

Impact of *Trichoderma*-based products on *Sclerotinia sclerotiorum*¹

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ABSTRACT

Trichoderma-based products are deployed on a large scale in regions where white mold is important. However, little is known about the contribution of alternating these products on sclerotia parasitism, disease incidence and plant yield. This study aimed to apply *Trichoderma*-based products sprayed alone or alternately, at the V2 and V4 phenological stages, in order to evaluate their effectiveness in controlling white mold in soybean. The treatments were: control (water); *Trichoderma harzianum* IBLF 006; *T. harzianum* BK-Th001; *T. harzianum* IBLF 006 followed by *T. harzianum* BK-Th001; and *T. harzianum* BK-Th001 followed by *T. harzianum* IBLF 006. Two field trials were conducted in different locations. Yield-related variables (thousand-grain weight and number of pods), grain yield, and white-mold-related variables (disease incidence; sclerotium parasitism, germination and viability; and number of apothecia) were evaluated on sclerotia originated from the field and produced in the laboratory. All the treatments with biocontrol agents reduced the incidence of white mold, when compared to the control, whereas the *Trichoderma* spray reduced the sclerotium germination. The sclerotia origin (laboratory or field) affected their ability to produce apothecia and cause the disease. The application of these biological control agents, regardless of sequence, reduced the sclerotia viability and disease incidence, and improved the yield.

KEYWORDS: *Trichoderma harzianum*, biological control agents, white mold, carpogenic germination.

RESUMO

Impacto de produtos à base de *Trichoderma* no manejo de *Sclerotinia sclerotiorum*

Produtos à base de *Trichoderma* são amplamente utilizados em regiões onde o mofo branco é relevante. No entanto, pouco se conhece sobre a contribuição da alternância entre esses produtos para o parasitismo de escleródios, incidência da doença e produtividade das plantas. Objetivou-se aplicar produtos à base de *Trichoderma* isoladamente ou em alternância, nos estádios fenológicos V2 e V4, a fim de avaliar sua eficácia no controle de mofo branco em soja. Os tratamentos foram: testemunha (água); *Trichoderma harzianum* IBLF 006; *T. harzianum* BK-Th001; *T. harzianum* IBLF 006 seguido de *T. harzianum* BK-Th001; e *T. harzianum* BK-Th001 seguido de *T. harzianum* IBLF 006. Dois experimentos de campo foram conduzidos em locais distintos. Avaliaram-se variáveis relacionadas à produtividade (massa de mil grãos e número de vagens), produtividade de grãos e variáveis relacionadas ao mofo branco (incidência da doença; parasitismo, germinação e viabilidade dos escleródios; e número de apotécios) em escleródios oriundos do campo e produzidos em laboratório. Todos os tratamentos com agentes de biocontrole reduziram a incidência do mofo branco, em comparação à testemunha, enquanto a pulverização com *Trichoderma* reduziu a germinação dos escleródios. A origem dos escleródios (laboratório ou campo) influenciou sua capacidade de produzir apotécios e causar a doença. A aplicação dos agentes de biocontrole, independentemente da sequência, reduziu a viabilidade dos escleródios e a incidência da doença e melhorou a produtividade.

PALAVRAS-CHAVE: *Trichoderma harzianum*, agentes de controle biológico, mofo branco, germinação carpogênica.

INTRODUCTION

Brazil is currently the leading global producer of soybean (USDA 2024). However, the expansion of the planted area has increased the need for integrated management methods to control diseases, weeds and insects (Bortolotto et al. 2015, Bueno et al. 2021).

Although higher yields may increase input costs, they can also cause quantitative and qualitative losses due to damage from biotic agents, such as plant diseases (Bennett et al. 2012).

A significant fungal pathogen that affects soybean is *Sclerotinia sclerotiorum* Lib de Bary (Willbur et al. 2019, Macena et al. 2020). This fungus

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infects plants through carpogenic or myceliogenic germination, leading to considerable losses in Brazil from an altitude of 600 m (Boland & Hall 1994, Meyer et al. 2016). Without control measures, yield losses may reach 70 % (Faria et al. 2022).

