Efficacy and Cost-Benefit Analysis of Fungicide Combinations against Soybean Rust and Cercospora Leaf Blight in Paraguay

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ABSTRACT

This study evaluated the efficacy, seed yield and economic returns of nine fungicide mixtures for the integrated management of Asian soybean rust (*Phakopsora pachyrhizi*) and Cercospora leaf blight (*Cercospora kikuchii*) under field conditions in Canindeyú, Paraguay, during the 2023/2024 and 2024/2025 seasons. Treatments included site-specific premixes combining QoI, DMI and SDHI modes of action, as well as protectant-only programs based on chlorothalonil or mancozeb applied sequentially at four timings. Disease severity was recorded over time to generate disease progress curves and the area under the disease progress curve (AUDPC); control efficacy was derived relative to the untreated control. Grain yield, thousand-grain weight, gross income, total treatment cost, net benefit and benefit–cost ratio (B/C) were also determined. Across seasons and diseases, multi-MOA premixes (QoI + DMI ± SDHI) consistently resulted in the lowest AUDPC values, providing the strongest suppression of the foliar disease complex compared with the untreated control. Soybean rust pressure differed between seasons, with later and lower severity in 2024/2025; therefore, treatment separation for rust was smaller in absolute terms in that season, while AUDPC rankings remained consistent. Yield and seed weight responses generally followed disease suppression, with the best premixes showing the highest numerical yields and thousand-seed weight. Although protectant-only programs provided intermediate disease reduction, their lower input costs often resulted in high B/C ratios, highlighting their value as low-cost benchmarks under certain scenarios. Overall, the results indicate that locally validated multi-MOA fungicide programs can provide effective and economically viable management of late-season soybean foliar diseases in Paraguay, under variable epidemic conditions.

KEYWORDS

Phakopsora pachyrhizi; *Cercospora* spp.; chemical control; disease progress curves; FRAC resistance management; benefit-cost ratio.

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) represents one of the agricultural pillars both in Paraguay and throughout South America, not only because of its high economic and nutritional value but also for the crucial role it plays in regional food security and rural development (Pagano & Miransari, 2016). The intensification of production has been accompanied by phytosanitary challenges that affect both yield and crop quality, among which foliar diseases of great impact stand out, soybean rust (SBR), caused by *Phakopsora pachyrhizi*, and Cercospora leaf blight (CLB), caused by species of the genus Cercospora (Yorinori *et al*., 2005; Hartman *et al*., 2015; Langenbach *et al*., 2016; Sautua *et al*., 2024; Fernandes *et al*., 2025). Under favorable environmental conditions, these diseases can significantly reduce the plant's active leaf area, compromising photosynthesis, assimilate translocation, and, consequently, final crop yield (Dalla Lana *et al*., 2015; Hartman *et al*., 2015; Hossain *et al*., 2024).

Soybean rust has emerged since the early twenty-first century as the most devastating fungal disease in soybean crops in tropical and subtropical regions, spreading rapidly in Brazil and reaching neighboring countries such as Paraguay (Yorinori *et al*., 2005; Meira *et al*., 2020). Its capacity for aerial dispersal, coupled with the pathogen's high adaptability, makes timely surveillance and implementation of control measures essential to avoid significant production losses, which in some cases may exceed 70 % of yield (Hartman *et al*., 2015; Hossain *et al*., 2024). Likewise, Cercospora leaf blight, although historically less studied than rust, has positioned itself as an emerging threat in intensive soybean production systems, acting synergistically with other foliar pathogens, reducing water-use efficiency, and causing grain-quality losses (Sautua *et al*., 2024; Shrestha *et al*., 2024).

The management of these diseases is based on integrated strategies in which the use of fungicides plays a leading role. Over the past two decades, there has been an increasing reliance on fungicides to control both soybean rust and leaf blight, with intensive use of products from the quinone outside inhibitors (QoI), demethylation inhibitors (DMI), succinate dehydrogenase inhibitors (SDHI), and multisite fungicide groups (Scherm *et al*., 2009; Meira *et al*., 2020; Machado *et al*., 2022). However, continuous use of single-site fungicides has been associated with sensitivity shifts and resistance issues in South America, and meta-analytic syntheses document temporal declines in fungicide performance for soybean rust after prolonged intensive use (Dalla Lana *et al*., 2018; Müller *et al*., 2021). In this context, fungicide premixes and programs combining active ingredients with different modes of action have been proposed as a promising strategy to improve technical performance and align with resistance-management principles by reducing reliance on any single target site (Barro *et al*., 2021; Machado *et al*., 2022; FRAC, 2024).

The use of fungicide mixtures not only aims to optimize the efficiency of foliar disease control but also responds to the need to manage the high resistance-development risks inherent to site-specific fungicides (QoI, DMI, and SDHI) (Barro *et al*., 2021; FRAC, 2024). In addition, integrating low resistance-risk multisite fungicides (e.g., mancozeb or chlorothalonil) as partners and/or alternation components is widely recommended to reduce selection intensity on single-site modes of action

(Machado *et al*., 2022; FRAC, 2024). Studies under high epidemic pressure have indicated that integrating different active ingredients in sequential or alternating applications can reduce disease progress, as reflected by lower area under the disease progress curve (AUDPC), and can improve agronomic yields (Edwards-Molina *et al*., 2019; Reis *et al*., 2022). In Paraguay, local surveys and field studies describe producer practices and evaluate fungicide programs under regional conditions, providing context for adoption and feasibility (Caballero-Mairesse *et al*., 2024).

Evaluating the efficacy and economic viability of these mixtures becomes even more critical in contexts where producer resources are limited and market competition demands maximization of yield per hectare without unsustainable increases in management costs (Alves *et al*., 2021; Reis *et al*., 2022). Previous results from countries with similar agroclimatic and pathological conditions, such as Brazil, have shown that strategic use of fungicide mixtures can maintain control levels above 80 %, which correlates directly with increased grain yield and net producer benefit (Reis *et al*., 2022; Dalla Lana *et al*., 2015). Therefore, generating multi-season field evidence in Paraguay is necessary to guide profitable fungicide programs that are also consistent with resistance-management stewardship principles. The objective of this study was to evaluate the efficacy and cost–benefit performance of fungicide premixes and multisite protectants against SBR and CLB in Paraguay across two growing seasons.

