

ARTÍCULO ORIGINAL

Compatibility of chemical fungicides with biological products for the control of soybean leaf blight caused by *Cercospora* sp.**Compatibilidad de fungicidas químicos con productos biológicos para el control del tizón foliar de la soja, causado por *Cercospora* sp.**Laura Elena Sawczuk Emhart¹  & Guillermo Andrés Enciso-Maldonado^{1,2,*} ¹Laboratorio de Fitopatología, Universidad Católica “Nuestra Señora de la Asunción” Unidad Pedagógica Hohenau, Hohenau, Itapúa, Paraguay.²Centro Multidisciplinario de Investigaciones Tecnológicas, Universidad Nacional de Asunción, San Lorenzo, Central, Paraguay.*Autor de correspondencia: guillermo.enciso@uc.edu.py.

Abstract: The increasing use of biological inputs in Paraguayan agriculture has created a need to evaluate their compatibility with chemical fungicides, given the limited available information. This study aimed to determine the compatibility between two commercial products, one based on *Trichoderma viride* and the other on *Bacillus amyloliquefaciens*, and five fungicide premixes used for soybean disease management, applied at different concentrations. The work was conducted under laboratory conditions in Paraguay and comprised three phases: (I) in vitro compatibility assessment between the biological agents and fungicides, (II) evaluation of the effect of compatible combinations on *Cercospora* sp. in fungicide-amended medium, and (III) determination of control efficacy on detached leaves. In Phase I, mancozeb maintained *T. viride* radial growth above 36 mm even at 1000 ppm, whereas Solatenol + prothioconazole and other triazole- and strobilurin-based mixtures completely inhibited *T. viride* growth from 50 ppm onwards. In Phase II, compatible combinations reduced the radial growth of *Cercospora* sp. on PDA from 4.1 cm in the untreated control to ≤ 0.1 cm with mancozeb + *T. viride* and to ≤ 0.2 cm with fungicide + *B. amyloliquefaciens* mixtures at 150–500 ppm, corresponding to inhibition levels $\geq 95\%$. In Phase III, preventive and curative applications of these combinations on detached leaves decreased disease severity from 40.5% in the inoculated control to $\leq 1.3\%$ in treated leaves. These results indicate that mancozeb + *T. viride* and selected fungicide mixtures combined with *B. amyloliquefaciens* can be safely integrated into soybean disease management programs in Paraguay.

Key words: Glycine max, *Trichoderma viride*, *Bacillus amyloliquefaciens*, biological control, chemical control.

Resumen: El aumento en el uso de insumos biológicos en la agricultura paraguaya exige evaluar su compatibilidad con fungicidas químicos. El objetivo de este estudio fue determinar la compatibilidad entre dos productos, uno a base de *Trichoderma viride* y otro de *Bacillus amyloliquefaciens*, y cinco premezclas de fungicidas usados en el manejo de enfermedades de la soja, aplicados a diferentes concentraciones. El trabajo se realizó en laboratorio y constó de tres fases: (I) evaluación in vitro de la compatibilidad entre agentes biológicos y fungicidas, (II) efecto de las combinaciones compatibles sobre *Cercospora* sp. en medio envenenado y (III) eficacia de control en hojas desprendidas. En la Fase I, mancozeb mantuvo el crecimiento radial de *T. viride* por encima de 36 mm incluso a 1000 ppm, mientras que Solatenol + prothioconazole y otras mezclas basadas en triazoles y estrobilurinas inhibieron completamente su crecimiento a partir de 50 ppm. En la Fase II, las combinaciones compatibles redujeron el crecimiento radial de *Cercospora* sp. en PDA de 4,1 cm en el testigo sin fungicida a $\leq 0,1$ cm con mancozeb + *T. viride* y a $\leq 0,2$ cm con fungicida + *B. amyloliquefaciens* a 150–500 ppm, con inhibiciones $\geq 95\%$. En la Fase III, aplicaciones preventivas y curativas en hojas desprendidas disminuyeron la severidad de 40,5 % en el testigo inoculado a $\leq 1,3\%$ en los tratamientos. Estos resultados indican que mancozeb + *T. viride* y ciertas mezclas con *B. amyloliquefaciens* pueden integrarse de forma segura en programas de manejo de enfermedades de la soja.

