]	77,09[(1)]	103,54[(2)]	1020,49[(1)]	1033,21[(2)]

*Considering the price of the bag U$ 23.34; [(1)]Early sowing; [(2)]Late sowing; Where: Negative control (Water); Positive control (Chemical fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens*; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx).

Treatment BCA 1_BCA 2 showed the highest profits in absolute and both sowing comparisons; an increase of U$ 332.61 in the first season and U$ 113.5 in the second season, compared to the second highest profit.

DISCUSSION

Soybean rust incidence and the level of severity of disease in field is guided by climatic factors as: temperature, rainfall, relative humidity, leaf wetness period, and UV radiation (Medeiros et al., 2008), inoculum pression that came from spontaneous plants infecting plants in the early season, and inoculum that came from other areas, causing epidemics in the later season (Dalla Lana et al., 2018). The correct management according to the developing stage of

the plant is essential to reduce the losses and/or damage related to disease development (Navarini et al., 2007).

We found that under lower rust inoculum pressure, the treatment BCA 1_BCA 2 (Bacillus amyloliquefaciens; Bacillus amyloliquefaciens followed by Bacillus subtilis) resulted in a yield rate increase of 21.2% (Figure 1), although it did not show any difference from other treatments for any other parameter, that represent an increase of U$ 340.65 (13.87 bags), from the second biggest yield ("melhorcambio", 2024). Some hypotheses can be assumed for the cause of this behavior is the concentration of the metabolites, as found by Ben Khedher et al., (2020) with surfactant concentration; applied in R5-R6 which is the most important stage in grain filling (Barro et al., 2021). The product used in this stage for the treatment BCA 1_BCA 2 was B. subtilis , this genus is known for a huge variety of metabolites produced, specially lipopeptides, ribosome produced or not (Kaspar et al., 2019).

Among these metabolites there are the exopolysaccharides and siderophores. These molecules can help the maintenance of the ionic balance in the plant, inhibit the colonization of pathogens thus avoid the transport of toxic ions (Radhakrishnan et al., 2017), mechanisms that might stimulate the plant and promote its growth, enabling an increase of the yield (Figure 1).

Another growth promoters may be produced by Bacillus genus are the phytohormones, like auxins where B. subtilis is capable of producing indole acetic acid (IAA), in high amounts under fermentation process (Prado et al., 2019), cytokinins (Chobotarov et al., 2017), gibberellins (Rizza and Jones, 2019) and abscisic acid (Shahzad et al., 2019): responsible to regulate cell growth and differentiation (Gomes and Scortecci, 2021); shoot formation, vascular proliferation and lateral root development (Wybouw and De Rybel, 2019); increase abiotic stress tolerance and plant height (Gao and Chu, 2020); and various kinds of stress, as saline or related to temperature (Jiang et al., 2019; Ma et al., 2015) respectively. At late sowing, although we still have the superiority of the treatment BCA 1_BCA 2 (Bacillus amyloliquefaciens , strain

; Bacillus amyloliquefaciens , strain followed by Bacillus subtilis) concerning the yield (Figure 1), there was no significant difference between the biological and chemical treatments. This might suggest that under a major inoculum pressure, the increase of yield was affected by the severity of the disease (Navarini et al., 2007). On the other hand, this yield boost represents a gain of 4 bags per hectare, which means U$98.24 per hectare, with no chemical products applied for disease control (“melhorcambio,” 2024).

The remain productivity parameter (Figure 2) did not present significant difference between the treatments in the early sowing, probably due to the low inoculum pressure (Godoy et al., 2018), whereas on the late sowing all treatments differed from the negative control, suggesting that the pressure directly affect these parameters (Godoy et al., 2018), except the pod number, which suggests that the products did not had any effect under flowering stimulation, and was only affected by the environment, the main factor that influences number of pods (Mundstock and Thomas, 2005).

The disease evaluations made, show the development of the disease in the field, as for rust, as for final cycle diseases (Fig. 3 and 6). Both show a buildup of the severity between the 3° to the 4° evaluation for all treatments on the 2 sowing periods, which is the crucial time for the disease control that harm the grain filling (Barro et al., 2021), for its pattern is shown in the three third of the canopy.

There is no disease severity difference between the treatments that justify the increase of the yield related to the control of the pathogen (Lana et al., 2015), therefore, another important element analogous to the yield increment is the initial concentration of the metabolites and cells present on the formulated product, an essential aspect in disease control (Bressan and Figueiredo, 2008; Yan and Khan, 2021). The concentration of autoinducer molecules and the cell density determines the activation of the quorum sensing (Kalamara et

al., 2018), that triggers the production of particles that might promote growth in the plant (Sansinenea, 2019), and the production of biofilms (Kalamara et al., 2018).

The formulation of the products presented by the leaflet does not show the amount of metabolites content presented on the product. Furthermore, the product Bioimune, has in its formulation the lipopeptides: iturine, fengicine and surfactin; and the enzymes: chitinases, lipases, proteases, and β-1, 3-glucanases (AGROFIT-Sistema de agrotóxicos fitossanitários, 2023). These lipopeptides have an antimicrobial effect, which are also involved in the production of biofilms (Penha et al., 2020), and act directly to the pathogen membrane (Sheppard et al., 1991). Those enzymes also degrade the cell wall (Tran et al., 2022) and avoid its germination (Wu et al., 2020). So, these molecules already present in the product applied in the field act directly into the pathogen, what costs less energy of the plant to control the fungus and can be used in the grain filling (Jinal and Amaresan, 2020).

The profit earned in BCA 1_BCA 2 (Table 1), lead us to the conclusion that in 22/23 crop, the foliar management disease could be made only with biologicals, and with higher profits than using only chemicals.

Conclusions

Our results indicate that biological control rotation works, but it depends on which biological we are rotating; and the inoculum pressure. Biologicals also increased the yield of soybean, representing gain to the grower in the early and late season, despite the statistically insignificant in late one; the rust inoculum in the late season might contribute to the yield decrease, where severity rises at R5-R6 impacting grain filling; metabolites and cell concentration can be the key factor to provoke yield increase; the economic analysis show that biological treatments application cost less than chemical one, and present same disease index,

in other words it its cheaper than chemical applications. Furthermore, Metabolomic analysis must be evaluated to understand the interaction between plant and microorganisms.

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7. Legends and figures

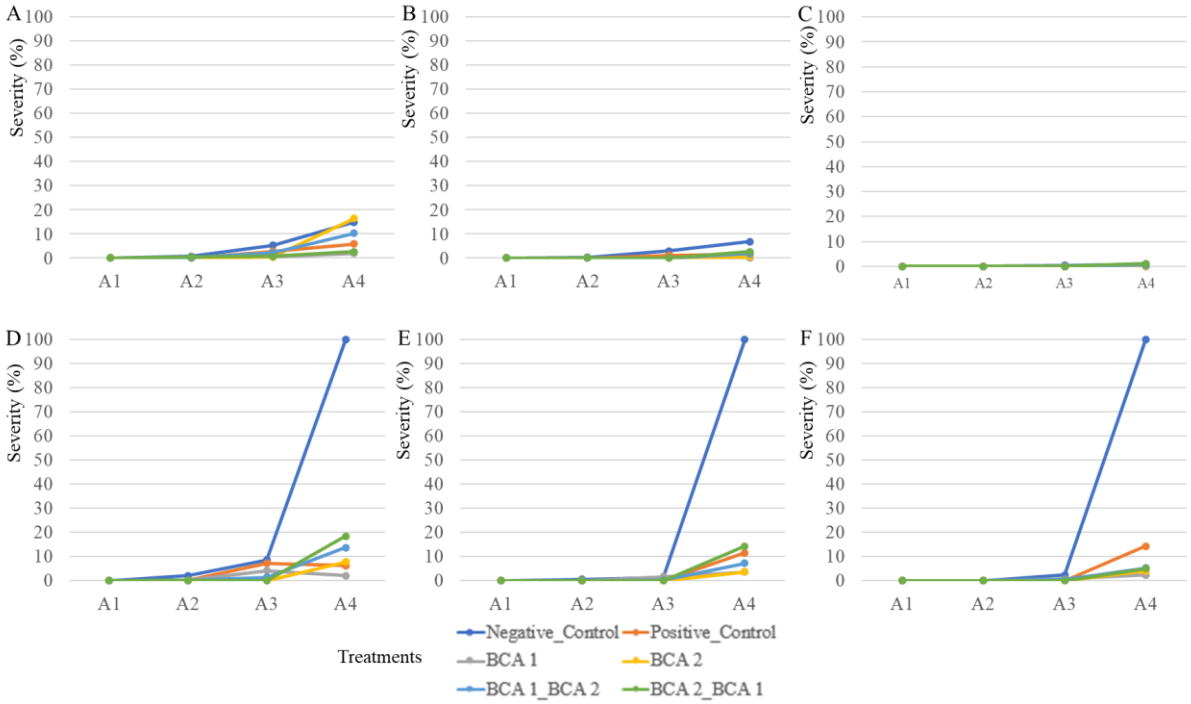


Figure 1. Severity of soybean rust (*Phakopsora pachyrhizi).* (A) Bottom third of the canopy, early sowing; (B) Middle Third of the canopy, early sowing; (C) Superior third of the canopy, early sowing; (D) Bottom third of the canopy, late sowing; (E) Middle third of the canopy, late sowing; (F) Superior third of the canopy, late sowing. Where: Negative control (Water);

Positive control (Chemical fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens*; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx).