MATERIALS AND METHODS

Field trials were conducted in two locations within the Canindeyú department, Paraguay. The first was carried out in La Paloma (coordinates: -24.1256, -54.5718) during the 2023/2024 growing season, with sowing on October 20th, 2023. The second took place in Agrícola Paraguaya (coordinates: -24.3805, -54.8207) during the 2024/2025 season, with sowing on September 28th, 2024. These sites are approximately 37.3 km apart. Soils at both locations are classified as *Rhodic Paleudalf*, with a clay loam texture, good drainage, slightly acidic pH, and moderate organic matter content.

Climatic conditions were monitored using local weather stations. In the 2023/2024 season, the average temperature was 26.8 °C (mean minimum: 20.8 °C; mean maximum: 31.28 °C), with a total rainfall of 757.4 mm during the experimental period. In the 2024/2025 season, the average temperature was 30.1 °C (mean minimum: 25.12 °C; mean maximum: 33.4 °C), and total precipitation was 472.74 mm.

A randomized complete block design was used, with four replicates per treatment and plot sizes of 24 m^2 (6 m x 4 m). In both growing seasons, the early-maturing soybean variety M 5947 RR2 PRO was used.

The treatments consisted of commercially formulated, registered fungicides representing the following mode-of-action groups: QoI (quinone outside inhibitors), DMI (demethylation inhibitors), SDHI (succinate dehydrogenase inhibitors), and multisite fungicides (Table 1). All products were purchased in their original, unopened containers from authorized agrochemical retailers in Canindeyú, Paraguay, and used according to label directions; no formulations were prepared by the research team. Manufacturers/registrants were: Corteva Agriscience (Aproach Power® EC; Viovan® EC; Vessarya® EC; and Dithane® NT 80/PM-80 NT WP), Bayer CropScience (Sphere Max® SC; Cripton Xpro® SC), Summit Agro (Planity® SC), and Syngenta (Bravonil 720 SC®). Fungicides were applied at the labelled commercial product rate using a CO_2-pressurized backpack sprayer at constant pressure, with a spray volume of 120 L ha^{-1}, fitted with M53MAG 015 cone nozzles and a pressure regulator, at 35, 50, 65, and 80 days after crop emergence. For reproducibility and comparison among studies, the dose of each active ingredient was also expressed as grams of active ingredient per hectare (g a.i. ha^{-1}), calculated from the formulation concentration and the applied field rate (mL ha^{-1} for liquid formulations; g ha^{-1} for dry formulations); product rates, formulation types (e.g., EC, SC, WP), active-ingredient concentrations, and calculated g a.i. ha^{-1} are provided in Table 1. Treatments T8 (chlorothalonil) and T9 (mancozeb) were implemented as protectant-only, low-cost benchmark programs, each consisting of four sequential applications of the same single active ingredient at each spray timing at the labelled rate (Table 1).

Table 1. Fungicide treatments, formulation rate, and active ingredient dose (g a.i. ha^{-1}).

Treatment	Commercial product (formulation)	Active ingredient(s) and formulation concentration	Formulated product rate	Active ingredient dose (g a.i. ha^{-1})
T1	Non-treated control	–	–	–
T2	Aproach Power® (EC)	picoxystrobin 90 g/L + cyproconazole 40 g/L	600 mL ha^{-1}	picoxystrobin 54 + cyproconazole 24
T3	Sphere Max® (SC)	trifloxystrobin 375 g/L + cyproconazole 160 g/L	200 mL ha^{-1}	trifloxystrobin 75 + cyproconazole 32
T4	Viovan® (EC)	picoxystrobin 100 g/L + prothioconazole 116.7 g/L	600 mL ha^{-1}	picoxystrobin 60 + prothioconazole 70
T5	Cripton Xpro® (SC)	trifloxystrobin 150 g/L + prothioconazole 175 g/L + bixafen 125 g/L	500 mL ha^{-1}	trifloxystrobin 75 + prothioconazole 87.5 + bixafen 62.5
T6	Vessarya® (EC)	picoxystrobin 100 g/L + benzovindiflupyr 50 g/L	600 mL ha^{-1}	picoxystrobin 60 + benzovindiflupyr 30
T7	Planity® (SC)	inpyrfluxam 60 g/L + tebuconazole 200 g/L	600 mL ha^{-1}	inpyrfluxam 36 + tebuconazole 120
T8	Bravonil 720 SC (SC)	chlorothalonil 720 g/L	1500 mL ha^{-1}	chlorothalonil 1080
T9	Dithane NT 80® (WP)	mancozeb 800 g/kg	1500 g ha^{-1}	mancozeb 1200

For each disease, the area under the disease progress curve (AUDPC) was calculated according to Shaner & Finney (1977). Soybean rust severity was assessed using the diagrammatic scale proposed by Godoy *et al*. (2006), while Cercospora leaf blight severity was evaluated using the scale by Martins *et al*. (2004). Severity was recorded at 42, 57, 72, 79, 86, and 93 days after emergence (DAE), corresponding to 7 days after the 1st, 2nd, and 3rd applications (42, 57, and 72 DAE) and subsequent late-season assessments spanning the period after the final (4th) application (79, 86, and 93 DAE). Although disease was monitored throughout the season, these assessment points were used for AUDPC because symptom expression and treatment separation became consistently detectable from this period onward, whereas earlier observations showed negligible severity and limited discrimination among treatments. Control efficacy was expressed as percentage reduction relative to the untreated control, calculated as Control efficacy (%) = $[1 - (AUDPC_i / AUDPC_0)] \times 100$, where $AUDPC_i$ is the value for treatment *i* and $AUDPC_0$ is the untreated control.