Palabras clave: Glycine max, *Trichoderma viride*, *Bacillus amyloliquefaciens*, control biológico, control químico, manejo integrado de enfermedades.

Introduction

Soybean (*Glycine max* [L.] Merr.) is the main agricultural crop in Paraguay during the spring–summer season and a key pillar of the national economy

(Sánchez et al., 2022). The country is currently the sixth largest producer and third largest exporter worldwide, with 11,074,900 tons harvested on 3,650,000 hectares and an average yield of 3,034 kg ha⁻¹ in 2023/2024 (Paraguayan Chamber of

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Exporters and Traders of Cereals and Oilseeds, 2025). Beyond its economic role, soybean is an essential source of protein and oil and has strategic importance for global food security (Pagano and Miransari, 2016).

However, soybean production potential faces significant constraints, notably plant diseases, which are among the main causes of yield reduction worldwide, with estimated losses ranging between 10% and 15% (Hartman et al., 2020). Among the so-called late-season diseases (LSDs), leaf blight caused by *Cercospora kikuchii* is particularly relevant due to its impact on seed quality and its tendency to accelerate plant senescence (Caballero-Mairesse et al., 2024; Enciso-Maldonado & Fernández-Gamarra, 2021). Traditionally, its management has relied on chemical fungicides from various groups, including demethylation inhibitors (DMIs), quinone outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs) (Mendoza-Duarte et al., 2024; de Mello et al., 2021; Hartman et al., 2015).

In response to increasing demands for more sustainable production and food safety, Paraguayan agriculture has shown a notable increase in the use of bioproducts as part of integrated disease management strategies in recent years. Among these, the microorganisms *Trichoderma viride* and *Bacillus amyloliquefaciens* have gained relevance due to their ability to promote plant growth, induce plant defenses, and act as biocontrol agents (Marchuk-Larrea et al., 2024; Luo, 2022). However, in production systems where the use of synthetic fungicides remains essential, it is necessary to assess the compatibility of these products to ensure the efficacy and sustainability of plant health management programs.

Several studies have shown that interactions between biological agents and chemical fungicides can vary. While some combinations are compatible and enhance disease control (Parraguirre-Lezama et al., 2025; Fernández et al., 2021), others have adverse effects on the growth and activity of beneficial microorganisms (Harsha et al., 2023; Rodríguez Prieto, 2020). These differences depend on fac-

tors such as the nature and concentration of active ingredients, the mode of action of the fungicides, and specific environmental conditions (Fernández-Gamarra et al., 2017).

In Paraguay, despite growing interest in bioproducts, there is limited available information on their interactions with synthetic fungicides under local conditions. This lack of studies hinders the safe and effective adoption of product combinations in the field, creating uncertainty in integrated disease management programs (Caballero-Mairesse et al., 2024). Therefore, it is essential to generate scientific knowledge that systematically evaluates the compatibility and efficacy of bioproduct–fungicide mixtures for the control of leaf blight caused by *Cercospora* spp., thereby contributing to the development of more sustainable, efficient agricultural practices aligned with the new quality and safety standards demanded by international markets.

In this context, the general objective of the present study was to evaluate the compatibility and efficacy of different mixtures of chemical fungicides under laboratory conditions for the control of *Cercospora* sp., providing information that can help optimize integrated disease management in soybean cultivation in Paraguay.

Materials and Methods

Location and study period

This study was conducted at the Plant Pathology Laboratory of the Hohenau Pedagogical Unit, Catholic University "Nuestra Señora de la Asunción", located at UTM coordinates Zone 21 X 634759.90 and Y 7006065.50 (Hohenau, Itapúa, Paraguay). The research was carried out from November 2024 to April 2025.

Pathogen isolation and identification

The *Cercospora* sp. isolate used (isolate CM-250) was provided by the Multidisciplinary Center for Technological Research (CEMIT-UNA) and the Plant Pathology Laboratory of the Catholic University, within the framework of the PINV01-152 Project funded by CONACYT. This isolate is currently undergoing molecular identification.