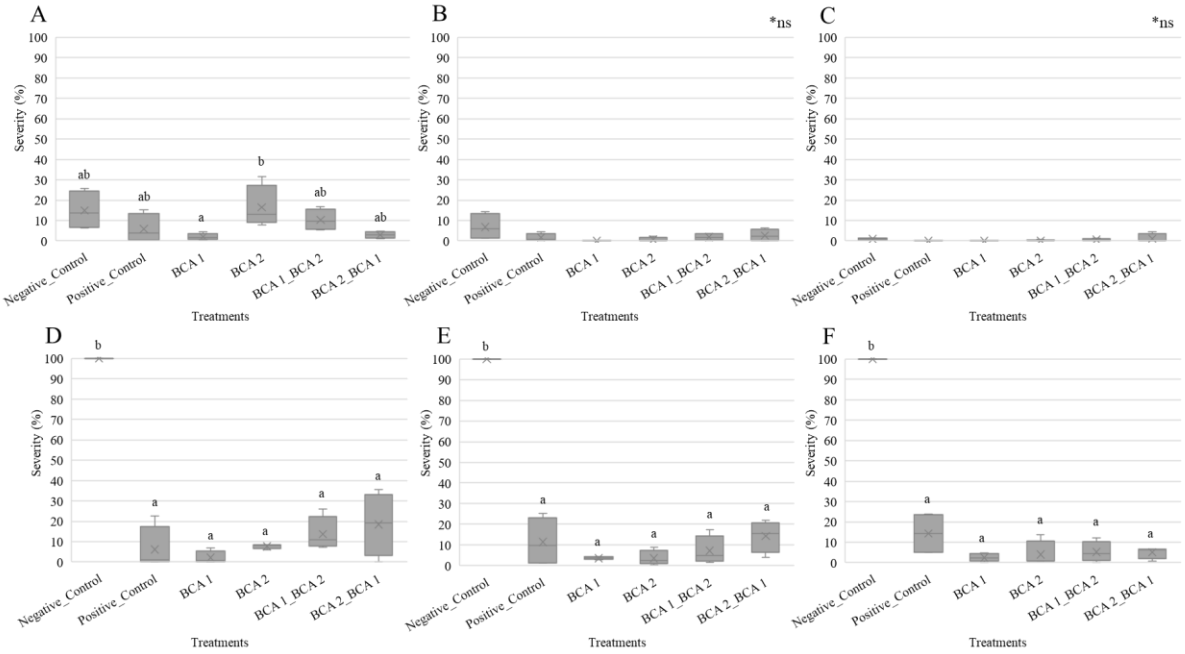


Figure 2. Severity of soybean rust (*Phakopsora pachyrhizi*) of the 4th evaluation (R6). (A) Bottom third of the canopy, early sowing; (B) Middle Third of the canopy, early sowing; (C) Superior third of the canopy, early sowing; (D) Bottom third of the canopy, late sowing; (E) Middle third of the canopy, late sowing; (F) Superior third of the canopy, late sowing. Where: Negative control (Water); Positive control (Chemical fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens*; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx). *ns (shows that there was no significant difference between treatments), **Averages followed by the same letter do not statistically different by the Tukey test ($p <= 0.05$).

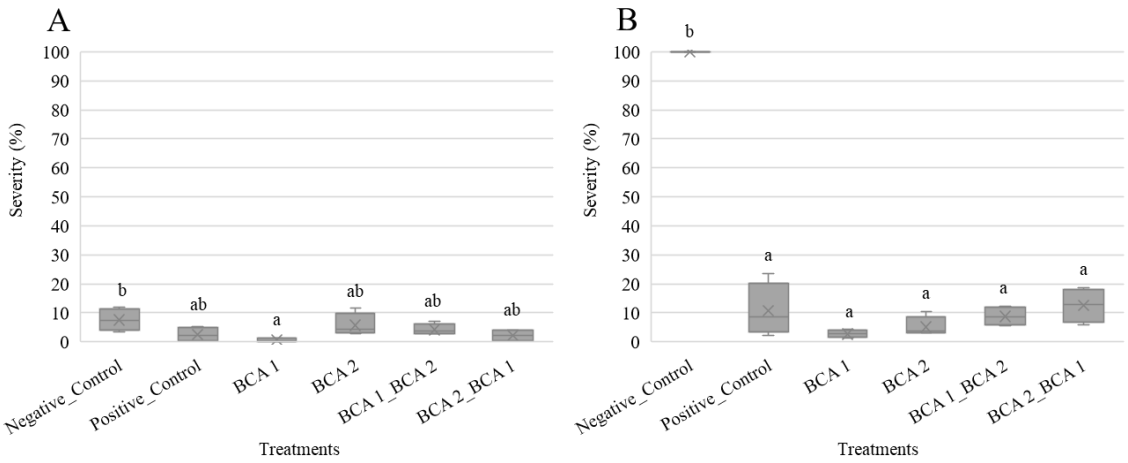


Figure 3. Average severity measured from individual assessment of the disease at the upper, middle, and bottom of the plant canopy for soybean rust (*Phakopsora pachyrhizi)* at early (A) and late (B) sowing. Where: Negative control (Water); Positive control (Chemical fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens*; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx).*Means followed by the same letter do not statistically different by the Tukey test (p <= 0.05).

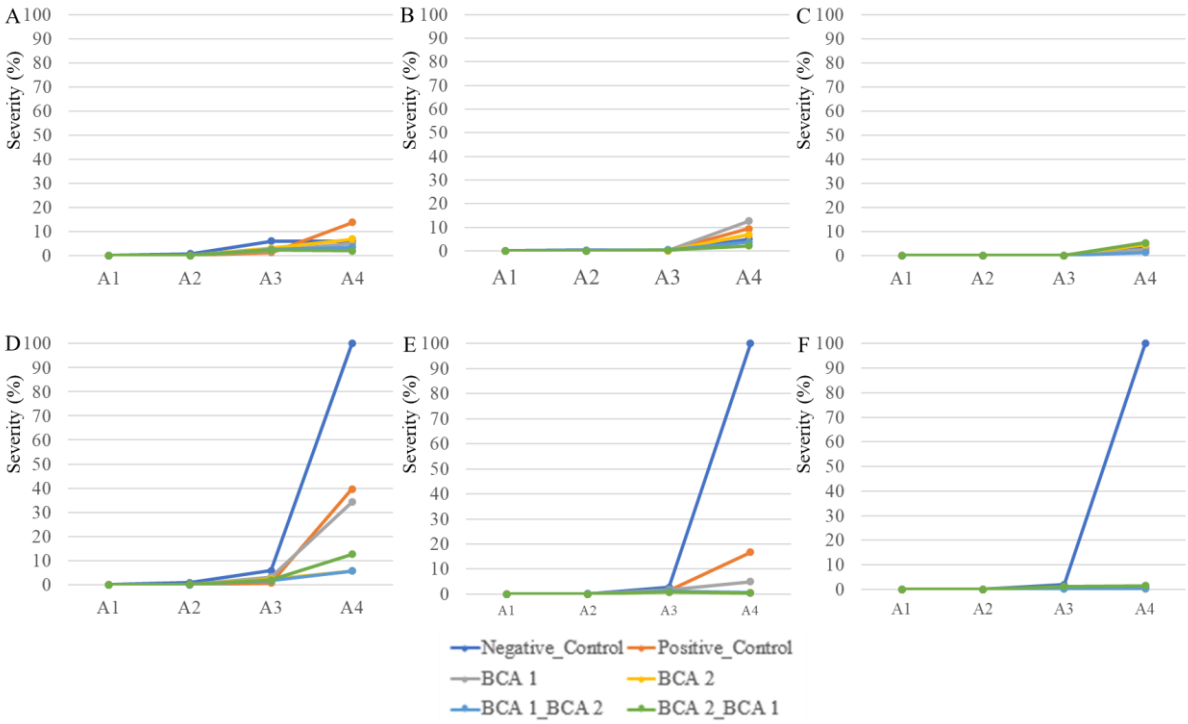


Figure 4. Severity of leaf blight (*Cercospora kikuchii* and *Septoria glycine*). (A) Bottom third of the canopy, early sowing; (B) Middle Third of the canopy, early sowing; (C) Superior third of the canopy, early sowing; (D) Bottom third of the canopy, late sowing; (E) Middle third of the canopy, late sowing; (F) Superior third of the canopy, late sowing. Where: Negative control (Water); Positive control (Chemical fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens* ; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx).

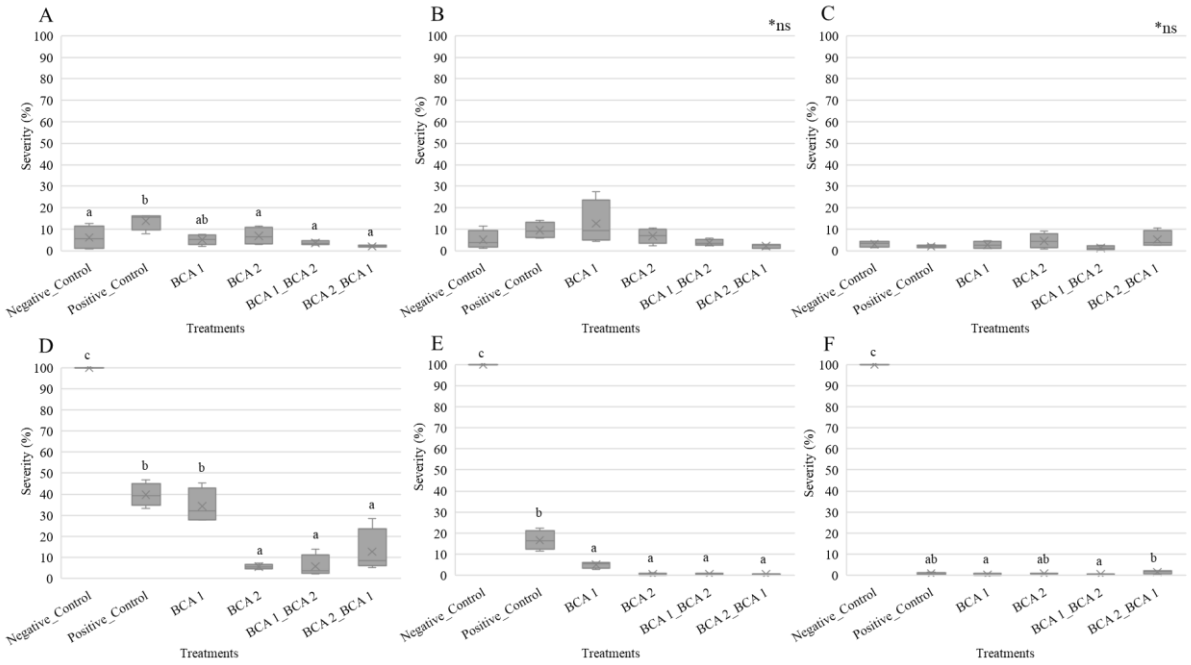


Figure 5. Severity of Final cycle diseases (*Cercospora kikuchii* and *Septoria glycine*)) of the 4° evaluation (R6). (A) Bottom third of the canopy, early sowing; (B) Middle Third of the canopy, early sowing; (C) Superior third of the canopy, early sowing; (D) Bottom third of the canopy, late sowing; (E) Middle third of the canopy, late sowing; (F) Superior third of the canopy, late sowing. Where: Negative control (Water); Positive control (Chemical fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens*; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx). *ns (shows that there was no significant difference between treatments), **Averages followed by the same letter do not statistically different by the Tukey test (p <= 0.05).