Productivity variables included thousand seed weight (TSW) and yield (kg ha^{-1}), adjusted to 13% grain moisture.

Data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test ($p \leq 0.05$) with R software (R Core Team 2025).

Economic analysis. A partial budget approach was used to quantify the profitability of each fungicide program in each season based on grain yield and fungicide product costs only. For each plot, grain yield (kg ha^{-1}) was standardized to 13% moisture and converted to gross income using the seasonal soybean price. Let Y_i be the yield of treatment i (kg ha^{-1}), Y_0 the yield of the untreated control, and P the soybean price (US\$ kg^{-1}).

Gross income (GI, US\$ ha^{-1}) was calculated as:

$$GI_i = Y_i \times P$$

Fungicide treatment cost (TC, US\$ ha^{-1}) was calculated as the total fungicide product cost per hectare across all sprays:

$TC_i = C\_fung,i$

where C_fung,i is the sum of the commercial product cost(s) applied per hectare, calculated from the purchase price per unit volume or mass and the applied field rate, and then summed across the number of applications. All treatments followed the same application schedule (four sprays at 35, 50, 65, and 80 DAE); therefore, fungicide costs were calculated for four applications for every treatment, including the protectant-only programs (T8 and T9).

Net benefit (NB, US\$ ha^{-1}) for each treatment was calculated as:

$NB_i = GI_i - TC_i$

Benefit/cost ratio (B/C) was calculated as:

$B/C_i = GI_i / TC_i$

To express the advantage of each fungicide program relative to the untreated control, we calculated:

Yield increase (ΔY, kg ha^{-1}): $\Delta Y_i = Y_i - Y_0$

Income gain (IG, %): $IG_i = [(NB_i - NB_0) / GI_0] \times 100$

and, when needed, the net benefit gain relative to the control (ΔNB, US\$ ha^{-1}): $\Delta NB_i = NB_i - NB_0$.

Because the untreated control had no fungicide costs ($TC_0 = 0$), $NB_0 = GI_0$.

Because application/operational costs (labor, fuel, machinery depreciation and/or contractor fees) were not recorded, they were not included in the economic analysis; thus, profitability metrics represent a partial budget based on fungicide product costs only. All profitability metrics were computed separately for each season using the corresponding soybean price and fungicide cost structure. Costs and prices are reported in US\$ ha^{-1}.

Results

Disease progress curves (DPCs) and area under the disease progress curve (AUDPC)

In the 2023/2024 season, all fungicide treatments significantly reduced soybean rust development compared to the untreated control (AUDPC 1,277.75). Consistent with the disease progress curves (Figure 1), rust severity remained negligible up to 57 DAE and then increased rapidly from 72 DAE onward; in the untreated control, mean severity rose from 30% (72 DAE) to 83% (93 DAE). The most effective treatments—picoxystrobin + prothioconazole (220), inpyrfluxam + tebuconazole (224.25) and trifloxystrobin + prothioconazole + bixafen (226.75)—each achieved over an 80 % reduction in disease progress and were statistically indistinguishable (Tukey's test, $p \leq 0.05$). These treatments also showed flatter DPCs, limiting final rust severity to ~19.0–19.5% at 93 DAE. Treatments based on picoxystrobin + benzovindiflupyr and picoxystrobin + cyproconazole were slightly less effective (AUDPC 245 and 272.5, respectively), with final severities of ~20.5% and 21.25% at 93 DAE, while multisite fungicides (chlorothalonil, 433.5; mancozeb, 396.5) still delivered over 60 % control. In agreement with their higher AUDPCs, these multisite treatments showed steeper late-season increases and ended at ~32.5% (chlorothalonil) and 28.5% (mancozeb) severity at 93 DAE.

