

# Sequential application of fungicides with different modes of action for the control of soybean diseases in Canindeyú, Paraguay

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## ABSTRACT

This study evaluated the impact of fungicide programs (FPs) on disease control in soybean during the 2021/2022 season in La Paloma, Katueté and Nueva Esperanza (Canindeyú, Paraguay). The area under the disease progress curve (AUDPC) was evaluated for the predominant diseases, defoliation, and yield. In addition, the control efficacy (CE) and the reduction in productivity (RP) were calculated. Analysis of variance was conducted on factors FP, Location, and their interaction, using Tukey's test at a 5 % error level for mean comparisons. Target spot (TS) and *Cercospora* leaf blight (CLB) were the predominant diseases in all locations. Asian soybean rust (ASR) occurred only in Nueva Esperanza and Katueté. FPs reduced AUDPC up to 64, 85 and 73 %, on average, for TS, ASR and CLB, respectively, leading to increased yield, on average, between 9 and 27 %, compared to the control. La Paloma achieved the highest yield (4651 kg ha<sup>-1</sup>) and the lowest defoliation (61 %), followed by Katueté (4330 kg ha<sup>-1</sup> with 70 % of defoliation), and Nueva Esperanza (3739 kg ha<sup>-1</sup> with 86 % of defoliation). This study provides strategies to optimize fungicide use in soybean management in Paraguay, highlighting sequential applications and rotation of modes of action.

**Keywords:** *Cercospora kikuchii*, *Corynespora cassiicola*, *Glycine max*, *Phakopsora pachyrhizi*

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## RESUMEN

Este estudio evaluó el impacto de programas de fungicidas (PFs) en el control de enfermedades en soja durante la temporada 2021/2022 en La Paloma, Katueté y Nueva Esperanza (Canindeyú-Paraguay). Se evaluó el área bajo la curva de progreso de la enfermedad (ABCPE), defoliación y rendimiento. Se calculó la eficacia de control (EC) y reducción de productividad (RP) mediante análisis de varianza, incluyendo PF, Localidad y su interacción, con la prueba de Tukey al 5 %. La mancha anillada (MA) y el tizón foliar por *Cercospora* (TFC) fueron las enfermedades predominantes en todas las localidades. La roya asiática de la soja (RAS) ocurrió solo en Nueva Esperanza y Katueté. Los PFs redujeron el ABCPE hasta 64, 85 y 73 %, en promedio, para MA, RAS y TFC, respectivamente, lo que llevó a un aumento del rendimiento promedio, entre el 9 y el 27 %, en comparación con el control. La Paloma logró el mayor rendimiento (4651 kg ha<sup>-1</sup>) y menor defoliación (61 %), seguido de Katueté (4330 kg ha<sup>-1</sup>, 70 %) y Nueva Esperanza (3739 kg ha<sup>-1</sup>, 86 %). Este estudio ofrece estrategias para optimizar el uso de fungicidas en soja en Paraguay, destacando aplicaciones secuenciales y rotación de modos de acción.

**Palabras clave:** *Cercospora kikuchii*, *Corynespora cassiicola*, *Glycine max*, *Phakopsora pachyrhizi*

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## INTRODUCTION

Soybean is the most important agricultural crop in Paraguay, holding a significant role in the national economy. Presently, the country produces 9.5 million tons of grains across 3.5 million hectares, making it the sixth-largest global producer and the third-largest exporter (SoyStats, 2021). Over the last two decades, soybean cultivation has emerged as a crucial contributor to the economy, fostering foreign currency inflow and employment

opportunities across various sectors such as industry and transportation (Cámara Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas, 2024).

Leaf diseases have a negative impact on soybean crops in Paraguay, potentially significantly affecting yields in some seasons (Wrather et al., 2010). The most important disease is Asian soybean rust (ASR) (*Phakopsora pachyrhizi*), which can cause yield losses of up to 60 % when

fungicides are not applied (Yorinori et al., 2005). Additionally, other foliar diseases (known as late-season soybean diseases) such as brown spot (*Septoria glycines*) and Cercospora leaf blight (CLB) (*Cercospora kikuchii*) can result in up to 15 % yield losses (Wrather et al., 2010). Moreover, Paraguayan farmers have recently reported that diseases like frog-eye leaf spot (*Cercospora sojina*) and target spot (TS) (*Corynespora cassiicola*) are occurring more frequently in soybean production fields (Caballero-Mairesse et al., 2024). The increase in the incidence and severity of these diseases may be associated with the expansion of the cultivated area under the no-till and monoculture system (Wrather et al., 2010), factors that favor the survival of pathogen inoculum in crop residues from one planting season to another. In addition, environmental factors critically influence the prevalence of soybean diseases. ASR thrives in conditions of 21–28 °C and over 75 % humidity, aiding spore germination and fungal penetration (Nunkumar et al., 2009). TS prefers warm, moist climates with 10–35 °C (optimum of 30 °C) temperatures and around 80 % humidity, enhancing spore dispersal and germination (Rondon and Lawrence, 2021). Similarly, CLB prospers in high humidity (80 %) and 23–27 °C, with sustained leaf wetness fostering pathogen spread in soybean fields (Lavilla et al., 2022).