Managing white mold requires both chemical and biological control methods, because no soybean cultivar is resistant to the disease, although susceptibility may vary (Sumida et al. 2015, Kandel et al. 2018). The main commercially available active ingredients in Brazil include fluazinam, methyl thiophanate, procymidone and carbendazim (Meyer et al. 2021). These fungicides are typically applied during the flowering stages (R1 and R2), with a 10-day interval between sprays (Lehner et al. 2017). However, these chemical controls primarily protect the flowers and do not effectively reduce the initial inoculum, namely the sclerotia already present in the area, which multiply from season to season (Garg et al. 2010, Peltier et al. 2012, Young & Werner 2012). Hence, the recommended chemical fungicide treatment is not sufficient to achieve the highest yield under conditions favorable to a disease epidemic. Therefore, it is imperative to improve the management techniques to reduce the inoculum and standardize the possible modes of action that could inhibit germination in the field by using biological control agents.

Several models using biological control agents rely on the mycoparasitic activity of overwintering structures such as sclerotia (Haddad et al. 2017, Rembinski et al. 2022, Silva et al. 2022a). These models range from field observations of the biological control agents activity and their colonization of sclerotia to controlled laboratory tests that assess the effects on germination and viability (Sumida et al. 2018, Silva et al. 2022b). Another important consideration concerns the conditions that lead to sclerotium germination - related to the source of initial inoculum and the pathogen population - as it can significantly influence the dynamics of the mycoparasitic processes associated with biological control agents, potentially varying from strong to weak inoculum sources (Foley et al. 2016, Taylor et al. 2018).

Sclerotia can be produced in the laboratory or collected from the field, although they develop under different conditions. Understanding these processes - both the epidemiology of the initial inoculum source and its interactions with the mycoparasitic activity

of biological control agents - is crucial for validating more efficient and promising methods to reduce the severity of white mold in the field.

Various biological control agents can reduce the losses caused by white mold in soybean, namely producing cell-wall-degrading enzymes, parasitizing sclerotia, inducing systemic defense responses, promoting plant growth and recruiting other beneficial organisms. Numerous studies have highlighted these benefits for *Trichoderma* spp. (Sumida et al. 2018, Macena et al. 2020, Silva et al. 2021, Singh et al. 2021), although there is limited knowledge regarding the use of different biological control agents and their synergistic effects. Additionally, using different types of beneficial microorganisms, even if they belong to the same species, may help to improve agricultural production and reduce the effects of diseases caused by the initial inoculum. These microorganisms might work together in ways that enhance plant health and increase yield.

To reach the sclerotia, it is usually recommended to spray biological control agents during the V2 (second node, totally developed trifoliolate leaf) and V4 (fourth node, totally developed trifoliolate leaf) phenological stages of a plant to characterize sclerotium viability (Meyer et al. 2016) or following soybean desiccation (Conte et al. 2022). At these phenological stages, the plant canopy is not fully developed, allowing the mycoparasite to reach the sclerotia on the soil (O'Sullivan et al. 2021). This application timing coincides with that of post-emergent herbicides (Silva et al. 2024), thus reducing production costs while employing a user-friendly approach by incorporating *Trichoderma*-based products into the agroecosystem (Adetunji & Varma 2020, Dutta et al. 2022).

Although the efficacy of *Trichoderma* sp. in the management of white mold has already been proven, similarly to what has been postulated for chemical control (Corkley et al. 2025), to the best of our knowledge, the role of alternating biological control agents on sclerotium parasitism, the incidence of white mold and plant yield have not been evaluated yet. Thus, this study aimed to evaluate the effects of applying two formulations of *Trichoderma harzianum*, sprayed alone or alternately, at the V2 and V4 phenological stages, on sclerotium parasitism, the incidence of white mold disease at R6 and crop yield parameters (i.e., thousand-grain weight, number of pods and yield), as well as the contribution of

the sclerotium origin (field and laboratory) for the product performance.