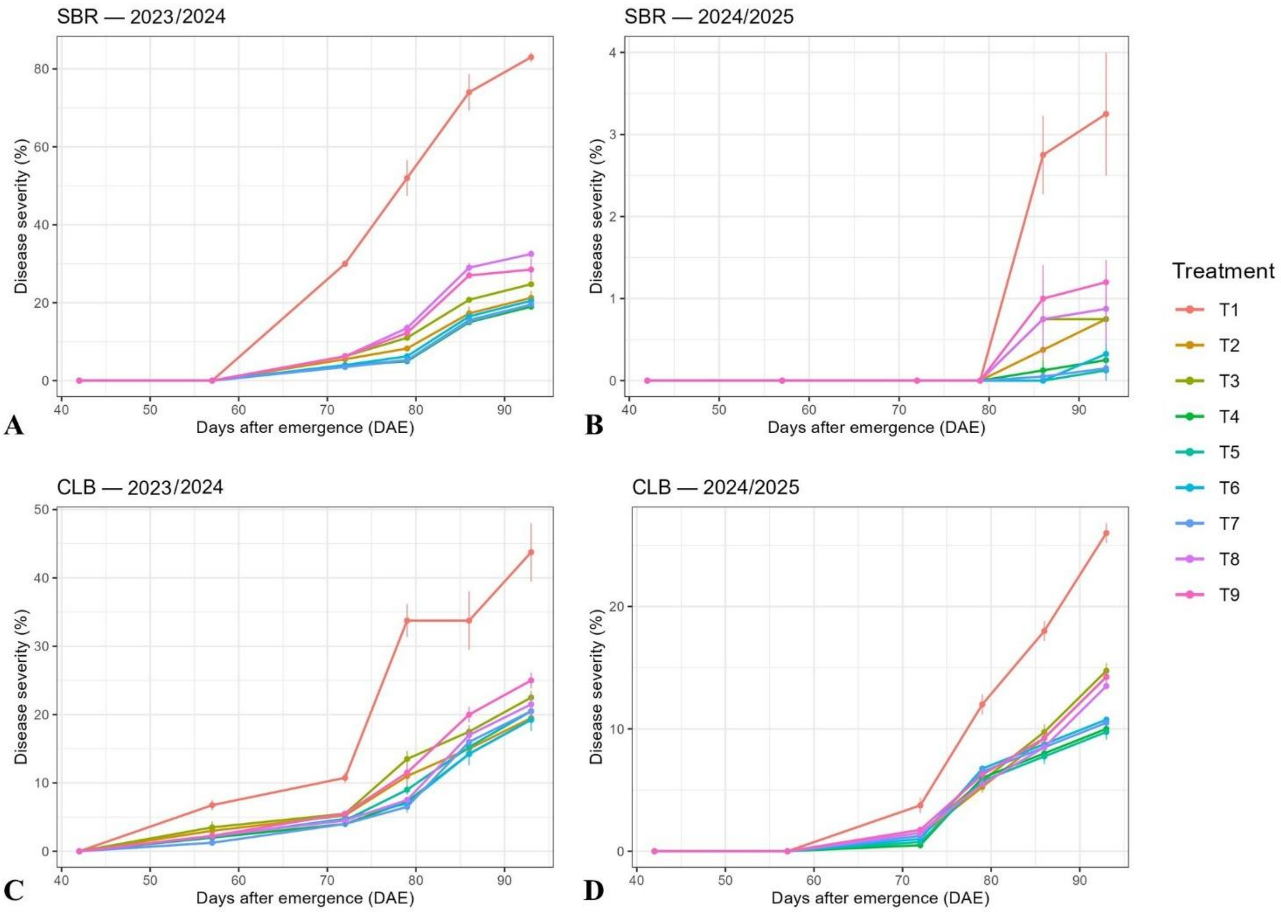

Figure 1. Disease progress curves (DPCs) for soybean rust (SBR) and Cercospora leaf blight (CLB) across two seasons (2023/2024 and 2024/2025). Panels (A) and (B) show SBR in 2023/2024 and 2024/2025, respectively, while panels (C) and (D) show CLB in 2023/2024 and 2024/2025, respectively. Points represent mean disease severity (%) ± SE (n = 4) at 42, 57, 72, 79, 86, and 93 days after emergence (DAE). Treatments were: T1, non-treated control; T2, picoxystrobin + cyproconazole; T3, trifloxystrobin + cyproconazole; T4, picoxystrobin + prothioconazole; T5, trifloxystrobin + prothioconazole + bixafen; T6, picoxystrobin + benzovindiflupyr; T7, inpyrfluxam + tebuconazole; T8, chlorothalonil; and T9, mancozeb (see Table 1 for formulation details and application rates).

During the 2024/2025 season, overall rust pressure was markedly lower and symptoms appeared late: the three leading systemic mixtures reduced AUDPC values to below 6 (0.5–5.25), whereas the untreated check reached 117.25 (Table 2). Disease progress curves showed near-zero severity through 79 DAE, with the untreated control reaching only 3.25% at 93 DAE; most treatments remained ≤1.2% at 93 DAE (and ≤0.25% for the best-performing systemic mixtures). Because absolute severities were low, treatment separation in the DPCs was necessarily limited; nevertheless, the AUDPC rankings and final severities consistently favored the best multi-MOA premixes over the untreated control.

Cercospora leaf blight followed a similar pattern. In 2023/2024, Picoxystrobin + Prothioconazole and Picoxystrobin + Benzovindiflupyr achieved the lowest AUDPCs (231.85 and 232.75, respectively), representing roughly a 65 % reduction relative to the control (663.25) (Table 2). The DPCs indicate that CLB symptoms were detectable earlier than rust (already at 57 DAE in the control) and increased from 6.75% (57 DAE) to 43.75% (93 DAE) in the untreated control. Other QoI + DMI or QoI + SDHI mixtures (e.g., trifloxystrobin + cyproconazole, 315; picoxystrobin + benzovindiflupyr, 232.75) and the multisite products maintained intermediate control (262.5–327.25). In line with AUDPC rankings, systemic mixtures generally limited final CLB severity to ~19–22.5% at 93 DAE, whereas mancozeb reached 25% at 93 DAE**.** In 2024/2025, the same systemic mixtures again outperformed all others, lowering AUDPC to approximately 130–147, while the untreated control reached 314.25 (Table 2). Disease progress curves showed CLB increasing from 3.75% (72 DAE) to 26% (93 DAE) in the untreated control, while the best treatments limited final severity to ~9.75–10.5% at 93 DAE.

Overall, mixtures of systemic fungicides with different modes of action consistently provided the deepest and most stable disease suppression across both seasons. Importantly, the DPCs mirrored the AUDPC results, showing delayed symptom build-up and reduced epidemic rates under the best systemic mixtures, whereas multisite fungicides provided intermediate suppression with more pronounced late-season increases. Multisite treatments, though less potent, still offered substantial reductions and may be favored when cost efficiency is a priority.

Table 2. Area Under the Disease Progress Curve (AUDPC) for Soybean Rust (*Phakopsora pachyrhizi*) and Cercospora Leaf Blight (*Cercospora kikuchii*) in Soybean, 2023/2024 and 2024/2025 Seasons.

Treatment	**AUDPC SBR 2023/2024**	**AUDPC SBR 2024/2025**	**AUDPC CLB 2023/2024**	**AUDPC CLB 2024/2025**
Picoxystrobin + Prothioconazole	220 d	2.25 $^{\text{NS}}$	231.85 cd	134.75 b
Inpyrfluxam + Tebuconazole	224.25 d	0.50	243.25 cd	146.12 b
Trifloxystrobin + Prothioconazole + Bixafen	226.75 d	0.75	257.25 bcd	131.25 b
Picoxystrobin + Benzovindiflupyr	245 d	1.00	232.75 d	149.62 b
Picoxystrobin + Cyproconazole	272.50 cd	5.25	268.62 bcd	155.70 b
Trifloxystrobin + Cyproconazole	331.25 bcd	8.00	315.00 bc	165.37 b
Chlorothalonil	433.50 bc	8.50	262.50 bcd	150.50 b
Mancozeb	396.50 b	11.50	327.25 b	164.50 b
Control	1 277.75 a	117.25	663.25 a	314.25 a
CV (%)	**12.16**	**34.00**	**12.89**	**11.88**

Different letters within each column indicate significant differences according to Tukey's test (p ≤ 0.05). NS = Not significant at the 5% significance level (Tukey's test, p ≤ 0.05).