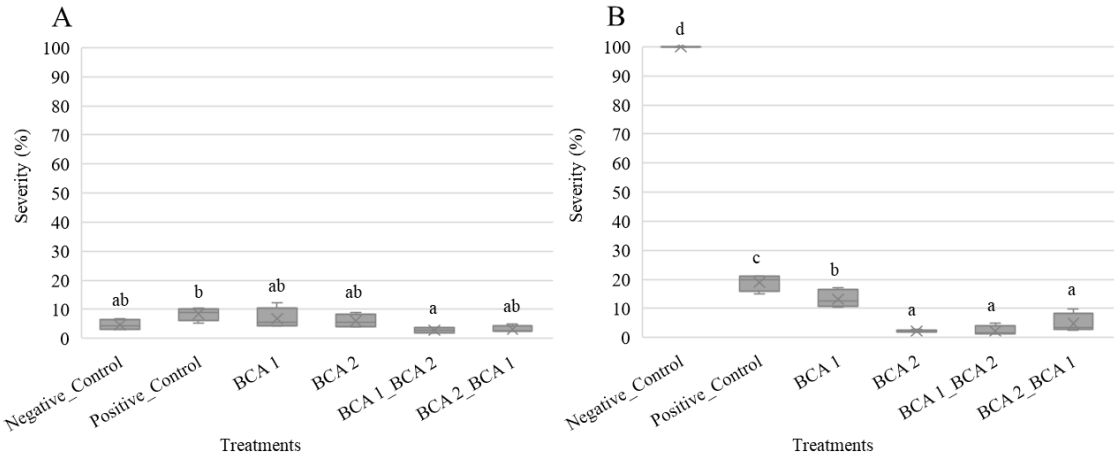


Figure 6. Average severity of the 3 thirds of the canopy of Final cycle diseases (*Cercospora kikuchii and Septoria glycine*). (A) Average of the severity from the canopy thirds, early sowing; (B) Average of the severity from the canopy thirds, late sowing. . Where: Negative control (Water); Positive control (Chemical fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens*; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx). *ns (shows that there was no significant difference between treatments), *Averages followed by the same letter do not statistically different by the Tukey test ($p <= 0.05$).

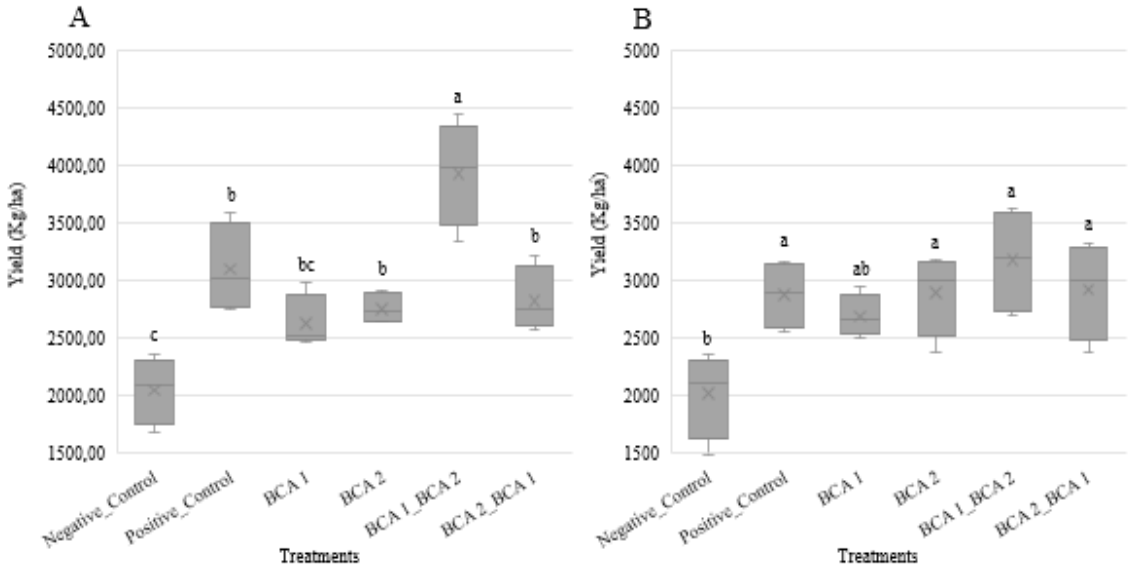
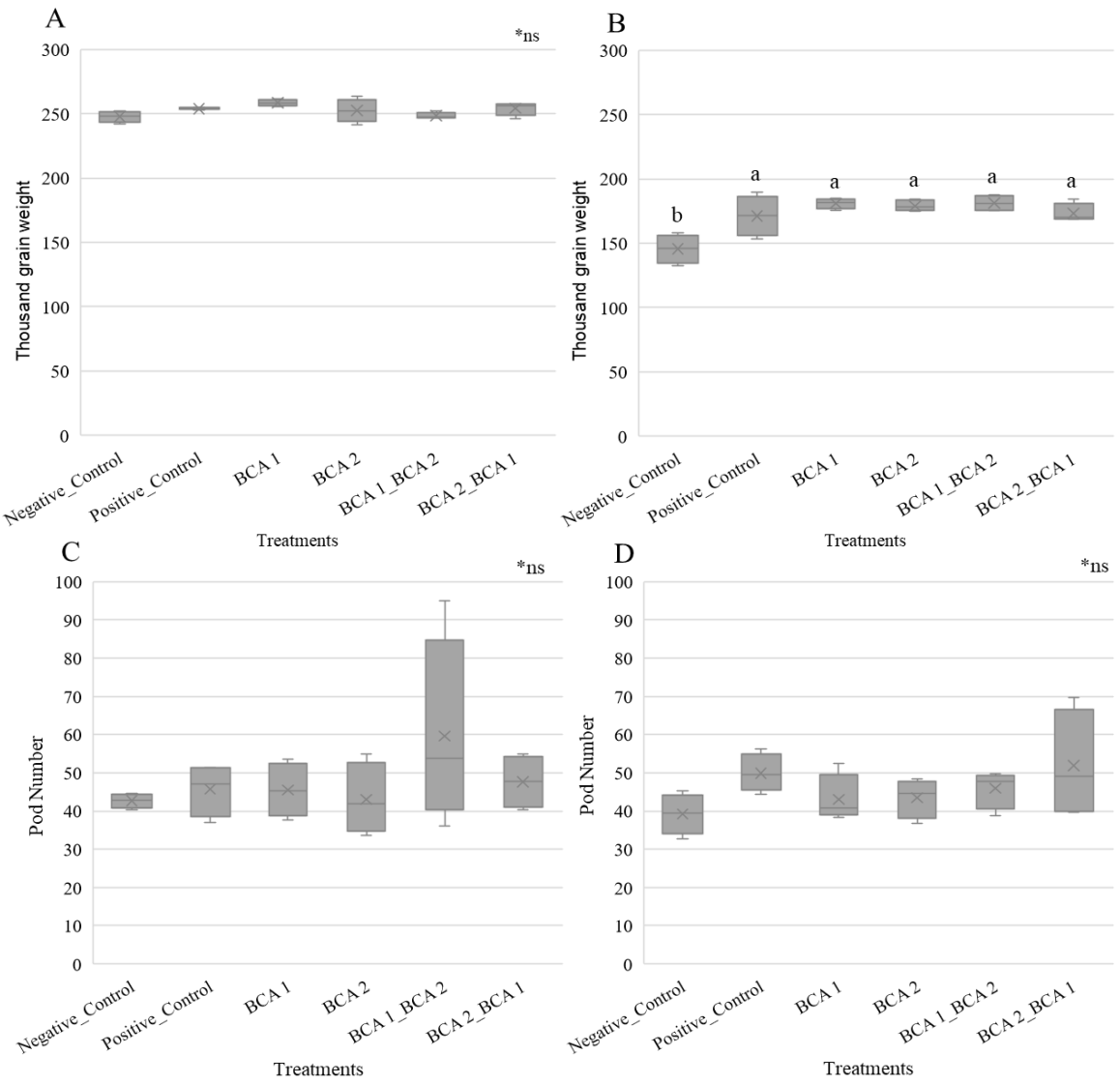


Figure 7. (A) Soybean yield in Kg/ha harvested in the early sowing; (B) Soybean yield in Kg/ha harvested in the late sowing. Where: Negative control (Water); Positive control (Chemical

fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens*; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx). *Averages followed by the same letter do not statistically different by the Tukey test (p <= 0.05).