MATERIAL AND METHODS

The *in vivo* experiment was conducted using *Trichoderma* sp. to control *Sclerotinia sclerotiorum* in two locations. These sites were chosen due to the confirmed prevalence of sclerotia with carpogenic germination before planting (Faria et al. 2022), where the average viability of the sclerotia was over 80 % in both areas. The Area 1 is located in Nepomuceno (21°19'08.9"S, 45°06'41.1"W and altitude of 964 m) and the Area 2 in Três Corações (21°34'41.6"S, 45°08'29.9"W and altitude of 972 m), both in the Minas Gerais state, Brazil.

The used cultivar was Brasmax Zeus IPRO, and sowing occurred on October 11, 2022, at the Area 1, and on October 15, 2022, at the Area 2, both managed under no-tillage with grass stubble. Planting was performed with 14 seeds m⁻¹, with row spacing of 0.5 m. The plots consisted of five treatments and four blocks, with each plot having an effective area of 18 m² (six lines spaced 0.5 m apart and with length of 6 m). The treatments were: control (water); *T. harzianum* 1 (*T. harzianum* IBLF 006, Ecotrich; 250 g ha⁻¹); *T. harzianum* 2 (*T. harzianum* BK-Th001, Natucontrol; 800 g ha⁻¹); *T. harzianum* 1_ *T. harzianum* 2 (Ecotrich followed by Natucontrol); and *T. harzianum* 2_ *T. harzianum* 1 (Natucontrol followed by Ecotrich). Each product was applied according to the product label for the biological target *S. sclerotiorum*. The applications took place at the phenological stages V2 (second node, totally developed trifoliolate leaf) and V4 (fourth node, totally developed trifoliolate leaf) (Fehr & Caviness 1977). A CO₂ pressurized pump was used to spray the products, aiming at the middle of the lines, and, consequently, the sclerotia present in the soil and the stubble. To isolate the effect of only *Trichoderma* in the system, Procimidona (ParrudoBR) was applied at a dose of 1 L ha⁻¹, to control *S. sclerotiorum* ascospore infection starting at R1 (beginning of flowering with the appearance of an open flower at any node of the main stem), and the application was repeated three more times with a 14-day interval between each treatment. To tackle foliar diseases, the products Carbendazim STK 500 SC-B (500 mL ha⁻¹) and Tebuconazole CCAB 200 EC (0.75 L ha⁻¹) were also sprayed for all treatments at

V2 and V4. Glyphosate (Roundup WG), at the dose of 2 kg ha⁻¹, was applied at V2, and thiamethoxam + lambda-cyhalothrin (Engeo Pleno S), at the dose of 200 mL ha⁻¹, was applied to control insects when necessary.

To assess the disease incidence, a total of 80 plants were checked for the presence of any white mold symptoms per plot from the central planting rows (the four rows in the middle) at R6. Yield (kg ha⁻¹) was calculated by determining the weight of the grains (g) collected from the main four lines of the center minus the moisture. Thousand-grain weight (g) was determined by weighting 100 grains eight times and transforming it into the weight of 1,000 grains. Finally, the number of pods per plant and the number of grains per pod were counted.

A test was conducted to assess the parasitism capacity of the fungi *T. harzianum* IBLF 006 and BK-Th001 on *S. sclerotiorum*. The experiment initially took place in the field, where sclerotia were collected from the area, as well as with sclerotia produced in the laboratory. The sclerotia were 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, where they were laid individually over an acrylic box (20 × 20 × 5 cm) with a lid, filled with autoclaved sand, and incubated in a growth chamber at 17 °C, to stimulate carpogenic germination. Each week, the number of apothecia on each sclerotium was counted until 60 days after the experiment was set up, to estimate the germination and number of apothecia per sclerotium (Meyer et al. 2016). The experiment was conducted in a randomized block design (n = 4).

R 2023.06.1 Build 524 (R Core Team 2022) was used for data analysis. The data were submitted to the Shapiro-Wilk normality test (p > 0.05), Bartlett's homogeneity test (p > 0.05) and Dixon's test (p ≤ 0.05). The Tukey test (using the "easynova" package in R) was used to compare means (p ≤ 0.05).