Control Efficacy (CE)

In both seasons, all fungicide treatments provided substantial suppression of SBR compared to the control. During 2023/2024, the three top-performing mixtures, Inpyrfluxam + Tebuconazole, Picoxystrobin + Prothioconazole, and Trifloxystrobin + Prothioconazole + Bixafen, each achieved over 82 % SBR control efficacy (CE), with no significant differences among them (82.25–82.78 %) (Table 3). Picoxystrobin + Benzovindiflupyr also performed well (80.83 %), while Picoxystrobin + Cyproconazole (78.68 %) and Trifloxystrobin + Cyproconazole (74.08 %) offered moderate CE. Multisite products Chlorothalonil and Mancozeb provided 66.08 % and 68.97 % CE, respectively.

In 2024/2025, SBR CE remained high across programs despite the low and late rust pressure observed in that season. Pre-mixed fungicides Trifloxystrobin + Prothioconazole + Bixafen led the trial with 98.39 % efficacy, closely followed by Inpyrfluxam + Tebuconazole (97.58 %) and Picoxystrobin + Benzovindiflupyr (96.77 %). Picoxystrobin + Prothioconazole maintained 92.74 % CE, whereas Picoxystrobin + Cyproconazole and Trifloxystrobin + Cyproconazole dropped to 83.06 % and 74.19 %, respectively. Multisite products remained effective, yielding 72.58 % (Chlorothalonil) and 62.90 % (Mancozeb).

Control efficay of CLB followed a parallel trend. In 2023/2024, Picoxystrobin + Prothioconazole and Trifloxystrobin + Prothioconazole + Bixafen each delivered approximately 65 % efficacy, significantly outperforming the untreated plots. Other systemic mixtures ranged from 52 to 61 % CE, while Chlorothalonil and Mancozeb achieved 60.41 % and 50.64 %, respectively (Table 3). The second season saw an overall increase in efficacy. Fungicides Trifloxystrobin + Prothioconazole + Bixafen reached 82.42 % CE, Inpyrfluxam + Tebuconazole 81.54 %, and Picoxystrobin + Prothioconazole 78.39 % (Table 3).

Table 3. Control efficacy (%) of fungicide treatments against soybean rust (SBR) and Cercospora leaf blight (CLB) in 2023/2024 and 2024/2025

Treatment	**CE SBR (%) 2023/2024**	**CE SBR (%) 2024/2025**	**CE CLB (%) 2023/2024**	**CE CLB (%) 2024/2025**
Picoxystrobin + Prothioconazole	82.78 a	92.74 a	65.03 a	78.39 a
Inpyrfluxam + Tebuconazole	82.45 a	97.58 a	64.89 a	81.54 a
Trifloxystrobin + Prothioconazole + Bixafen	82.25 a	98.39 a	63.31 a	82.42 a
Picoxystrobin + Benzovindiflupyr	80.83 ab	96.77 a	61.20 a	85.80 a
Picoxystrobin + Cyproconazole	78.68 bc	83.06 a	59.48 ab	69.20 a
Trifloxystrobin + Cyproconazole	74.08 c	74.19 a	52.49 bc	62.93 a
Chlorothalonil	66.08 d	72.58 a	60.41 a	59.66 a
Mancozeb	68.97 d	62.90 a	50.64 c	55.38 a
Control	0.00 e	0.00 b	0.00 d	0.00 b
CV (%)	**1.85**	**20.6**	**5.6**	**11.3**

Different letters within each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

Grain yield (kg ha^{-1}) and thousand grain weight (TGW)

Across both seasons, fungicide applications significantly increased soybean grain yield compared to the control. In 2023/2024, all systemic and multisite treatments outperformed the control (2 249.75 kg ha^{-1}), with average yields ranging from 2 677.00 to 3 070.75 kg ha^{-1}. Picoxystrobin + Prothioconazole, Inpyrfluxam + Tebuconazole, Trifloxystrobin + Prothioconazole + Bixafen, and Picoxystrobin + Benzovindiflupyr each achieved yields above 2 987 kg ha^{-1} and did not differ significantly among themselves (Tukey's test, $p \leq 0.05$). Treatments combining systemic actives with multisite compounds (Chlorothalonil, Mancozeb) and the QoI + DMI and QoI + SDHI mixes also produced yields (2 677–2 764 kg ha^{-1}) significantly higher than the check.

During 2024/2025 season, yields were uniformly higher, reflecting both improved disease control and favorable growing conditions. All treatments delivered yields between 3 466.67 and 3 689.81 kg ha^{-1}, compared to 3 333.33 kg ha^{-1} in the untreated plots (Table 4). The combination of Trifloxystrobin + Prothioconazole + Bixafen led the trial with 3 663.94 kg ha^{-1}, although it did not differ significantly from other systemic or multisite treatments (Tukey's test, $p \leq 0.05$) (Table 4).

In the 2024/2025 season, several variables showed limited statistical separation among treatments (e.g., yield; Table 4), with all means sharing the same Tukey grouping. This result is consistent with the epidemiological context observed during that season: disease pressure was low and appeared late, especially for soybean rust, for which final severities remained very low and AUDPC values were close to zero in most treated plots. Under these conditions, the expected yield response to fungicide protection is inherently small, and treatment effects can be masked by plot-to-plot variability. Moreover, the 2024/2025 rust dataset exhibited high relative variability (CV = 34% for AUDPC), which further reduces statistical power to detect differences among treatment means even when numerical differences exist. Therefore, for the 2024/2025 season we interpret differences among treatments primarily as numerical trends rather than statistically confirmed superiority, and we avoid describing any program as "leading" or "outperforming" others when mean separation is not supported by the multiple-comparison test.