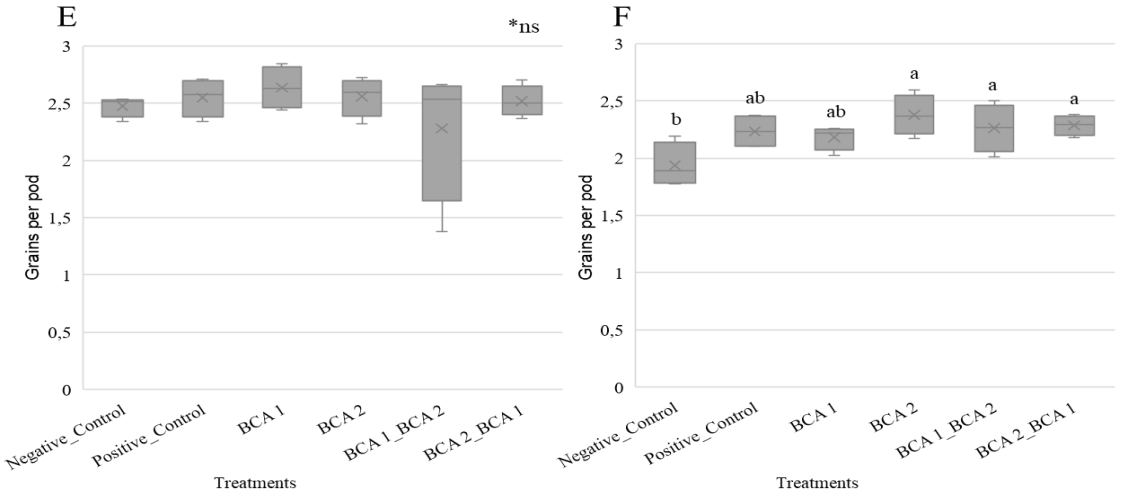


Figure 8. (A) Soybean thousand grain weight in grams, collected from three plants per plot, harvested in the early sowing; (B) Soybean thousand grain weight in grams in the late sowing; (C) Number of pods in the early sowing; (D) Number of pods, late sowing; (E) Number of grains per pod, early sowing; (F) Number of grains per pod, late sowing. . Where: Negative control (Water); Positive control (Chemical fungicides treatment); BCA 1 (*Bacillus amyloliquefaciens*; *Bacillus amyloliquefaciens* (Twixx, Agrivalle)); BCA_2 (*Bacillus subtilis* (Bioimune, Vittia)); BCA_1_BCA_2 (Twixx_ Bioimune); and BCA_2_BCA_1 (Bioimune_ Twixx). *ns (shows that there was no significant difference between treatments), **Averages followed by the same letter do not statistically different by the Tukey test (p <= 0.05).

BIBLIOGRAPHY

AGROFIT-Sistema de agrotóxicos fitossanitários [WWW Document], 2024. . Ministério da Agricultura, Pecuária e Abastecimento. URL https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed 2.13.24).

Albu, S., Schneider, R. W., Price, P. P., and Doyle, V. P. 2016. Cercospora cf. flagellaris and Cercospora cf. sigesbeckiae are associated with Cercospora leaf blight and purple seed stain on soybean in North America. Phytopathology 106:1376-1385.

Ashaolu, T.J., 2020. Applications of soy protein hydrolysates in the emerging functional foods: a review. Int J Food Sci Technol. https://doi.org/10.1111/ijfs.14380

Barro, J.P., Alves, K.S., Godoy, C. V., Dias, A.R., Forcelini, C.A., Utiamada, C.M., de Andrade Júnior, E.R., Juliatti, F.C., Grigolli, F.J., Feksa, H.R., Campos, H.D., Chaves, I.C.P.V., Araújo Júnior, I.P., Roy, J.M.T., Nunes Júnior, J., Belufi, L.M.R., Carneiro, L.C., Silva, L.H.C.P., Canteri, M.G., Goussain Júnior, M.M., Senger, M., Meyer, M.C., Dias, M.D., Müller, M.A., Martins, M.C., Debortoli, M.P., Tormen, N.R., Furlan, S.H., Konageski, T.F., Carlin, V.J., Venâncio, W.S., Del Ponte, E.M., 2021. Performance of dual and triple fungicide premixes for managing soybean rust across years and regions in Brazil: A meta-analysis. Plant Pathol 70, 1920–1935. https://doi.org/10.1111/ppa.13418

Ben Khedher, S., Boukedi, H., Laarif, A., Tounsi, S., 2020. Biosurfactant produced by Bacillus subtilis V26: a potential biological control approach for sustainable agriculture development. Organic Agriculture 10, 117–124. https://doi.org/10.1007/s13165-020-00316-0

Bressan, W., Figueiredo, J.E.F., 2008. Efficacy and dose-response relationship in biocontrol of Fusarium disease in maize by Streptomyces spp. Eur J Plant Pathol 120, 311–316. https://doi.org/10.1007/s10658-007-9220-y

Brown, M.T., Shim, S., Da Silva, C.R., Waibel, K.G., Telenko, D.E.P., 2024. Assessing Foliar Fungicides for Soybean Disease Management and Yield in Indiana. Plant Health Prog 25, 43–48. https://doi.org/10.1094/PHP-05-23-0046-RS

Chobotarov, A., Volkogon, M., Voytenko, L., Kurdish, I., 2017. Accumulation of phytohormones by soil bacteria Azotobacter Vinelandii and Bacillus subtilis under the influence of nanomaterials. Journal of Microbiology, Biotechnology and Food Sciences 7, 271–274. https://doi.org/10.15414/jmbfs.2017/18.7.3.271-274

Godoy, C. V., Carlos M. Utiamada, Maurício C. Meyer, Hercules D. Campos, Ivani de O. N. Lopes, Alfredo R. Dias, C.C.D., Cláudia B. Pimenta, David S. Jaccoud Filho, Eder N. Moreira, Edson P. Borges, Edson R. de Andrade Junior, Fabiano V. Siqueri, Fernanda C.

Juliatti, Fernando C. Juliatti, Fernando Favero, Heraldo R. Feksa, Ivan Pedro Araújo Júnior, José Fernando J. Grigolli, José Nunes Junior, Luana M. de R. Belufi, Luciana C. Carneiro, Luís Henrique C. P. da Silva, Luiz Nobuo Sato, Marcelo G. Canteri, Marcelo R. Volf, Marcio Goussain, Moab D. Dias, Mônica Paula Debortoli, Mônica C. Martins, Ricardo S. Balardin, Silvânia H. Furlan, Tiago Madalosso, Valtemir J. Carlin, Wilson S. Venancio, 2018. Eficiência de fungicidas para o controle da ferrugem-asiática da soja, Phakopsora pachyrhizi, na safra 2017/2018:Resultados sumarizados dos ensaios cooperativos. Londrina-PR.

Claudio M. Mundstock, André Luís Thomas, 2005. SOJA: Fatores que afetam o crescimento e o rendimento de grãos. Porto Alegre.

Dalla Lana, F., Paul, P.A., Godoy, C. V., Utiamada, C.M., Da Silva, L.H.C.P., Siqueri, F. V., Forcelini, C.A., Jaccoud-Filho, D.D.S., Miguel-Wruck, D.S., Borges, E.P., Juliatti, F.C., Campos, H.D., Nunes, J., Carneiro, L.C., Canteri, M.G., Ito, M.F., Meyer, M.C., Martins, M.C., Balardin, R.S., Furlan, S.H., Carlin, V.J., Del Ponte, E.M., 2018. Meta-analytic modeling of the decline in performance of fungicides for managing soybean rust after a decade of use in Brazil. Plant Dis 102, 807–817. https://doi.org/10.1094/PDIS-03-17-0408-RE

FAO. World food and agriculture - statistical yearbook 2020. Rome: FAO. (2020).

Gao, S., Chu, C., 2020. Gibberellin metabolism and signaling: Targets for improving agronomic performance of crops. Plant Cell Physiol. https://doi.org/10.1093/pcp/pcaa104

Gomes, G.L.B., Scortecci, K.C., 2021. Auxin and its role in plant development: structure, signalling, regulation and response mechanisms. Plant Biol. https://doi.org/10.1111/plb.13303

Hossain, M.M., Sultana, F., Yesmin, L., Rubayet, M.T., Abdullah, H.M., Siddique, S.S., Bhuiyan, M.A.B., Yamanaka, N., 2023. Understanding Phakopsora pachyrhizi in soybean:

comprehensive insights, threats, and interventions from the Asian perspective. Front Microbiol. https://doi.org/10.3389/fmicb.2023.1304205

Ishiwata, Y.I., Furuya, J., 2020. Evaluating the contribution of soybean rust-resistant cultivars to soybean production and the soybean market in Brazil: A supply and demand model analysis. Sustainability (Switzerland) 12. https://doi.org/10.3390/su12041422

Jiang, Z., Zhou, X., Tao, M., Yuan, F., Liu, L., Wu, F., Wu, X., Xiang, Y., Niu, Y., Liu, F., Li, C., Ye, R., Byeon, B., Xue, Y., Zhao, H., Wang, H.N., Crawford, B.M., Johnson, D.M., Hu, C., Pei, C., Zhou, W., Swift, G.B., Zhang, H., Vo-Dinh, T., Hu, Z., Siedow, J.N., Pei, Z.M., 2019. Plant cell-surface GIPC sphingolipids sense salt to trigger Ca2+ influx. Nature 572, 341–346. https://doi.org/10.1038/s41586-019-1449-z

Jinal, N.H., Amaresan, N., 2020. Evaluation of biocontrol Bacillus species on plant growth promotion and systemic-induced resistant potential against bacterial and fungal wilt-causing pathogens. Arch Microbiol 202, 1785–1794. https://doi.org/10.1007/s00203-020-01891-2

Juliatti, F.C., Mesquita, A.C.O., Teixeira, F.G., Beloti, I.F., Mota, L.C.B.M., Fonseca, L.J., de Sousa, L.A., Souza, M.S.X. de A., Silva, N.S., Zancan, N.L.B., de Morais, T.P., 2019. Characterization of soybean genotypes showing partial resistance to soybean rust. Summa Phytopathol 45, 313–319. https://doi.org/10.1590/0100-5405/190552

Kalamara, M., Spacapan, M., Mandic-Mulec, I., Stanley-Wall, N.R., 2018. Social behaviours by Bacillus subtilis: quorum sensing, kin discrimination and beyond. Mol Microbiol. https://doi.org/10.1111/mmi.14127

Karlsson, J.O., Parodi, A., van Zanten, H.H.E., Hansson, P.A., Röös, E., 2021. Halting European Union soybean feed imports favours ruminants over pigs and poultry. Nat Food 2, 38–46. https://doi.org/10.1038/s43016-020-00203-7

Kaspar, F., Neubauer, P., Gimpel, M., 2019. Bioactive Secondary Metabolites from Bacillus subtilis: A Comprehensive Review. J Nat Prod. https://doi.org/10.1021/acs.jnatprod.9b00110

Lana, F.D., Ziegelmann, P.K., De Maia, A.H.N., Godoy, C. V., Del Ponte, E.M., 2015. Meta-analysis of the relationship between crop yield and soybean rust severity. Phytopathology 105, 307–315. https://doi.org/10.1094/PHYTO-06-14-0157-R

Ma, Y., Dai, X., Xu, Y., Luo, W., Zheng, X., Zeng, D., Pan, Y., Lin, X., Liu, H., Zhang, D., Xiao, J., Guo, X., Xu, S., Niu, Y., Jin, J., Zhang, H., Xu, X., Li, L., Wang, W., Qian, Q., Ge, S., Chong, K., 2015. Erratum: COLD1 Confers Chilling Tolerance in Rice (Cell (2015) 160 (1209-1221)). Cell. https://doi.org/10.1016/j.cell.2015.06.046

Medeiros, E., Ponte, D., Paul, ;, Esker, D., 2008. Piracicaba, Braz.), v.65, special issue, Sci. Agric.