RESULTS AND DISCUSSION

The biological control agent treatments had a significant effect on all considered variables. They

reduced the incidence of white mold by 48.9-94 % ($p = 0.001$; Figure 1); however, there was no difference between the *Trichoderma* treatments.

The incidence of white mold is determined by multiple factors, primarily moisture and temperature. The optimal conditions for carpogenic germination occur under high moisture levels and temperatures ranging from 10 to 20 °C (Wu & Subbarao 2008). This stage is crucial for causing epidemics in crops (Purdy 1979). The average minimum temperature recorded at the Lavras weather station from October

2022 to February 2023 was 17 °C (Brasil 2024). This temperature is conducive to apothecium germination, which increases the incidence of white mold, especially in the absence of biological control agents, regardless of whether they are in crop rotation.

Table 1 shows the germination and parasitism of sclerotia (field and laboratory) in the Areas 1 and 2. In both areas, the biological control agents significantly reduced the germination ($p < 0.001$) of the sclerotia collected from the field and laboratory, when compared with the control group. In the

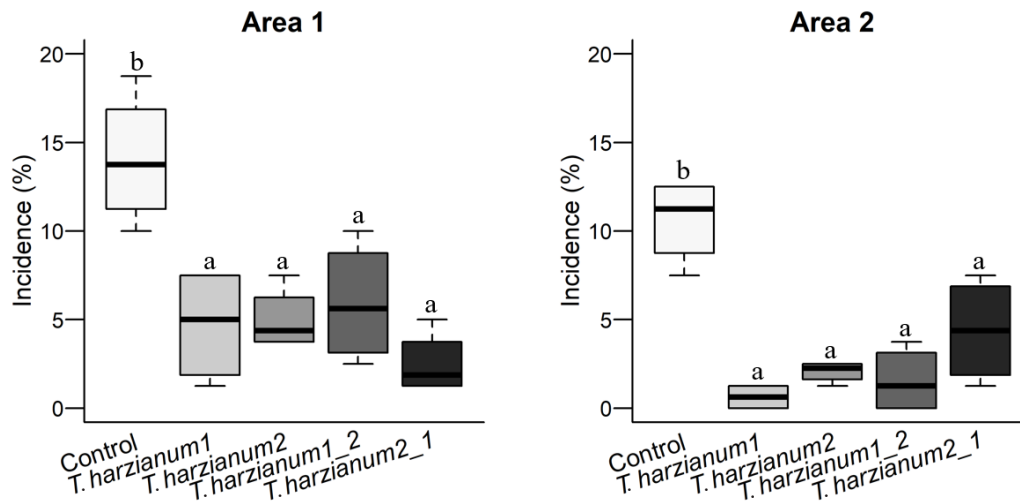


Figure 1. White mold incidence (%), considering the presence or absence of white mold symptoms, regardless of its severity ($n = 4$; 80 plants plot⁻¹), in two field trials (Areas 1 and 2), according to different treatments: control (water); *Trichoderma harzianum* 1 (*T. harzianum* IBLF 006, Ecotrich, Ballagro); *T. harzianum* 2 (*T. harzianum*, Natucontrol, Biotrop); *T. harzianum* 1_2 (Ecotrich_Natucontrol); and *T. harzianum* 2_1 (Natucontrol_Ecotrich). Means followed by the same letter are not significantly different (Tukey test; $p \leq 0.05$).

Table 1. Rates of germination and parasitism of the sclerotia after 60 days.