Thousand-grain weight (TGW) followed a similar pattern of enhancement in both seasons. In 2023/2024, treatments with Picoxystrobin + Prothioconazole and Inpyrfluxam + Tebuconazole achieved the heaviest seeds (130.5 g), significantly above the control (126.25 g), while other treatments ranged from 122.25 to 129.25 g. In 2024/2025, TGW decreased slightly across all plots but remained higher under systemic mixtures (112.5–115.5 g) than in the check (112.25 g) (Table 4).

Table 4. Soybean grain yield and thousand-grain weight under different fungicide treatments, 2023/2024 and 2024/2025 seasons

Treatment	Yield (kg ha^{-1}) 2023/2024	Yield (kg ha^{-1}) 2024/2025	TGW (g) 2023/2024	TGW (g) 2024/2025
Picoxystrobin + Prothioconazole	3 070.75 a	3 513.89 a	130.5 ^{NS}	115.5 ^{NS}
Inpyrfluxam + Tebuconazole	3 029.25 a	3 564.81 a	130.5	115.5
Trifloxystrobin + Prothioconazole + Bixafen	3 014.50 a	3 663.94 a	126.25	112.5
Picoxystrobin + Benzovindiflupyr	2 987.75 a	3 511.57 a	122.25	107.25
Picoxystrobin + Cyproconazole	2 801.50 a	3 689.81 a	129.25	114.25
Chlorothalonil	2 764.75 ab	3 585.65 a	122.25	107.25
Trifloxystrobin + Cyproconazole	2 701.00 ab	3 466.67 a	127.5	112.5
Mancozeb	2 677.00 ab	3 639.81 a	129.25	114.25
Control	2 249.75 b	3 333.33 a	126.25	112.25
CV (%)	**7.64**	**6.48**	**5.64**	**6.4**

Different letters within each column indicate significant differences according to Tukey's test ($p \leq 0.05$). NS = Not significant at the 5% significance level (Tukey's test, $p \leq 0.05$).

Economic analysis

In the 2023/2024 season, all fungicide treatments translated into higher grain yields, gross incomes and net benefits compared to the untreated control (2 249.75 kg ha^{-1}; US$ 701.17 ha^{-1}) (Table 5). Picoxystrobin + Prothioconazole delivered the highest yield (3 070.75 kg ha^{-1}) and gross income (US$ 957.05 ha^{-1}), resulting in a net benefit of US$ 864.05 ha^{-1} and a benefit–cost ratio of 10.29. Although prone to slightly lower yields (2 764.75–2 987.75 kg ha^{-1}), treatments with Chlorothalonil and Mancozeb achieved excellent economic returns (B/C 16.41 and 10.08, respectively) thanks to their low application costs. Trifloxystrobin + Prothioconazole + Bixafen and Picoxystrobin + Benzovindiflupyr also combined solid disease control with attractive net benefits (US$ 831.52 and US$ 835.18 ha^{-1}, B/C 8.70 and 9.70) (Table 5). Overall, the return on investment was highest for multisite products, while systemic mixtures delivered the greatest absolute gains in yield and income.

In the 2024/2025 season, baseline yields were higher and SBR pressure was lower and later, so yield differences among treatments were comparatively small (control 3 333.33 kg ha^{-1}). Yields ranged from 3 466.67 to 3 689.81 kg ha^{-1}, resulting in gross incomes above US$ 1 155 ha^{-1}. Picoxystrobin + Cyproconazole showed the highest numerical yield (3 689.81 kg ha^{-1}) and the highest net benefit (US$ 1 152.14 ha^{-1}; B/C 14.81), followed by Chlorothalonil (US$ 1 141.42 ha^{-1}; B/C 21.22). Trifloxystrobin + Prothioconazole + Bixafen and Picoxystrobin + Prothioconazole maintained high profitability (net benefits ~US$ 1 077–1 113 ha^{-1}; B/C 10.31–11.46). However, when expressed as income gain relative to the untreated control, gains were modest (≈ −5.3% to +3.7%), reflecting that in a low-pressure season the fungicide cost can offset much of the yield benefit in a partial-budget framework.

Table 5. Economic performance of fungicide treatments on soybean, 2023/2024 and 2024/2025 seasons.

Treatment	Yield (kg ha^{-1})	Gross income (US\$ ha^{-1})	Total cost (US\$ ha^{-1})	Cost (US\$ kg^{-1})	Yield increase (kg ha^{-1})	Net margin (kg ha^{-1})	Net margin (US\$ ha^{-1})	Net benefit (US\$ ha^{-1})	Income gain (%)	Benefit –cost ratio
					2023/2024					
Picoxystrobin + Prothioconazole	3 070.75	957.05	93	0.0303	821	522.6	162.88	864.05	23.23	10.29
Inpyrfluxam + Tebuconazole	3 029.25	944.12	135	0.0446	779.5	346.34	107.95	809.12	15.4	6.99
Trifloxystrobin + Prothioconazole + Bixafen	3 014.50	939.52	108	0.0358	764.75	418.22	130.35	831.52	18.59	8.7
Picoxystrobin + Benzovindiflupyr	2 987.75	931.18	96	0.0321	738	429.98	134.01	835.18	19.11	9.7
Picoxystrobin + Cyproconazole	2 801.50	873.13	76.8	0.0274	551.75	305.33	95.16	796.33	13.57	11.37
Chlorothalonil	2 764.75	861.68	52.5	0.019	515	346.55	108.01	809.18	15.4	16.41
Trifloxystrobin + Cyproconazole	2 701.00	841.81	64.8	0.024	451.25	243.33	75.84	777.01	10.82	12.99
Mancozeb	2 677.00	834.33	82.8	0.0309	427.25	161.58	50.36	751.53	7.18	10.08
Control	2 249.75	701.17	0	0	0	0	0	701.17	—	—
					2024/2025					
Picoxystrobin + Prothioconazole	3 513.89	1 171.30	94	0.0268	180.56	-101.44	-33.81	1 077.30	-3.04	12.46
Inpyrfluxam + Tebuconazole	3 564.81	1 188.27	136	0.0382	231.48	-176.52	-58.84	1 052.27	-5.3	8.74
Trifloxystrobin + Prothioconazole + Bixafen	3 663.94	1 221.31	108	0.0295	330.61	6.61	2.2	1 113.31	0.2	11.31
Picoxystrobin + Benzovindiflupyr	3 511.57	1 170.52	96	0.0273	178.24	-109.76	-36.59	1 074.52	-3.29	12.19
Picoxystrobin + Cyproconazole	3 689.81	1 229.94	77.8	0.0211	356.48	123.08	41.03	1 152.14	3.69	15.81
Chlorothalonil	3 585.65	1 195.22	53.8	0.015	252.32	90.92	30.31	1 141.42	2.73	22.22
Trifloxystrobin + Cyproconazole	3 466.67	1 155.56	65	0.0187	133.34	-61.66	-20.55	1 090.56	-1.85	17.78
Mancozeb	3 639.81	1 213.27	82.8	0.0227	306.48	58.08	19.36	1 130.47	1.74	14.65
Control	3 333.33	1 111.11	0	0	0	0	0	1 111.11	—	—