Meyer, M.C., Bueno, A. de F., Mazaro, S.M., Silva, J.C. da, 2022. Bioinsumos NA Cultura Da Soja. Embrapa Soja.

Meyer, M.C., Godoy, C. V., Utiamada, C.M., Campos, H.D., medeiros, F.H.V. de, Oliveira, M.C.N. de, Mazaro, S.M., senger, M., Bonani, J.C., Carvalho, A.G. de, Brustolin, R., Nunes Junior, J., Dias, A.R.; F.L.H., Goussain Junior, M.M., Gomes, L.M.M.R., Farias, A. de, ChaveS, I.C.P. de V., Martins, M.C., Belufi, L.M. de R., Konageski, F.T., Konageski, T.F., Stefanelo, M.S., Grigolli, J.F.J., 2023. Avaliação da associação de fungicidas químicos e biológicos no controle de doenças foliares da soja, safra 2022/2023: resultados sumarizados da rede de experimentos cooperativos 198, 1–15.

Navarini, L., Dallagnol, L.J., Balardin, R.S., Moreira, M.T., Meneghetti, R.C., Madalosso, M.G., 2007. Chemical Control of Soybean Rust (Phakopsora pachyrhizi Sidow) in soybean crop, Summa Phytopathologica.

Oilseeds: World Markets and Trade, 2024.

Padial-Domínguez, M., Espejo-Carpio, F.J., Pérez-Gálvez, R., Guadix, A., Guadix, E.M., 2020. Optimization of the emulsifying properties of food protein hydrolysates for the production of fish oil-in-water emulsions. Foods 9. https://doi.org/10.3390/foods9050636

Penha, R.O., Vandenberghe, L.P.S., Faulds, C., Soccol, V.T., Soccol, C.R., 2020. Bacillus lipopeptides as powerful pest control agents for a more sustainable and healthy agriculture: recent studies and innovations. Planta. https://doi.org/10.1007/s00425-020-03357-7

Prado, D.Z. do, Okino-Delgado, C.H., Zanutto-Elgui, M.R., Silva, R.B.G. da, Pereira, M.S., Jahn, L., Ludwig-Müller, J., Silva, M.R. da, Velini, E.D., Fleuri, L.F., 2019. Screening of Aspergillus, Bacillus and Trichoderma strains and influence of substrates on auxin and phytases production through solid-state fermentation. Biocatal Agric Biotechnol 19. https://doi.org/10.1016/j.bcab.2019.101165

Preço da soja hoje [WWW Document], 2024. URL https://www.melhorcambio.com/soja-hoje#:~:text=O%20valor%20da%20saca%20da,em%20R%24%20115%2C82. (accessed 2.13.24).

Primiano, I.V., Amorim, L., 2023. Soybean and grapevine rusts accelerate the defoliation rates of host plants. Plant Pathol. https://doi.org/10.1111/ppa.13841

Radhakrishnan, R., Hashem, A., Abd Allah, E.F., 2017. Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. Front Physiol 8. https://doi.org/10.3389/fphys.2017.00667

Rizza, A., Jones, A.M., 2019. The makings of a gradient: spatiotemporal distribution of gibberellins in plant development. Curr Opin Plant Biol. https://doi.org/10.1016/j.pbi.2018.08.001

Sansinenea, E., 2019. Bacillus spp.: As plant growth-promoting bacteria, in: Secondary Metabolites of Plant Growth Promoting Rhizomicroorganisms: Discovery and

Applications. Springer Singapore, pp. 225–237. https://doi.org/10.1007/978-981-13-5862-3_11.

Sautua, F. J., Searight, J., Doyle, V. P., Scandiani, M. M., and Carmona, M. A. 2020. Cercospora cf. nicotianae is a causal agent of Cercospora leaf blight of soybean. Eur. J. Plant Pathol. 156:1227-1231.

Shahzad, R., Khan, A.L., Waqas, M., Ullah, I., Bilal, S., Kim, Y.H., Asaf, S., Kang, S.M., Lee, I.J., 2019. Metabolic and proteomic alteration in phytohormone-producing endophytic Bacillus amyloliquefaciens RWL-1 during methanol utilization. Metabolomics 15. https://doi.org/10.1007/s11306-018-1467-0

Sheppard, J.D., Jumarie, C., Cooper, D.G., Laprade, R., 1991. Ionic channels induced by surfactin in planar lipid bilayer membranes, Biochimica et Biophysica Acta.

Sistema de agrotóxicos fitossanitários, A., 2023. Bula Bio-imune/Multi-Attack/Multi-Guard/FungiOuro/Biologic Aerum [WWW Document]. Agrofit. URL https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed 10.29.23).

Soares, A.P.G., Guillin, E.A., Borges, L.L., Da Silva, A.C.T., De Almeida, Á.M.R., Grijalba, P.E., Gottlieb, A.M., Bluhm, B.H., De Oliveira, L.O., 2015. More Cercospora species infect soybeans across the Americas than meets the eye. PLoS One 10. https://doi.org/10.1371/journal.pone.0133495.

Tran, C., Cock, I.E., Chen, X., Feng, Y., 2022. Antimicrobial Bacillus: Metabolites and Their Mode of Action. Antibiotics. https://doi.org/10.3390/antibiotics11010088

Wu, J.J., Huang, J.W., Deng, W.L., 2020. Phenylacetic Acid and Methylphenyl Acetate From the Biocontrol Bacterium Bacillus mycoides BM02 Suppress Spore Germination in Fusarium oxysporum f. sp. lycopersici. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.569263

Wybouw, B., De Rybel, B., 2019. Cytokinin – A Developing Story. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2018.10.012.

Yan, L., Khan, R.A.A., 2021. Biological control of bacterial wilt in tomato through the metabolites produced by the biocontrol fungus, Trichoderma harzianum. Egypt J Biol Pest Control 31. https://doi.org/10.1186/s41938-020-00351-9.

CHARPTER 2 – WHITE MOLD

Does alternating different *Trichoderma*-formulated products control *Sclerotinia sclerotiorum*?

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Abstract

White mold, caused by *Sclerotinia sclerotiorum*, is a significant plant disease that affects over 400 different host plants. In soybean crops, it tends to appear in regions with an altitude higher than 600 meters, with high moisture and rainfall up to 250 mm. The disease can be managed through cultural control, using grasses and cover crops, chemical control, with fungicides, and biological control, using biological control agents (BCAs), such as *Trichoderma* sp. It has different ways of acting against plant pathogens, specifically against sclerotia. In the present work, *Trichoderma*-based products were sprayed separately at the V2 and V4 phenological stages to evaluate their effectiveness in controlling white mold in soybean. Two field trials were conducted in different areas during the same season. We evaluated productivity parameters such as yield (kg/ha), thousand grains weight (g), pod number, and grains per pod. We also assessed disease parameters, including the incidence of white mold (reproductive stage of R6), sclerotia parasitism, sclerotia germination, and the number of apothecia from sclerotia produced in field and laboratory conditions. Although there was no statistical difference in productivity between the BCAs treatments, all were better than the

control. There was also no significant difference in the weight of a thousand grains, the number of pods, and grains per pod, except between BCA 3 and the control in Area 2 for the weight of a thousand grains. Regarding the disease parameters, all BCA treatments were found to be different from the control in terms of incidence of white mold, while parasitism and germination of sclerotia were enhanced with *Trichoderma* spray. The application of these BCAs is essential in the production system, as they can potentially reduce the initial inoculum, colonize the stubble, and accumulate gains for the next season, in addition to increasing the yield.

Keywords: Biological Control Agents, *Trichoderma harziaunum* White mold, Yield increase, carpogenic germination.

1. Introduction

Brazil is currently the leading global producer of soybeans (USDA, 2023). The estimated yield for the upcoming 2023/24 season is projected to be around 317.5 million tons, grown on more than 78 million hectares of land (CONAB, 2023). However, with the expansion of the cultivation area, there is a greater need for integrated management methods to control diseases, weeds, and insects (Bortolotto et al. in 2015; Bueno et al. in 2021). Although the increased yield can lead to higher input costs, it can also result in quantitative and qualitative losses due to damage from biotic agents' actions (Bennett et al. in 2012).

One of Brazil's major fungal pathogens affecting soybean crops is Sclerotinia sclerotiorum (Willbur et al., 2019; Macena et al., 2020) It infects the plant through carpogenic or myceliogenic germination, causing significant losses in Brazil at elevations higher than 600m (Boland and Hall, 1994; Meyer et al., 2016). White mold can cause damage of up to 2-6.7 bags/ha in various Brazilian states, even with chemical fungicides (Reis et al., 2020). Without control measures, nearly 70% of losses can be incurred (Meyer et al., 2022).

The management of white mold involves both chemical and biological control methods since no soybean cultivars are resistant to this fungus, although their susceptibility may vary (Kandel et al., 2018; Sumida et al., 2015). The main active ingredients commercialized in Brazil are fluazinam, methyl thiophanate, procymidone, and carbendazim (Reis et al., 2020). Normally, during flowering, they are sprayed at R1 and R2 with a 10-day interval between sprays. (Lehner et al., 2017). Additionally, using different fungicides with different modes of action helps reduce the selection of resistant pathogens populations (Pethybridge et al., 2019; Meyer et al., 2020)

One of the ways to control plant pathogens is through biological control with Trichoderma spp fungus. This is a multi-mechanism biological control agent (BCA) that can reduce the damage caused by white mold in soybean. It does so by producing cell wall-degrading enzymes, parasitizing the sclerotia, inducing systemic defense response, and promoting growth. Several studies have described these benefits of Trichoderma spp (Geraldine et al., 2013; Sumida et al., 2018; Macena et al., 2020; Pratap Singh et al., 2021;). It is used in the V2 and V4 phenological stages of the plant for sclerotia viability characterization (Meyer et al., 2016) or in time of after dessecation of soybean (Conte et al., 2022), where the canopy is not fully developed or the dead plant, and the spray can reach the sclerotia remains in the soil (O'Sullivan et al., 2021). Thus, using the same timing sprayed of post-emergent herbicide (Canuto da Silva, et al., 2024) and desiccant herbicide in soybean and consequently, reducing the coast of production besides use friendlly approach when insert Trichoderma-based products in agroecossystm (Adetunji et al., 2020; Dutta et al., 2022)..