Table 1. Radial growth of *Trichoderma viride* after 7 days of incubation and colony-forming units (CFU) of *Bacillus amyloliquefaciens* after 4 days of incubation in PDA medium amended with various fungicides at different concentrations. Values represent mean colony diameter (mm). A value of 0 indicates complete absence of growth (no colony development was observed). F-values correspond to the F statistic from the analysis of variance (ANOVA) for the effect of treatments on each response variable. ns: non-significant; * $p < 0.05$; ** $p < 0.01$. Letters indicate statistical groups; means followed by the same letter in a column do not differ significantly (Tukey's HSD test, $p \leq 0.05$).

Fungicide	Concentration (ppm)	Radial growth of <i>T. viride</i> (mm)	CFU of <i>B. amyloliquefaciens</i>
Solatenol + Prothioconazole	0	39.3 c	162.0 c
Solatenol + Prothioconazole	50	0.0 a	99.3 b
Solatenol + Prothioconazole	150	0.1 a	32.7 a
Solatenol + Prothioconazole	500	0.0 a	30.3 a
Solatenol + Prothioconazole	1000	0.0 a	25.0 a
Picoxystrobin + Benzobindiflupyr	0	38.8 c	158.5 c
Picoxystrobin + Benzobindiflupyr	50	4.5 a	32.5 a
Picoxystrobin + Benzobindiflupyr	150	3.5 a	30.8 a
Picoxystrobin + Benzobindiflupyr	500	0.8 a	13.2 a
Picoxystrobin + Benzobindiflupyr	1000	0.0 a	11.5 a
Trifloxystrobin + Prothioconazole	0	39.3 c	13.3 a
Trifloxystrobin + Prothioconazole	50	0.0 a	10.7 a
Trifloxystrobin + Prothioconazole	150	0.0 a	6.0 a
Trifloxystrobin + Prothioconazole	500	0.0 a	5.3 a
Trifloxystrobin + Prothioconazole	1000	0.0 a	4.3 a
Mancozeb	0	38.6 c	129.3 bc
Mancozeb	50	36.3 c	0.0 a
Mancozeb	150	36.7 c	0.0 a
Mancozeb	500	36.7 c	0.0 a
Mancozeb	1000	36.3 c	0.0 a
Epoxiconazole + Fluxapyroxad + Pyraclostrobin	0	38.4 c	142.7 bc
Epoxiconazole + Fluxapyroxad + Pyraclostrobin	50	23.9 b	16.0 a
Epoxiconazole + Fluxapyroxad + Pyraclostrobin	150	0.0 a	8.5 a
Epoxiconazole + Fluxapyroxad + Pyraclostrobin	500	0.0 a	8.5 a
Epoxiconazole + Fluxapyroxad + Pyraclostrobin	1000	0.0 a	6.8 a
F-value		85.4**	11.1**

Phase 1: In vitro compatibility evaluation

In this phase, the compatibility between five commercial fungicide mixtures (Solatenol + prothioconazole, picoxystrobin + benzovindiflupyr, trifloxystrobin + prothioconazole, mancozeb, epoxiconazole + fluxapyroxad + pyraclostrobin) at concentrations of 0, 50, 150, 500, and 1000 ppm, and commercial bioproducts based on *Trichoderma viride* (Trifisol 100 WP, IGT S.A.) and *Bacillus amyloliquefaciens* (GrowAmylo, Arion Group S.A.) was evaluated. The 0 ppm concentrations served as negative controls (Table 1).

Stock solutions of fungicides were prepared using the C1V1 = C2V2 formula and diluted in potato dextrose agar (PDA, Liofilmchem, Italy), without antibiotics or other additives. The medium was sterilized in an autoclave at 121 °C and 1 atm for 20 minutes, and 25 mL were poured into sterile Petri dishes.

For *T. viride* compatibility testing, pure colonies were first obtained from the formulated product and grown on PDA medium. After 21 days of incubation, 5 mm diameter mycelial plugs were cut and placed in Petri dishes containing fungicide-amended PDA. Plates were incubated at 28 ± 1 °C for seven days, with daily measurements of radial mycelial growth using a graduated ruler. The final radial growth was recorded on day seven.

For *B. amyloliquefaciens*, serial dilutions were prepared up to 10^{-6} from an initial 10,000 ppm suspension. Each dilution was surface-spread onto PDA plates amended with fungicides by pipetting 100 μ L into the center and spreading with a sterile Drigalski spatula. Plates were incubated at 28 ± 1 °C for four days, after which colony-forming units (CFU) were counted.