Experimental site	Treatments	Germination (%)		Parasitism (%)	
		Field	Lab	Field	Lab
Area 1	Control	85.27 a*	22.42 a	8.10 a	0.00 a
	<i>T. harzianum</i> 1	5.075 b	5.62 b	2.85 a	2.50 a
	<i>T. harzianum</i> 2	5.32 b	2.50 b	0.00 a	0.00 a
	<i>T. harzianum</i> 1_2	10.58 b	0.00 b	0.00 a	3.12 a
	<i>T. harzianum</i> 2_1	15.65 b	0.00 b	0.00 a	2.27 a
	P-value	< 0.001	< 0.001	0.2471	0.7212
Area 2	Control	100 a	10.55 a	0.00 b	0.00 a
	<i>T. harzianum</i> 1	13.33 b	0.00 b	22.91 a	12.50 a
	<i>T. harzianum</i> 2	5.00 b	0.00 b	0.00 b	0.00 a
	<i>T. harzianum</i> 1_2	24.79 b	2.50 b	25.41 a	6.81 a
	<i>T. harzianum</i> 2_1	16.66 b	0.00 b	0.00 b	2.77 a
	P-value	< 0.001	< 0.001	0.0032	0.1905

Control: water; *Trichoderma harzianum* 1: *T. harzianum* IBLF 006, Ecotrich, Ballagro; *T. harzianum* 2: *T. harzianum*, Natucontrol, Biotrop; *T. harzianum* 1_2: Ecotrich_Natucontrol; *T. harzianum* 2_1: Natucontrol_Ecotrich. Germination: all the sclerotia that produced apothecium; parasitism: all the parasitized sclerotia. * Means followed by the same letter are not significantly different (Tukey test; $p \leq 0.05$).

Area 1, the biological control agents did not affect the parasitism of the field ($p = 0.247$) and laboratory ($p = 0.7212$) sclerotia. In the Area 2, *T. harzianum* 1 and *T. harzianum* 1_2 resulted in parasitism of the sclerotia collected from the field. In contrast, the laboratory sclerotia did not show significant parasitism ($p = 0.1905$).

Based on the data, the sclerotia from the field had a higher germination rate than those from the laboratory, what may be attributed to the adaptation to the local environment, including to the temperature (Huang & Kozub 1991). Although all the biological control agent treatments reduced the carpogenic germination, the *T. harzianum* strain was seldom recovered from the sclerotia, while other microbes were frequently observed. This indicates that microbial succession may have occurred. Initially,

T. harzianum may have reduced the pathogen viability, but it did not persist on the sclerotia, allowing saprophytic communities to take over the sclerotia debris. Then, a microbial community with higher mycoparasitic relationship may have taken over (Sharma et al. 2020). An alternative mechanism that might have occurred is microbial selection, which alters the soil community (Umadevi et al. 2018). Microbial succession may contribute to the ability of the soil to suppress diseases (Wang et al. 2019) and thus reduce the incidence of white mold among the biological control agent treatments, if compared to the control group (Figure 1).

The number of apothecia (Figure 2) was reduced in the Areas 1 and 2 ($p < 0.001$), regardless of the sclerotia origin, after the biological control agent treatments, when compared to the control.