This study aims to evaluate the following: (i) the effects of applying Trichoderma harzianum on white mold disease and crop parameters; (ii) the synergy resulting from the alternate use of the formulated products; (iii) the viability of the sclerotia; and (iv) the efficacy of controlling white mold by combining the products.

2. Materials and Methods

2.1 "*in vivo*" test

The in vivo experiment with Sclerotinia sclerotiorum was conducted in two different locations where the disease was prevalent and sclerotia confirmed for virulence before planting, according to (Faria et al., 2022). The first area (Area 1) was at Nepomuceno-MG (coordinates 21°19'08.9"S 45°06'41.1"W) and the second (Area 2) at São Bento Abade-MG (21°34'41.6"S 45°08'29.9"W), both in Brazil. The cultivar used was Brasmax Zeus IPRO. Sowing at Area 1 occurred on 11/10/2022 and for Area 2 on 15/10/2022, sown on sorghum and maize stubble, respectively. Planting was done with 14 seeds per meter, with a row spacing of 0.5 meters. The plots consisted of five treatments and four blocks, with each plot having an effective area of 18m². The treatments were: Control (water); BCA 1 (Trichoderma harzianum IBLF 006, Ecotrich); BCA 2 (Trichoderma harzianum Natucontrol); BCA 1_BCA 2 (Ecotrich followed by Natucontrol); and BCA 2_BCA 1 (Natucontrol followed by Ecotrich). Procimidona (ParrudoBR) was applied at a dose of 1L/ha for the control of S. sclerotiorum starting at R1 for all treatments and also the products Carbendazim (Carbendazim STK 500 SC-B) 500ml/ha, Tebuconazole (Tebuconazole CCAB 200 EC) 0.75L/ha to control foliar diseases. Glyphosate (Roundup WG), at the dose of 2Kg/ha, was applied to control weed at V2, and thiamethoxam + lambda-cyhalothrin (Engeo Pleno S), at the dose of 200ml/ha to control insects when necessary.

To assess the experiments, the incidence of white mold was quantified by evaluating 80 plants of the main planting lines (the four lines of the middle) at R6. The parameters: yield (kg/ha), was measured by the weight of the grains collected from the main 4 lines (useful plot) of the center of the plot with the discount of the moisture; thousand-grain weight, measured by weighting one hundred grains 8 times and transforming it into one thousand grains weight; number of pods, measured by counting the number of pods of the main stem; and number of

grains per pod, measured by dividing the number of grains by the number of pods of the main stem.

2.2 Sclerotia parasitism

A test of parasitism capacity on the fungus Sclerotinia sclerotiorum was conducted. The experiment initially took place in the field, where sclerotia were collected from the area as well as produced in the laboratory to assess parasitism, under both conditions. The sclerotia were then placed inside bags made of aphid-proof mesh, with only one layer covering the sclerotia. A tray containing B horizon soil was placed in the middle of the soybean planting rows, and the sclerotia were placed on the tray. The tray was covered with the stubble of the last crop, concealing the sclerotia. Ten days after the second application, the sclerotia were removed from the field and taken to the laboratory. In the laboratory, a Gerbox with autoclaved sand was placed in a BOD at 17°C, with the sclerotia inside to stimulate the carpogenic germination of the fungus. Weekly, the number of apothecia on each sclerotium was counted until 60 days after the experiment was set up (Meyer et al., 2016). Cases of hyperparasitism by fungi or colonization by bacteria that rendered the sclerotia non-viable were also counted. The experiment was conducted with a design consisting of four blocks within the BOD, with each shelf representing one block.

2.3 Statistical analysis

The data were tested according to the Shapiro-Wilk normality test (p-value > 0.05), Bartlett's homogeneity test (p-value > 0.05), and Dixon's test (p-value ≤ 0.05), with the removal of outliers when necessary. For the comparison of means, Tukey’s test was performed for the mentioned variables (p-value ≤ 0.05). The software used was the R program 2023.06.1 Build 524.

3 Results

3.1 Disease parameters

The results obtained for the disease parameters in the first area, show us a lower incidence in the BCAs treatments, where *T. harzianum* 1 (66.6% lower), *T. harzianum* 2 (58.2% lower), the combinations *T. harzianum*1_2 (48.9% lower) and *T. harzianum* 2_1 (82.3% lower) compared to control in Area 1 ($p = 0.0014$), in the second area as the first, all treatments exhibited lower incidence than control ($p < 0.001$), where *T. harzianum* 1, (94% lower); *T. harzianum* 2, (84.9% lower); *T. harzianum* 1_ 2, (84.9% lower); and *T. harzianum* 2_1 (58.5% lower) incidence for each.

The germination of the sclerotia (Figure 2) collected from the field was high, but it was reduced by the spray of the BCAs for both areas. In area 1 the treatments reduced the germination by more than 75%: *T. harzianum* 1, (94.1%); *T. harzianum* 2, (93.8%); *T. harzianum* 1_ 2, (87.6%); and *T. harzianum* 2_1 (81.6%). Area 2, *T. harzianum* 1, (86.6%); *T. harzianum* 2, (95%); *T. harzianum* 1_ 2, (76.7%); and *T. harzianum* 2_1 (83.3%) respectively. The parasitism (Figure 2) on the other hand occurred only in treatments *T. harzianum* 1 and *T. harzianum* 1_2, but at a low percentage.

Sclerotia produced in the laboratory (Figure 3) on the other hand presented low germination and low parasitism. For both areas, the highest percentage of germination occurred in the control, but it did not exceed 23%, also there was germination under the treatment *T. harzianum* 1_2 for both areas, and in area 1 *T. harzianum* 1. The parasitism of these sclerotia occurred at treatments *T. harzianum* 1, *T. harzianum* 1_2, and *T. harzianum* 2_1, where the highest parasitism was in area 2 under the treatment *T. harzianum* 1 with 12.5% parasitism.

The number of apothecium (Figure 4), for both areas and both (sclerotia collected from the field and produced in the laboratory), the treatments with BCAs, were better than the control

one, and statistically differed from it. Where area 1 ($p < 0.001$); area 2 ($p < 0.001$) for sclerotia from the field; area 1 ($p < 0.001$) and, area 2 ($p < 0.001$) for sclerotia from the laboratory. Additionally, the number of apothecia produced by the sclerotia collected from the field was 73% higher than the number produced by the laboratory sclerotia, which means 11 more sclerotia.

3.2 Crop parameters

The crop parameters obtained after the harvest were: pod number, a thousand grans weight, and yield. For the pod number, there was no significant difference between the treatments for area 1 ($p = 0.1645$) and area 2 ($p = 0.3624$).

A thousand grains weight on the other hand (Figure 5), in area 1 there was no significant difference between the treatments ($p = 0.1432$), but in area 2 *T. harzianum* 1 showed higher weight than the control and *T. harzianum* 2 ($p = 0.0134$), the other treatments were ambiguous.

Finally, for the yield, the BCA *T. harzianum* 2_1 had a significant increase in the yield compared to *T. harzianum* 1_2, which for instance was better than the control, along with *T. harzianum* 1 and *T. harzianum* 2 for area 1 ($p = <0.001$); these numbers mean an increase of: *T. harzianum* 1, (21.13%); *T. harzianum* 2, (26.1%); *T. harzianum* 1_ 2, (19.11%); and *T. harzianum* 2_1 (81.6%), where the BCA *T. harzianum* 2_1 produced 24 bags more than control. For area 2 we had significant difference between the treatments and the control, where statistically *T. harzianum* 1 as the highest yield, followed by *T.harzianum* 2 and *T. harzianum* 1_2, and the lowest, the control. These numbers mean a increase on the yield of: *T. harzianum* 1, (34,1%); *T. harzianum* 2, (20,81%); *T. harzianum* 1_ 2, (22,03%); and *T. harzianum* 2_1 (31,97%), where the BCA *T. harzianum* 1 produced 29 bags more than control

4. Discussion

Sclerotinia sclerotiorum the causal agent of white mold disease, is a moisture and temperature-dependent pathogen. Its optimal carpogenic germination rates occur under 10-20°C, and high moisture conditions (Wu and Subbarao, 2008), which is the stage responsible for causing an epidemic in crops (Purdy, 1979). In the present work, the rates of carpogenic germination of the sclerotia collected from the field for the control was higher than the treatment ones (Figure 4); on the other hand, there was not much difference between the carpogenic germination of the laboratory sclerotia (Figure 6). According to INMET - Instituto Nacional de Meteorologia, (2024), the data collected from Lavras weather station, the average minimum temperature from October/22 to February/23 was 17 degrees, the optimum temperature for apothecia germination, where the sclerotia from the field were previously under this temperature, and the laboratory ones were not. The superior germination rate might be due to the accumulation of the formation of the sclerotia collected from the field under optimum temperature, and its adaptation to the local environment (Huang and Kozub, 1991).

The sclerotia parasitism thought (Figures 4 and 6), did show a parasitism percentage for treatments BCA 1 and BCA 1_BCA 2, in both sclerotia collected from the field and produced in lab. The main mechanism of action of Trichoderma against Sclerotinia sclerotiorum is hyperparasitism through hyphal penetration (Montalvão et al., 2023) and the production of chitinolytic enzymes (Guilger-Casagrande et al., 2019), which was not showed in all treatments and did not follow the yield pattern (Figure 1). One alternative mechanism that can be present in this situation is microbial selection, altering the community of the soil (Umadevi et al., 2018); this may lead to a suppressiveness of the soil against the disease (Wang et al., 2019), causing a reduction of the incidence of the disease between the biological treatments and the control (Figure 3).

Since the treatment control was treated with fungicides, the statistical difference between the control and the other treatments indicates a growth-promoting action by Trichoderma; It has the capability to enhance growth in plants by matching direct and indirect mechanisms, including microbial recruitment (Hang et al., 2022).