Phase 2: Inhibitory Effect on *Cercospora* sp.

The inhibitory effect of combinations previously identified as compatible or partially compatible was evaluated using dual culture assays on fungicide-amended PDA plates. Plates containing *Cercospora* growth on non-amended media and those treated with 0 ppm served as controls (Table 2).

For *T. viride*, a 5 mm mycelial plug of the bio-

control agent was placed in one corner of the plate, with a 5 mm plug of *Cercospora* sp. placed in the opposite corner, both obtained from 21-day-old active cultures. Plates were incubated at 28 ± 1 °C under a 12-hour light/dark photoperiod for seven days prior to evaluation.

For *B. amyloliquefaciens*, a plug of *Cercospora* sp. was placed at the center of the plate and surrounded by ten 20 μ L drops of bacterial suspension at 10^5 CFU/mL, evenly distributed. Plates were first incubated in the dark for 24 hours, followed by a 12 h light/12 h dark cycle at 28 ± 1 °C. Evaluations were performed on day seven.

Phase 3: Detached leaf assay

Healthy soybean leaves (cv. M 5957 IPRO) were collected from the middle-lower third of plants grown in pots containing a 1: 1 mixture of compost and coarse sand. The substrate was thermally sterilized using a metal drum heated by direct fire. Leaves were disinfected by sequential washes: 2% sodium hypochlorite (1 min), 70% ethanol (30 s),

Table 2. Mycelial growth of *Cercospora* sp. under different concentrations of mancozeb in the presence of *T. viride* after 7 days of incubation. Values represent mean radial diameter (cm) of *Cercospora* sp. **Significant at 1% probability level ($p < 0.01$). Means followed by the same letters do not differ statistically according to Tukey's test at 5% probability level.

Treatment	Concentration (ppm)	Radial diameter (cm)
Control (<i>Cercospora</i> only)	—	4.1 b
Mancozeb + <i>T. viride</i>	0	0.6 a
Mancozeb + <i>T. viride</i>	50	0.7 a
Mancozeb + <i>T. viride</i>	150	0.1 a
Mancozeb + <i>T. viride</i>	500	0.0 a
Mancozeb + <i>T. viride</i>	1000	0.0 a
F-value		43.2**

and three rinses in sterile distilled water.

Disinfected leaves were placed on moistened paper towels inside plastic trays, with petioles wrapped in moistened cotton to maintain turgor.

Inoculation with *Cercospora* sp. was performed by spraying a propagule suspension adjusted to 1×10^6 using a Neubauer chamber. For the preventive treatment, selected fungicide mixtures were applied 24 hours before pathogen inoculation. For the curative treatment, fungicide application was conducted 24 hours after inoculation.

Leaves were incubated under controlled temperature ($25 \pm 2^\circ\text{C}$), photoperiod (12 h light/dark), and high humidity conditions to favor infection. Foliar damage was assessed using the diagrammatic scale proposed by Martins (2004), which quantifies severity as a percentage of leaf area affected by the pathogen and distinguishes between aggregated and random distribution patterns.

Phase I was conducted using a completely randomized design with a $2 \times 5 \times 5$ factorial arrangement and three replicates per treatment. In this design, factor A corresponded to two biological products (*Trichoderma viride* and *Bacillus amyloliquefaciens*), factor B to five fungicide mixtures (Solatenol + prothioconazole, picoxystrobin + benzovindiflupyr, trifloxystrobin + prothioconazole, mancozeb, and epoxiconazole + fluxapyroxad + pyraclostrobin), and factor C to five fungicide concentrations (0, 50, 150, 500, and 1000 ppm). Each experimental unit consisted of two Petri dishes in Phase II and one plastic tray containing three soybean leaflets in Phase III. Collected data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test ($p < 0.05$) with specialized statistical software. All statistical analyses were performed using Infostat version 2020 (Di Rienzo et al., 2020)