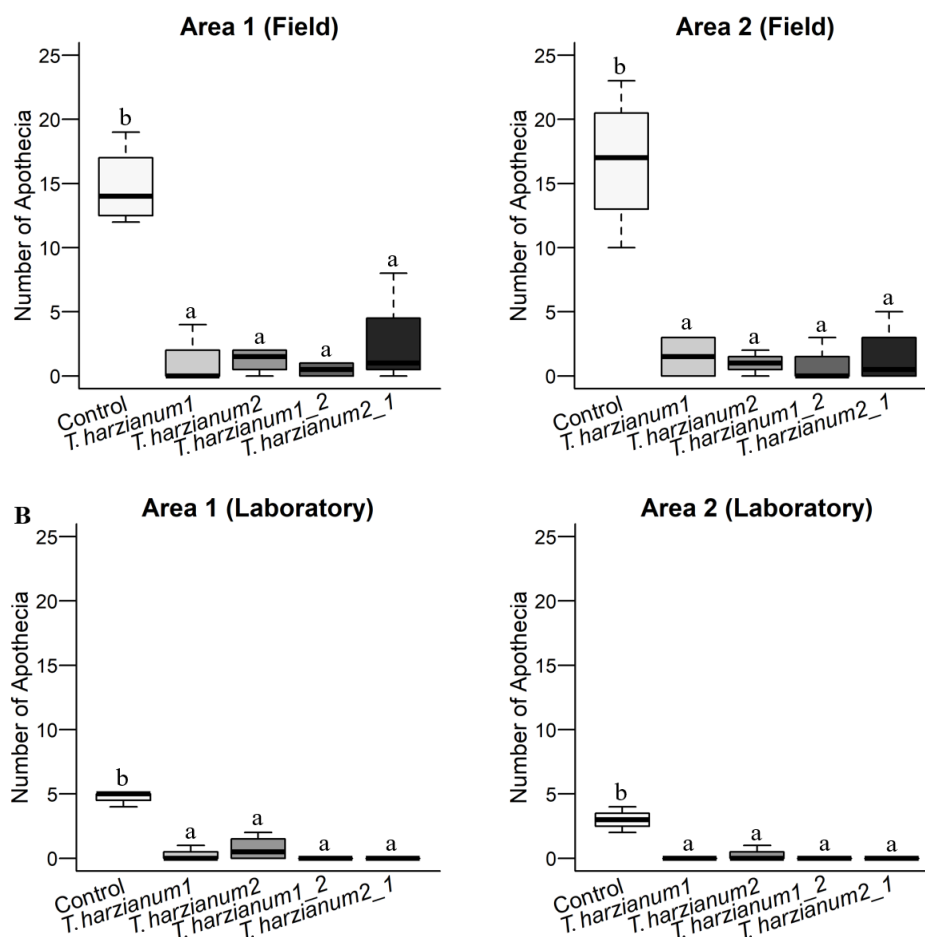


Figure 2. Number of apothecia produced from sclerotia originated from a population obtained in the field (A) or produced in the laboratory (B), in field trials carried out in the Areas 1 and 2. Control: water; *Trichoderma harzianum* 1: *T. harzianum* IBLF 006, Ecotrich, Ballagro; *T. harzianum* 2: *T. harzianum*, Natucontrol, Biotrop; *T. harzianum* 1_2: Ecotrich_Natucontrol; *T. harzianum* 2_1: Natucontrol_Ecotrich. Means ($n = 4$) followed by the same letter are not significantly different (Tukey test; $p \leq 0.05$).

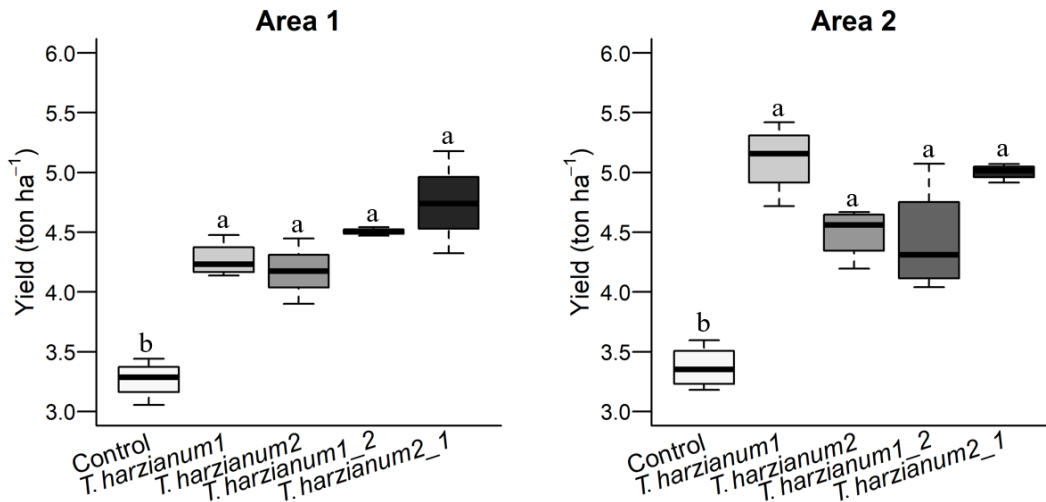


Figure 3. Soybean yield in two field trials (Areas 1 and 2), according to *Trichoderma*-based treatments. Control: water; *Trichoderma harzianum* 1: *T. harzianum* IBLF 006, Ecotrich, Ballagro; *T. harzianum* 2: *T. harzianum*, Natucontrol, Biotrop; *T. harzianum* 1_2: Ecotrich_Natucontrol; *T. harzianum* 2_1: Natucontrol_Ecotrich. Means (n = 4) followed by the same letter are not significantly different (Tukey test; $p \leq 0.05$).