5. Conclusions

This work allows us to conclude: sclerotia collected from the field produced more apothecia than the sclerotia produced in laboratory, due to the temperature at which they were formed and the adaptability of the sclerotia from the area; There was no difference in the rotation of the products tested, probably because of their mode of action that works on sclerotia; As parasitism was low, but sclerotia unviability was high the application of *Trichoderma* in conditions not favorable for it, probably provoked a microbial recruiting that acted over the sclerotia and avoided the sclerotia germination.

6. Acknowledgments

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7. Legends and figures

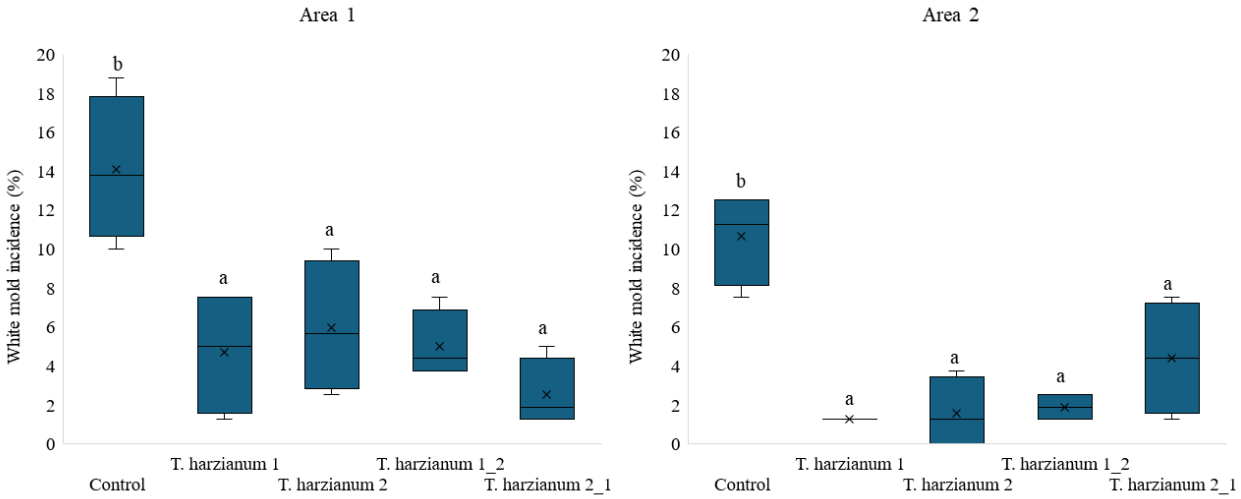

Figure 1. White mold incidence (%), taken from 80 plants per plot, considering the presence or absence. Where: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma* harzianum (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich. *Averages followed by the same letter are not statistically different by the Tukey test (p <= 0.05).

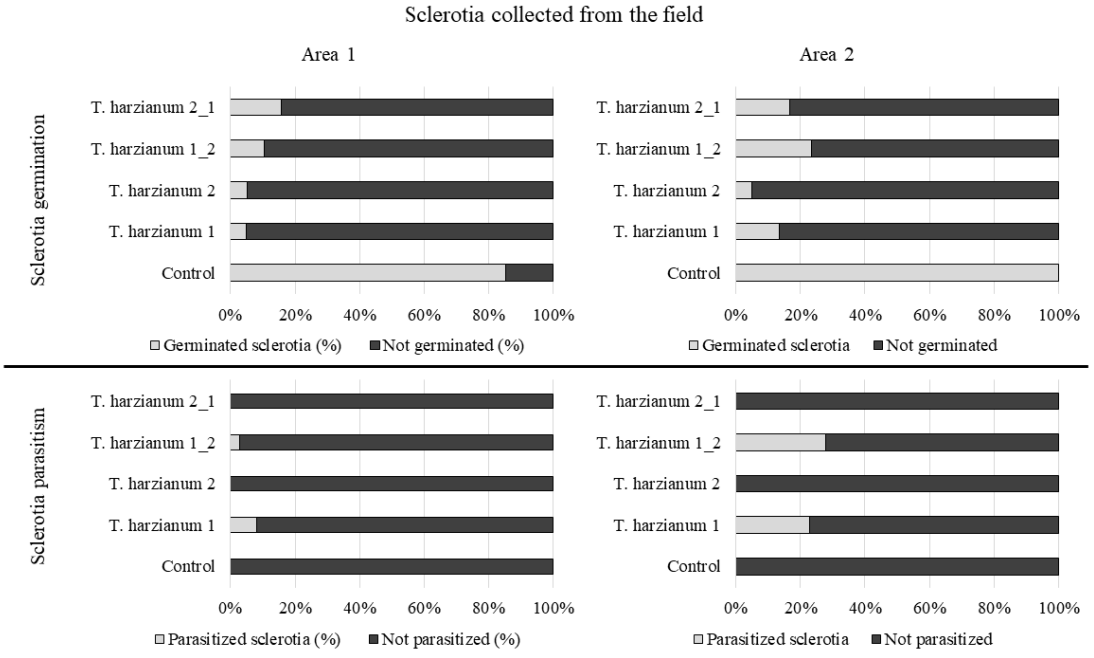

Figure 2. Germination (%) and parasitism (%) of sclerotia collected from the field. Where: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma* harzianum (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich.

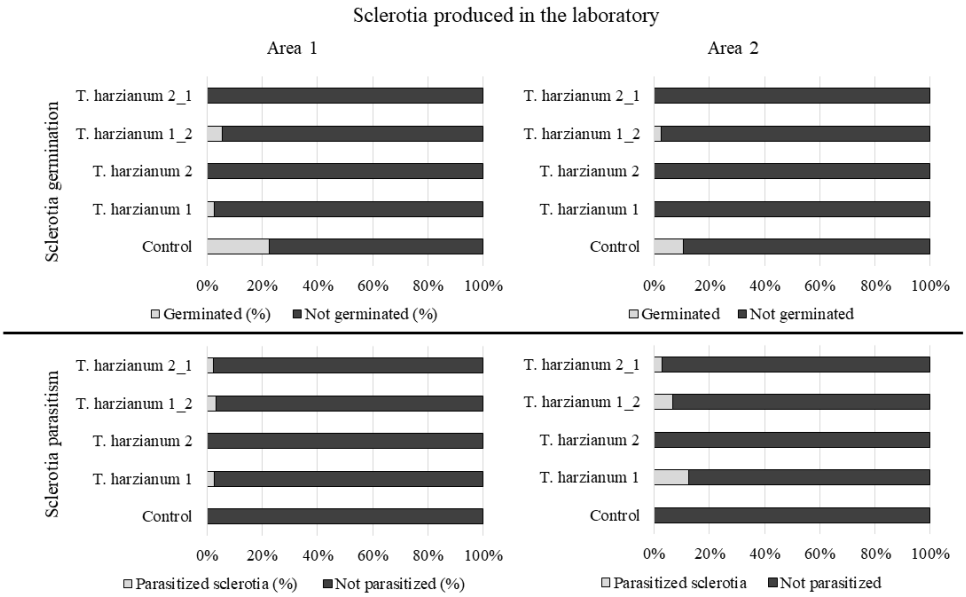

Figure 3. Germination (%) and parasitism (%) of sclerotia produced in the laboratory. Where: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma* harzianum (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich.

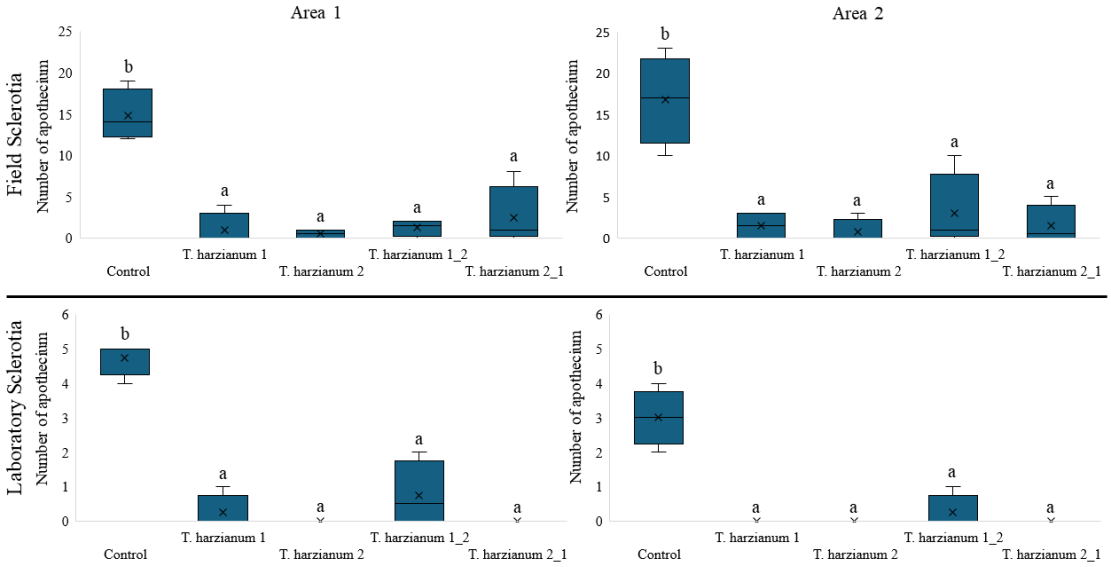

Figure 4. Number of apothecia grown from the sclerotia collected from the field and produced in the laboratory. Where: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma* harzianum (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich.