Statistical Analysis

Results and Discussion

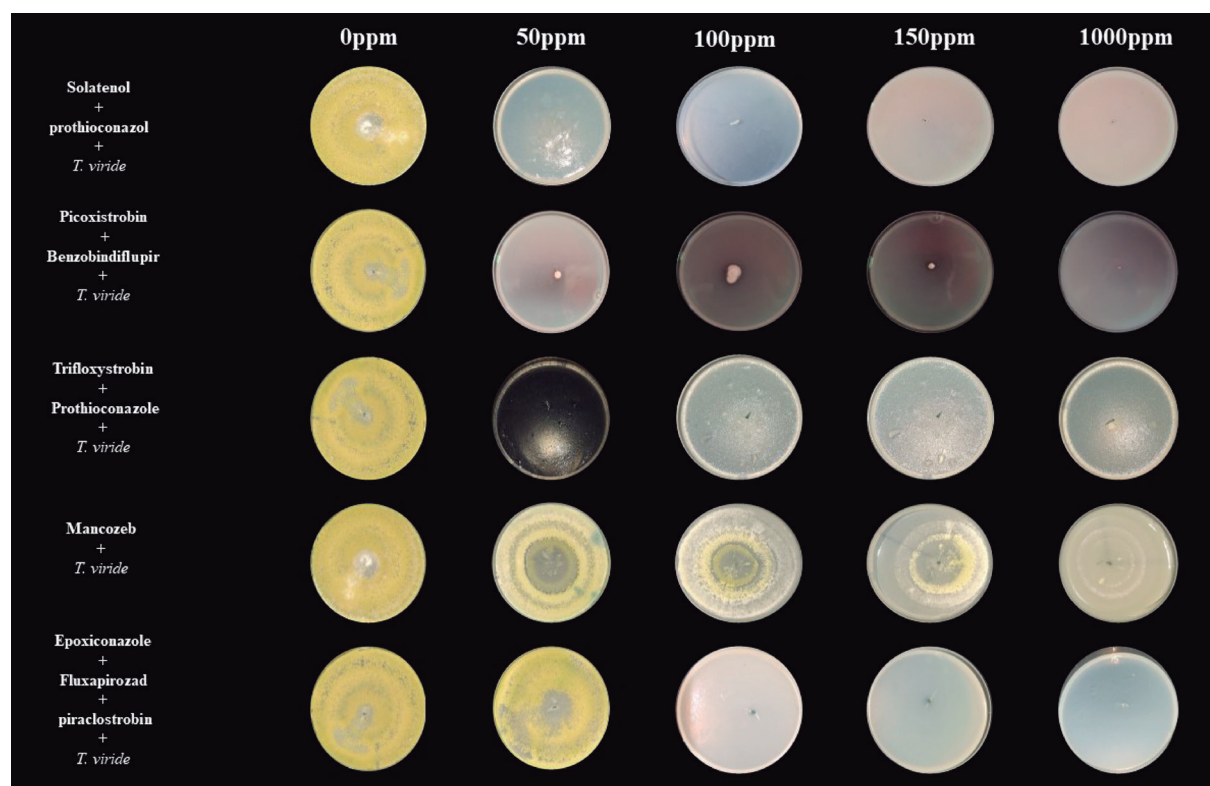


Figure 1. Effect of increasing concentrations of various fungicide mixtures on the radial growth of *Trichoderma viride*.