Cost (US\$ kg^{-1}) = Total cost ÷ Yield; Yield increase (kg ha^{-1}) = Treatment yield − Control yield; Net margin (kg ha^{-1}) = Yield increase − (Total cost ÷ Price per kg); Net margin (US\$ ha^{-1}) = Net margin (kg) × Price per kg = (Net benefit$_i$ − Net benefit$_0$); Net benefit (US\$ ha^{-1}) = Gross income − Total cost; Income gain (%) = \($(NB_i - NB_0) \div GI_0$\) × 100; Price of soybean: US\$ 0.312 kg^{-1}

DISCUSSION

This study compares the performance of different fungicide treatments (commercial premixes and multisite protectants) and analyses their economic viability considering observed yield responses and input costs. Beyond ranking treatments by AUDPC, our results have direct implications for FRAC-class resistance management in South American soybean systems, where repeated exposure of *Phakopsora pachyrhizi* populations to single-site fungicides can increase the frequency of less-sensitive genotypes and has been associated with declines in field performance over time (Dalla Lana *et al.*, 2018; Müller *et al.*, 2021; FRAC, 2024). A key finding from the trials is that treatments combining active ingredients from different chemical groups, specifically those including strobilurins (QoI), demethylation inhibitors (DMI), and succinate dehydrogenase inhibitors (SDHI), consistently achieved superior control of disease severity, as demonstrated by lower values of the AUDPC for both SBR and CLB. This pattern is consistent with regional evidence indicating that multi-MOA premixes and well-structured programs often outperform narrower MOA options under soybean foliar disease pressure (Scherm *et al.*, 2009; Barro *et al.*, 2021; Machado *et al.*, 2022). In our trials, premixes such as T5 (trifloxystrobin + prothioconazole + bixafen), T4 (picoxystrobin + prothioconazole), and T7 (inpyrfluxam + tebuconazole) produced the lowest AUDPC values across both seasons, indicating strong potential to interfere with multiple stages of the pathogen infection cycle through preventive and residual activity (Barro *et al*., 2021). Importantly, these mixtures also differ functionally in the resistance pressure they impose adding a third, independent single-site mode of action (SDHI) to a QoI + DMI program can distribute selection across multiple targets rather than repeatedly "pushing" the same target site. In practical terms, QoI + DMI + SDHI programs (e.g., T5) provide a broader biochemical barrier than QoI + DMI programs (e.g., T2), which is expected to be more consistent with resistance-management stewardship when deployed within an anti-resistance strategy (Barro *et al*., 2021; FRAC, 2024). A similar ranking for CLB suggests that these programs can also help stabilize control across the foliar disease complex under field conditions.

Integrated interpretation of the foliar disease complex (SBR + CLB). Because the trials targeted a late-season foliar disease complex rather than a single pathosystem, treatment performance should be interpreted jointly for SBR and CLB. In 2023/2024, disease progress patterns suggested a temporal offset between pathogens: CLB symptoms were detectable earlier, whereas SBR remained negligible until later in the season and then increased rapidly. This timing difference implies that an "ideal" fungicide program must provide early protective/residual activity to suppress CLB while still maintaining persistence to protect against late SBR development.

Although the best premixes ranked among the lowest AUDPC values for both diseases, the magnitude of suppression suggests a differential efficacy profile between pathogens. Under 2023/2024 conditions, the top premixes produced very large reductions in SBR progress (i.e., deep suppression relative to the untreated control), whereas CLB reductions were more moderate, indicating that CLB was comparatively harder to suppress to the same extent under the same spray schedule and field epidemic conditions. This differential response is important for interpretation: a program can appear "excellent" against SBR while still leaving meaningful residual CLB pressure, which may contribute to remaining canopy injury and yield/quality impacts in mixed epidemics.

Regarding synergy or antagonism between diseases or their control, the present study did not include a pathogen manipulation design (e.g., single-disease inoculations) or interaction modeling that would allow inference of true biological synergy/antagonism. Therefore, we do not claim synergy or antagonism as a demonstrated outcome. However, the broadly consistent ranking of treatments for both pathogens suggests that yield protection in these trials is most parsimoniously interpreted as the result of additive suppression of total foliar disease burden, rather than a compound effect operating exclusively through one pathogen.

Finally, the 2024/2025 season provides an important contextual nuance: SBR pressure was markedly lower and symptoms appeared late, which limits statistical separation for SBR and implies that between-treatment differences in agronomic/economic outcomes in that season should be interpreted primarily through the combined disease complex (and particularly CLB) and seasonal conditions, rather than SBR control alone.