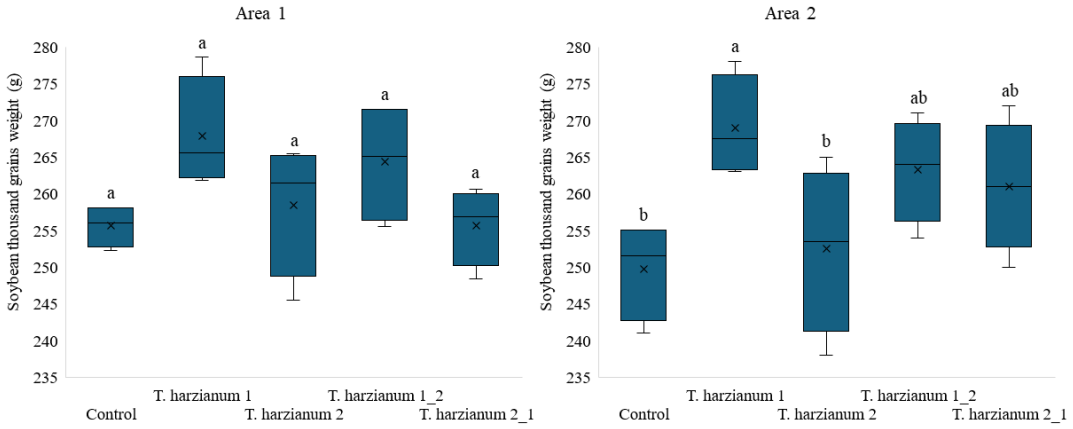

Figure 5. Soybean thousand-grain weight in grams, collected from 3 plants per plot. Where: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma* harzianum (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich.

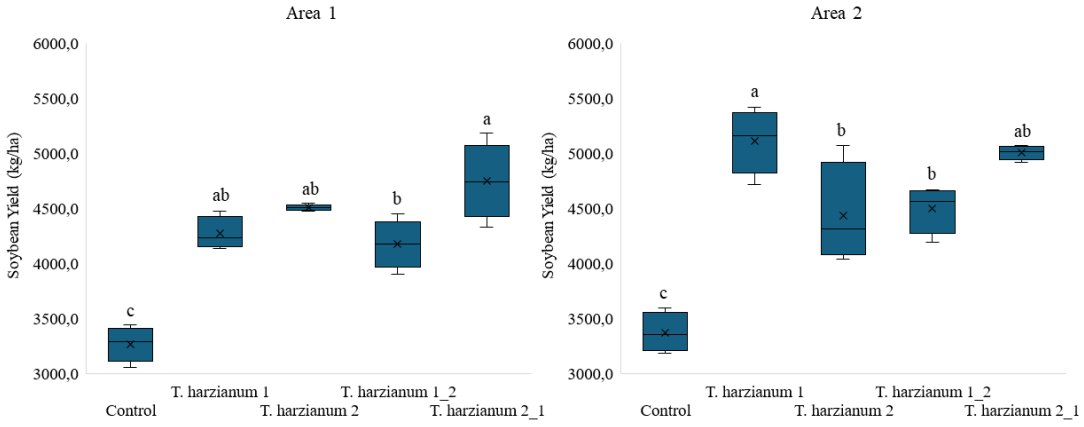

Figure 6. Soybean yield, measured in soybean harvest. Where: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma* harzianum (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich.

BIBLIOGRAPHY

AGROFIT-Sistema de agrotóxicos fitossanitários. 2024. https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed February 13, 2024).

Adetunji, C. O., & Varma, A. (2020). Biotechnological application of trichoderma: A powerful fungal isolate with diverse potentials for the attainment of food safety, management of pest and diseases, healthy planet, and sustainable agriculture. Trichoderma: Agricultural Applications and Beyond, 257-285.

Bennett, A. J., Bending, G. D., Chandler, D., Hilton, S., & Mills, P. (2012). Meeting the demand for crop production: the challenge of yield decline in crops grown in short rotations. Biological reviews, 87(1), 52-71.

Boland GJ, Hall R. Index of plant hosts of sclerotinia sclerotiorum. Canadian Journal of Plant Pathology 1994;16:93–108. https://doi.org/10.1080/07060669409500766.

Bortolotto, O. C., Pomari-Fernandes, A., de Freitas Bueno, R. C. O., de Freitas Bueno, A., da Cruz, Y. K., Sanzovo, A., & Ferreira, R. B. (2015). The use of soybean integrated pest management in Brazil: a review. Agronomy Science and Biotechnology, 1(1), 25-25.

BUENO, A. D. F., Panizzi, A. R., Hunt, T. E., Dourado, P. M., Pitta, R. M., & Gonçalves, J. (2021). Challenges for adoption of integrated pest management (IPM): the soybean example. Neotropical Entomology, 50, 5-20.

Canuto da Silva, L., de Faria, A. F., Guimarães, R. A., Afridi, M. S., de Medeiros, F. H. V., & de Medeiros, F. C. L. (2024). How can an in vitro incompatibility of Trichoderma-based products and herbicides impact the parasitism and control of white mold (Sclerotinia sclerotiorum (Lib.) De Bary)?. Crop Health, 2(1), 1-16.

Conte, E. D., Dal Magro, T., Dal Bem, L. C., Dalmina, J. C., Matté, J. A., Schenkel, V. O., & Schwambach, J. (2022). Use of Trichoderma spp. in no-tillage system: Effect on soil and soybean crop. Biological Control, 171, 104941.

Dutta, P., Deb, L., & Pandey, A. K. (2022). Trichoderma-from lab bench to field application: Looking back over 50 years. Frontiers in Agronomy, 4, 932839.

Emidio Rizzo Bonato, Ana Lidia Variani Bonato. A SOJA NO BRASIL: História e Estatística. Londrina: 1987.

Faria AF de, Schulman P, Conrado Meyer M, Diniz Campos H, Cruz-Magalhães V, Vieira Godoy C, et al. Seven years of white mold biocontrol product's performance efficacy

on Sclerotinia sclerotiorum carpogenic germination in Brazil: a meta-analysis. 2022. https://doi.org/https://dx.doi.org/10.2139/ssrn.4120095.

Guilger-Casagrande M, Germano-Costa T, Pasquoto-Stigliani T, Fraceto LF, Lima R de. Biosynthesis of silver nanoparticles employing Trichoderma harzianum with enzymatic stimulation for the control of Sclerotinia sclerotiorum. Sci Rep 2019;9. https://doi.org/10.1038/s41598-019-50871-0.

Hang X, Meng L, Ou Y, Shao C, Xiong W, Zhang N, et al. Trichoderma-amended biofertilizer stimulates soil resident Aspergillus population for joint plant growth promotion. NPJ Biofilms Microbiomes 2022;8. https://doi.org/10.1038/s41522-022-00321-z.

HUANG HC, KOZUB GC. Temperature requirements for carpogenic germination of sclerotia of Sclerotinia sclerotiorum isolates of different geographic origin. Botanical Bulletin of Academia Sinica 1991;32:279–86.

INMET - Instituto Nacional de Meteorologia. 2024. https://portal.inmet.gov.br/ (accessed February 14, 2024).

Juliatti F, A. Rezende A, Cezar Marinho Juliatti B, P. Morais T. Trichoderma as a Biocontrol Agent against Sclerotinia Stem Rot or White Mold on Soybeans in Brazil: Usage and Technology . Trichoderma - The Most Widely Used Fungicide, IntechOpen; 2019. https://doi.org/10.5772/intechopen.84544.

Kandel R, Chen CY, Grau CR, Dorrance AE, Liu JQ, Wang Y, et al. Soybean resistance to white mold: Evaluation of soybean germplasm under different conditions and validation of QTL. Front Plant Sci 2018;9. https://doi.org/10.3389/fpls.2018.00505.

Macena, A. M., Kobori, N. N., Mascarin, G. M., Vida, J. B., & Hartman, G. L. (2020). Antagonism of Trichoderma-based biofungicides against Brazilian and North American isolates of Sclerotinia sclerotiorum and growth promotion of soybean. BioControl, 65, 235-246.

Meyer MC, Campos HD, Godoy C V., Utimada C. M. Ensaios cooperativos de controle biológico de mofo-branco na cultura da soja-safras 2012 a 2015 2016.

Montalvão SCL, Marques E, Martins I, da Silva JP, de Mello SCM. Suppression of the phytopathogens Sclerotinia sclerotiorum and Sclerotium rolfsii by Trichoderma spp. Biologia (Bratisl) 2023. https://doi.org/10.1007/s11756-023-01457-9.

O'Sullivan, C. A., Belt, K., & Thatcher, L. F. (2021). Tackling control of a cosmopolitan phytopathogen: Sclerotinia. Frontiers in Plant Science, 12, 707509.

Pethybridge, S. J., Gugino, B. K., & Kikkert, J. R. (2019). Optimizing fungicide timing for the management of white mold in processing snap bean in New York. Crop protection, 125, 104883.

Purdy LH. Sclerotinia sclerotiorum: History, Diseases and Symptomatology, Host Range, Geographic Distribution, and Impact. Gainesville: 1979.

Rahman MME, Hossain DM, Suzuki Kazuki, Shiiya A, Suzuki Kazushi, Dey TK, et al. Suppressive effects of Bacillus spp. on mycelia, apothecia and sclerotia formation of Sclerotinia sclerotiorum and potential as biological control of white mold on mustard. Australasian Plant Pathology 2016;45:103–17. https://doi.org/10.1007/s13313-016-0397-4.

Sumida CH, Canteri MG, Peitl DC, Tibolla F, Orsini IP, Araújo FA, et al. Controle químico e biológico da podridão branca da haste na cultura da soja. Ciencia Rural 2015;45:760–6. https://doi.org/10.1590/0103-8478cr20140198.

Umadevi P, Anandaraj M, Srivastav V, Benjamin S. Trichoderma harzianum MTCC 5179 impacts the population and functional dynamics of microbial community in the rhizosphere of black pepper (Piper nigrum L.). Brazilian Journal of Microbiology 2018;49:463–70. https://doi.org/10.1016/j.bjm.2017.05.011.

Wang Z, Li Y, Zhuang L, Yu Y, Liu J, Zhang L, et al. A Rhizosphere-Derived Consortium of Bacillus subtilis and Trichoderma harzianum Suppresses Common Scab of Potato and Increases Yield. Comput Struct Biotechnol J 2019;17:645–53. https://doi.org/10.1016/j.csbj.2019.05.003.

Willbur, J., McCaghey, M., Kabbage, M., & Smith, D. L. (2019). An overview of the Sclerotinia sclerotiorum pathosystem in soybean: impact, fungal biology, and current management strategies. Tropical Plant Pathology, 44, 3-11.

Wu BM, Subbarao K V. Effects of soil temperature, moisture, and burial depths on carpogenic germination of Sclerotinia sclerotiorum and S. minor. Phytopathology 2008;98:1144–52. https://doi.org/10.1094/PHYTO-98-10-1144.