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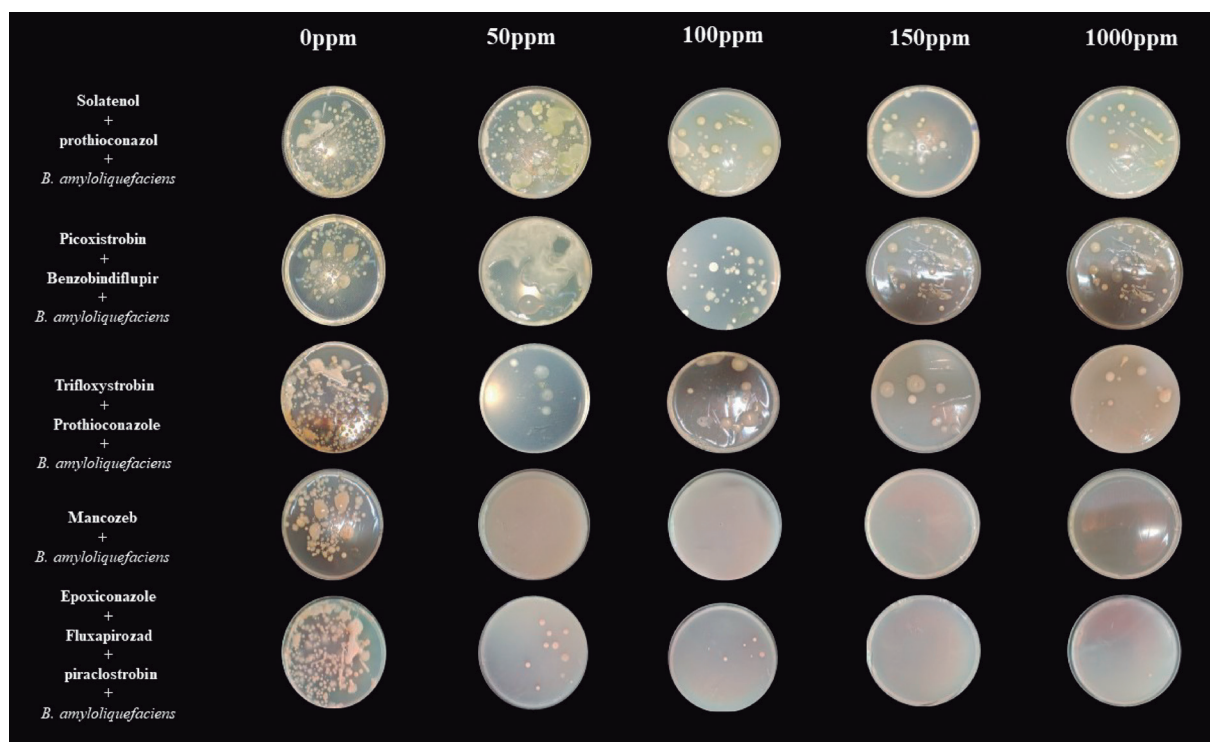


Figure 2. Effect of increasing concentrations of various fungicide mixtures on the growth of *Bacillus amyloliquefaciens*.

Phase 1: Compatibility between chemical fungicides and biological agents

The *in vitro* evaluation revealed a highly significant interaction ($p < 0.01$) between fungicide types and their concentrations on the radial growth of *Trichoderma viride* (Table 1, Fig. 1). Mancozeb was the only fungicide that did not inhibit the growth of the biological agent at any of the tested concentrations (50–1000 ppm), maintaining radial diameters similar to the untreated control (approximately 36 mm). This result is consistent with previous findings, which indicate full compatibility of this multi-site fungicide with *Trichoderma* spp. (Dalvi, 2022; Maheshwary et al., 2020).

From a practical standpoint, this indicates that mancozeb-based spray programs can incorporate *T. viride* either in tank mixtures or in closely timed applications without compromising the viability of the biocontrol agent, facilitating its inclusion in existing soybean fungicide schedules.

In contrast, the fungicide mixtures Solatenol +

prothioconazole, trifloxystrobin + prothioconazole, and epoxiconazole + fluxapyroxad + pyraclostrobin exhibited complete inhibition of *T. viride* growth even at the lowest concentration tested (50 ppm), with values close to zero, indicating significant incompatibility. This behavior is typical of fungicides with specific modes of action, such as triazoles and strobilurins (Castellanos-González, 2015).

For picoxystrobin + benzobindiflupyr, an intermediate response was observed. Although complete inhibition occurred at higher concentrations (500 and 1000 ppm), some limited yet detectable growth was recorded at lower concentrations (50 and 150 ppm), suggesting partial and concentration-dependent incompatibility.

Regarding *Bacillus amyloliquefaciens*, significant differences in bacterial viability were observed depending on the fungicide mixture and its concentration (Table 1, Fig. 2). Solatenol + Prothioconazole showed partial compatibility at low concentration (50 ppm), with a drastic reduction in viability from 150 ppm onwards.

Similarly, picoxystrobin + benzobindiflupyr showed limited compatibility only at 50 ppm. In contrast, mancozeb exhibited total incompatibility from the lowest concentration, reflecting a severe impact on bacterial cell integrity (Prabu, 2019). These results suggest that to more efficiently exploit the effect of *B. amyloliquefaciens*, the dose could be increased when applying in a mixture with fungicides.

Phase 2: Inhibitory Effect on *Cercospora* sp.