Additionally, the number of apothecia produced by the sclerotia collected from the field was 73 % higher than that produced by the sclerotia from the laboratory. This finding supports the view that field-collected sclerotia demonstrate a greater ability to develop white mold, when compared with sclerotia that originate from the laboratory.

As shown in Figure 3, the *T. harzianum* 1 and *T. harzianum* 2 treatments increased the yield, when compared with the control group. For the Area 1 ($p < 0.001$), the yield increase ranged from 19.11 to 81.6 %, with an increase of up to 21 60-kg bags per hectare, in relation to the control. In the Area 2, there was also a significant effect ($p < 0.001$) between the treatments and the control, with *T. harzianum* 1 (34.1 %) promoting the highest yield; an increase of 27 60-kg bags, if compared to the control.

While a fungicide has a singular role (to protect plants against *S. sclerotiorum* ascospore infection), biological control agents can have a dual role and also act as a biostimulant (Nieto-Jacobo et al. 2017). Notably, *T. harzianum* possesses growth-promoting properties that enhance plant growth through both direct and indirect mechanisms, such as microbial recruitment and improved yield (Hang et al. 2022). *Trichoderma* spp. produces extracellular cell-wall-degrading enzymes such as β -1,3-glucanase, chitinases and proteases (Vázquez-Garcidueñas et al. 1998), which act synergistically with the physical penetration of sclerotia on the reduction of the

pathogen's overwintering structure (Geraldine et al. 2013). Furthermore, *Trichoderma* spp. solubilizes phosphorus, making this nutrient more available to plants and consequently reinforcing their growth and increased yield (Bononi et al. 2020, Galeano et al. 2025). Therefore, the increased yield can be a combination of both disease biocontrol and growth promotion.

Finally, based on the obtained results, it was observed that alternating the application of *T. harzianum*-based products did not affect the incidence of white mold or plant yield. These findings may have a direct implication on the practical adoption of biological control agents, because growers will have more freedom in their choice. Indeed, based on local availability or price constraints, a grower can opt to alternate biological control agents in each application without detrimental effects on the biocontrol performance or plant yield. Furthermore, it is imperative to use the local sclerotia population to assess the contribution of biological control agents on disease management. A laboratory population may not explain such benefits, due to its higher susceptibility to natural parasitism.

CONCLUSIONS

1. Treatments with *Trichoderma harzianum* reduced the incidence of white mold in 48.9-82.3 % (Area 1) and 58.5-94 % (Area 2); however, the

- combined treatments did not reduce the white mold incidence;
2. Sclerotia germination was reduced by *T. harzianum*, whereas non-viability was increased. Parasitism was observed only with the *T. harzianum* 1 (*T. harzianum* IBLF 006, Ecotrich, Ballagro) and *T. harzianum* 1_2 (Ecotrich_Natucontrol) treatments;
 3. The field sclerotia presented a higher germination rate than the laboratory ones;
 4. The affinity of *T. harzianum*, regarding germination, does not change depending on the sclerotia origin (laboratory or field), but the rate changes depending on the origin. Field sclerotia represent a good inoculum for white mold development;
 5. The number of apothecia was affected by the biological control agent treatments: the field sclerotia produced 73 % more apothecia than the laboratory ones;
 6. Applying *T. harzianum* increased the yield by about 27 60-kg bags, when compared to the control.

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Impact of Trichoderma-based products on Sclerotinia sclerotiorum¹

Impacto de produtos à base de Trichoderma no manejo de Sclerotinia sclerotiorum

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