Data from the second season (2024/2025) revealed an increase in overall control efficacy, upwards of 98% control for T5 and 97.58% for T7, underscoring that timing and environmental conditions can strongly influence apparent field performance. Notably, "dilution of selection pressure" should not be interpreted as a guarantee: if one component is compromised by reduced sensitivity, the mixture may effectively behave as a reduced-number-of-modes program, increasing selection pressure on the remaining effective target sites. This reinforces FRAC guidance that mixtures must be used with appropriate alternation/rotation of MOAs and with limits on the number of applications per season for any single FRAC group, rather than repeated back-to-back use of the same combination (FRAC, 2024).

A closer look at treatments with protectant-only fungicides, such as T8 (chlorothalonil) and T9 (mancozeb), shows that while they can delay symptom onset by providing a protective barrier and reducing spore germination, they generally do not prevent progressive epidemic development once infection is established. In our trials, both chlorothalonil and mancozeb registered higher AUDPC values than the single-site premixes, with efficacy ranging between 50% and 73%. From a resistance-management perspective, this intermediate technical performance should be interpreted alongside their key benefit: multisite fungicides are low-risk options for resistance selection because they affect multiple cellular targets, making them valuable for reducing selection intensity on single-site classes when integrated appropriately (Machado *et al*., 2022; FRAC, 2024). Because T8 and T9 were evaluated as stand-alone treatments in this study, our results should not be interpreted as evidence of tank-mix performance; rather, they support the stewardship rationale for incorporating multisites as partners or alternation tools in broader spray programs to help preserve single-site efficacy.

The agronomic benefits of effective disease control are clearly illustrated by the grain yield responses observed in the field. In the 2023/2024 season, treatments such as T4, T7, and T5 enabled yields that exceeded 3000 kg ha^{-1}, whereas control plots only reached about 2250 kg ha^{-1}. Yield improvement is especially valued in the context of late-season diseases, which tend to compromise assimilate translocation by reducing the photosynthetically active leaf area. In this regard, the integrated use of fungicide premixes with multiple modes of action that maintain foliar health has allowed the soybean crop to sustain a stable production level even under conditions of moderate to high disease pressure (Hartman *et al*., 2015).

Furthermore, aside from the final grain yield, the quality of the harvest, as reflected by the TGW was positively affected by the more efficacious treatments. Fungicide mixtures that provided superior protection against foliar diseases preserved the integrity of the crop's canopy during the grain-filling period, which in turn led to heavier, higher-quality seeds. Treatments with T4 and T7 recorded the highest TGW values compared to those with lower control efficacy such as T8, which did not manage to maintain adequate foliar protection throughout the crop cycle. This supports a management logic in which robust MOA programs reduce epidemic growth rate and extend functional canopy duration, thereby improving both yield and seed quality while enabling more sustainable use of the available FRAC classes.

A critical component of the study is the economic analysis, which goes beyond mere agronomic performance to assess the financial viability of the fungicide regimes for producers operating under constrained budgets. In the 2023/2024 season, the treatment with T4 (picoxystrobin + prothioconazole) registered a net benefit of US$ 864.05 ha^{-1} and a benefit/cost (B/C) ratio of 10.29. This indicates that for every dollar invested, the economic return was more than ten-fold. Treatments with T5 and T7 also demonstrated strong profitability indices, reinforcing the notion that integrating multiple modes of action not only ensures technical disease control but also translates into higher net incomes for soybean producers. From a regional stewardship perspective, this is relevant because economic feasibility is often the limiting factor for adopting rotation/alternation schemes; demonstrating that effective multi-MOA programs can remain profitable increases the likelihood that growers will adopt resistance-management recommendations rather than relying on repeated applications of a single MOA.

During the 2024/2025 season, gross incomes were higher mainly because overall yields were higher, but the net advantage over the untreated control was smaller because the control yield was also high under the season's conditions. Notably, even protectant-only fungicides such as T8 (chlorothalonil) and T9 (mancozeb) remained economically attractive under these conditions, with net benefits above US$ 1 130 ha^{-1} and chlorothalonil achieving the highest B/C ratio (21.22), while mancozeb also delivered a favorable return (B/C 13.65). When income gain is expressed relative to the untreated control (IG), gains ranged from slightly negative to modestly positive (e.g., ~+3.7% for T2 and ~+2.7% for T8), highlighting that under reduced disease pressure, product cost becomes a stronger determinant of profitability than marginal differences in technical efficacy. This also has implications for resistance management: when disease pressure is low, it may be strategically advantageous to rely more on low-risk multisites and reduce the number of single-site applications, thereby lowering seasonal selection pressure on QoI/DMI/SDHI classes without compromising the partial-budget outcome (Barro, 2022).

Finally, it is important to clarify that while multi-MOA premixes and multisite integration are widely recommended as resistance-management strategies, our study did not assess in vitro sensitivity, pathogen population structure, or temporal shifts in resistance frequencies. Therefore, we present the resistance-management rationale based on established stewardship principles rather than as a demonstrated resistance outcome of these trials (FRAC, 2024; Müller *et al*., 2021). Overall, our findings align with regional evidence that effective disease suppression and economic returns can be achieved while supporting stewardship through deliberate use of multiple FRAC classes, avoidance of repeated single-MOA exposure, and strategic incorporation of low-risk multisites (Scherm *et al*., 2009; Dalla Lana *et al*., 2018; Machado *et al*., 2022).

CONCLUSION

The combined results of efficacy, yield, and economic analyses provide compelling evidence that fungicide mixtures, particularly those combining QoI, DMI, and SDHI modes of action, represent a beneficial practice for managing late-season foliar diseases in soybean. For Paraguay, where production intensification and market competitiveness are of utmost importance, these findings offer a technical and economical pathway to protect yields and extend the durability of fungicidal chemistries.

AUTHORS' CONTRIBUTION

MJMD led the conceptualization and methodological design; conducted the investigation, carried out validation and statistical analyses, and contributed to writing – review & editing; ACZC, NRVC, MAFG, MMO and AAA each performed investigation, validation, and writing – review & editing; and GAEM provided visualization, overall supervision, and prepared the original draft.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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