The combination of mancozeb with *T. viride* exhibited an almost complete inhibitory effect on the growth of *Cercospora* sp., particularly at 500 and 1000 ppm. Even lower doses (50 and 150 ppm) significantly reduced mycelial growth compared to the control. These results agree with previous studies highlighting the potential of mancozeb and *Trichoderma* spp. in effectively suppressing pathogens such as *Cercospora* (Sautua et al., 2020; Noorulla et al., 2013).

The evaluation of mycelial growth of *Cercospora* sp. in the presence of *B. amyloliquefaciens* and compatible fungicides revealed significant differences between treatments (Table 3). The combinations of Solatenol + prothioconazole and

picoxystrobin + benzovindiflupyr in the presence of *B. amyloliquefaciens* showed a clear inhibitory effect on pathogen growth. The greatest inhibitory effect was recorded at higher concentrations (150 and 500 ppm), where nearly total or total suppression of mycelial growth was observed compared to controls.

These strong inhibitory effects suggest that the compatible combinations identified here can serve as a core component of integrated *Cercospora* leaf blight management, allowing agronomists to design spray programs that combine biological agents with selected fungicides to maintain high levels of protection while reducing the need for repeated applications of single-site fungicides.

These findings are consistent with prior in vitro studies showing that *Trichoderma viride* and *T. harzianum* can significantly inhibit the growth of *Cercospora musae*, with inhibition rates of 88.51% and 82.31%, respectively.

Similarly, *Bacillus subtilis* strains have also shown antagonistic effects, with one isolate achieving 42.55% growth inhibition. These results, obtained through dual culture techniques in Petri dishes, underscore the biocontrol potential of both fungal and bacterial agents in managing *Cercospora*

Table 3. Mycelial growth of *Cercospora* sp. under different concentrations of compatible fungicides in the presence of *Bacillus amyloliquefaciens* after 4 days of incubation. Values represent mean radial diameter (cm) of *Cercospora* sp. **Significant at 1% probability level ($p < 0.01$). Means followed by the same letters do not differ statistically according to Tukey's test at 5% probability level.

Treatment	Concentration (ppm)	Radial diameter (cm)
Control (<i>Cercospora</i> only)	–	3.7 b
Solatenol + Prothioconazole + <i>B. amyloliquefaciens</i>	0	3.2 b
Solatenol + Prothioconazole + <i>B. amyloliquefaciens</i>	50	0.8 a
Solatenol + Prothioconazole + <i>B. amyloliquefaciens</i>	150	0.2 a
Solatenol + Prothioconazole + <i>B. amyloliquefaciens</i>	500	0.0 a
Picoxystrobin + Benzobindiflupyr + <i>B. amyloliquefaciens</i>	0	3.2 b
Picoxystrobin + Benzobindiflupyr + <i>B. amyloliquefaciens</i>	50	0.3 a
Picoxystrobin + Benzobindiflupyr + <i>B. amyloliquefaciens</i>	150	0.0 a
F-value		47.7**

Compatibility of chemical fungicides with biological products for the control of soybean leaf blight caused by *Cercospora* sp.

Table 4. Severity of leaf blight caused by *Cercospora* sp. on leaves treated with combinations of biocontrol agents and fungicides, applied preventively and curatively. Values represent mean disease severity (%). **Significant at 1% probability level ($p < 0.01$). Means followed by the same letters do not differ statistically according to Tukey’s test at 5% probability level.

Treatment	Severity (%)
Absolute control	0.0 a
Inoculated control	40.5 b
Preventive <i>T. viride</i>	0.0 a
Preventive <i>T. viride</i> + Mancozeb	0.0 a
Preventive Mancozeb	0.0 a
Curative <i>T. viride</i>	0.0 a
Curative <i>T. viride</i> + Mancozeb	0.0 a
Curative Mancozeb	0.0 a
Preventive <i>B. amyloliquefaciens</i>	0.8 a
Preventive <i>B. amyloliquefaciens</i> + Picoxystrobin + Benzobindiflupyr	0.0 a
Preventive <i>B. amyloliquefaciens</i> + Solatenol + Prothioconazole	1.1 a
Preventive Picoxystrobin + Benzobindiflupyr	0.5 a
Preventive Solatenol + Prothioconazole	0.5 a
Curative <i>B. amyloliquefaciens</i>	1.3 a
Curative <i>B. amyloliquefaciens</i> + Picoxystrobin + Benzobindiflupyr	0.0 a
Curative <i>B. amyloliquefaciens</i> + Solatenol + Prothioconazole	0.0 a
Curative Picoxystrobin + Benzobindiflupyr	0.8 a
Curative Solatenol + Prothioconazole	0.8 a
F-value	1190.2**

spp. infections (Noorulla et al., 2013).

In operational terms, this means that *B. amyloliquefaciens* should not be tank-mixed with mancozeb and, when combined with Solatenol + prothioconazole or picoxystrobin + benzovindiflupyr, lower fungicide doses or sequential applications are preferable to preserve bacterial populations and ensure consistent field performance.

Phase 3: Severity of leaf blight on detached leaves

The total control achieved by combinations such as *T. viride* + mancozeb and *B. amyloliquefaciens* + Picoxystrobin + Benzobindiflupyr or Solatenol +

Prothioconazole suggests a synergistic or at least additive interaction between biological control agents and selected fungicides. These results align with previous studies showing that compatible chemical and biological agents can act in concert to improve disease suppression (Harman et al., 2010; Shores et al., 2010).

The mechanisms by which *B. amyloliquefaciens* exerts foliar disease control likely include the production of antifungal lipopeptides (such as surfactin, iturin, and fengycin), secretion of hydrolytic enzymes (e.g., chitinases and proteases), and induction of systemic resistance (ISR) in the host plant. These metabolites can disrupt fungal cell walls and

inhibit spore germination or mycelial growth, while ISR primes plant defenses for faster and stronger responses upon pathogen attack (Koumoutsis et al., 2004; Chen et al., 2009).

On the other hand, *Trichoderma viride* can control foliar pathogens through multiple modes of action, including the production of antifungal secondary metabolites, competition for nutrients and infection sites, and the activation of induced systemic resistance in plants. While traditionally studied for its role in soil and root disease control, its foliar application has shown potential to directly inhibit pathogens through mycoparasitism and the secretion of volatile organic compounds (VOCs), as well as to strengthen plant defense signaling pathways (Marchuk-Larrea et al., 2024).

Notably, standalone applications of *T. viride*, mancozeb, and *B. amyloliquifaciens*, in both preventive and curative modes, also led to a marked reduction in blight severity, confirming their antagonistic or protective capacity under controlled conditions. However, the combinations provided equal or superior control, emphasizing their relevance in integrated disease management (IDM) programs (Table 4).

The comparable effectiveness of preventive and curative treatments in many of the evaluated strategies highlights their operational flexibility. This is particularly relevant for field conditions, where disease onset may vary, and responsive management is critical (Mendoza-Duarte et al., 2024).

Despite these promising results, some limitations must be acknowledged. This phase was conducted under controlled laboratory conditions using detached leaves, which do not fully replicate the complexity of field environments, including fluctuating climatic variables, microbiome interactions, and plant physiological responses. Additionally, the longevity and stability of biocontrol agent activity on leaf surfaces under natural conditions were not evaluated.

Future studies should validate these results in field trials, evaluating persistence and colonization of *T. viride* and *B. amyloliquifaciens* under varying environments, elucidate induced resistance mecha-

nisms in soybean, test compatibility with other agrochemicals and cultivars, and thus strengthen the evidence base for large-scale integrated disease management.

Conclusions

This study demonstrated the potential for integrating biocontrol agents with certain chemical fungicides for the management of leaf blight caused by *Cercospora* sp. in soybean. *Trichoderma viride* exhibited full compatibility with mancozeb, while *Bacillus amyloliquifaciens* was compatible with low concentrations of Solatenol + Prothioconazole and Picoxystrobin + benzobin-diflupyr. The compatible combinations significantly reduced *Cercospora* sp. growth under *in vitro* conditions and showed high efficacy in both preventive and curative applications on soybean leaves. In practical terms, these combinations represent concrete options that agronomists and growers can test in pilot plots and, if confirmed under field conditions, incorporate into integrated disease management programs for soybean in Paraguay. These findings suggest that further studies should evaluate whether higher doses of *B. amyloliquifaciens* improve their performance when combined with fungicides, reducing exclusive reliance on synthetic fungicides and promoting more responsible and efficient agricultural practices.

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Author contributions

All authors contributed equally to the preparation of this article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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