The management of soybean diseases can be achieved through different strategies. In order to reduce the inoculum of *P. pachyrhizi*, it is recommended to apply sanitary vacuum (which diminishes the number of volunteer plants out of season), to use early-cycle cultivars, and to sow at the recommended start of the season (Godoy et al., 2016). Meanwhile, for both rust and late-season diseases, resistant cultivars could be employed, monitoring them from the beginning of their development to determine the best time for chemical control: before or after symptoms appear (Hartman et al., 2015).

In Paraguay, the use of fungicides is the primary strategy employed for soybean disease management, with particular attention given to ASR due to its economic impact. Site-specific fungicide premixes are used, combining two or three different modes of action: sterol biosynthesis inhibitors (DMIs), quinone outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs). Additionally, these fungicides are applied in conjunction with multisite and protective fungicides (mancozeb, chlorothalonil, copper oxychloride) starting from 35 days after emergence (DAE), and subsequently at intervals of approximately 15 to 21 days, until completing four to five applications

(Mendoza-Duarte et al., 2023; Enciso-Maldonado et al., 2021).

The present study aimed to evaluate the efficiency of programs based on sequential applications of fungicides with different modes of action on soybean diseases, and their impact on productivity in Canindeyú, Paraguay, during the 2021/2022 season.

## MATERIALS AND METHODS

### Locations and experimental period

The trials were conducted in three locations within the Department of Canindeyú (La Paloma [-24.126860, -54.539684], Katueté [-24.258429, -54.811421], and Nueva Esperanza [-24.545126, -54.858601]) during the 2020/2021 growing season, in production plots where no-till cultivation has been practiced for over ten years. The soil in this region has a predominantly clayey texture, with an altitude ranging from 350 to 370 meters above sea level. The climate is subtropical humid mesothermal, with an average temperature of 23 °C and an annual precipitation of 1300 mm (Dirección de Meteorología e Hidrología, 2021).

### Field experiment

The trials consisted of the sequential application of fungicides with different modes of action for the control of soybean diseases, henceforth will call fungicide programs (FP) (Table 1). In each location, 13 FP and a control distributed under a complete randomized block design with four repetitions were evaluated. The experimental unit consisted of experimental plots of 24 m<sup>2</sup> (8 m long and 3 m wide). A useful plot was delimited within the experimental unit from which the measurement variables were taken. To determine the useful plot, 1 m was removed from each end and 0.5 m from each side of the experimental unit, resulting in a total of 12 m<sup>2</sup> (6 m long and 2 m wide).

### Agronomic management of experimental plots

Prior to the installation of the trials, field desiccation was carried out to reduce weed populations, using 30 g ha<sup>-1</sup> of halauxifen-methyl 11.5 % + Diclosulam 58 % (Texaro®, Corteva Agriscience Paraguay S. A.) combined with 2 L ha<sup>-1</sup> of glyphosate 60.8 % (Panzer Gold®, Corteva Agriscience Paraguay S. A.). Subsequently, ten days after the first application, a sequential

**Table 1.** Sequence of fungicides with different modes of action applied at 35, 50, 65, and 80 days after emergence (DAE) in La Paloma, Katueté, and Nueva Esperanza (Canindeyú, Paraguay), season 2021/2022

FP <sup>a</sup>	Fungicide application timing			
	35 DAE	50 DAE	65 DAE	80 DAE
FP0	Control	Control	Control	Control
FP1	Viovan® <sup>1</sup>	Vessarya® + Dithane®	Viovan® + Dithane®	
FP2	Aproach® Power <sup>2y</sup>	Viovan®	Vessarya® + Dithane®	Viovan® + Dithane®
FP3	Viovan®	Vessarya® + Dithane®	Viovan® + Dithane®	Aproach® Power
FP4	Viovan®	Vessarya®	Viovan®	Aproach® Power
FP5	Viovan®	Vessarya® + Dithane®	Viovan® + Dithane®	Aproach® Power + Dithane®
FP6	Viovan® + Dithane® <sup>3</sup>	Vessarya® + Dithane®	Viovan® + Dithane®	Aproach® Power
FP7	Vessarya® <sup>4</sup> + Tebuconazole 43 SC <sup>5</sup>	Viovan® + Dithane®	Aproach® Power + Dithane®	
FP8	Vessarya® + Dithane®	Viovan® + Dithane®	Aproach® Power + Dithane®	
FP9	Vessarya® + Cypress® <sup>6</sup>	Viovan® + Dithane®	Aproach® Power + Dithane®	
FP10	Mazen® <sup>7</sup> + Cypress®	Viovan® + Dithane®	Aproach® Power + Dithane®	
FP11	Mazen® Forte <sup>8</sup>	Mazen® + Cypress®	Aproach® Power + Dithane®	
FP12	Cripton® Xpro <sup>9</sup>	Cripton® Xpro + Dithane®	Aproach® Power + Dithane®	
FP13	Ativum® <sup>10</sup> + Dithane®	Ativum® + Dithane®	Aproach® Power + Dithane®	

<sup>a</sup>Fungicide program; <sup>1</sup>Picoxystrobin 10.00 % + Prothioconazole 11.67 % (600 cc ha<sup>-1</sup>); <sup>2</sup>Picoxystrobin 9.00 % + Cyproconazole 4.00 % (600 cc ha<sup>-1</sup>); <sup>3</sup>Mancozeb 80.00 % (1500 cc ha<sup>-1</sup>); <sup>4</sup>Picoxystrobin 10.25 % + Benzovindiflupyr 5.13 % (600 cc ha<sup>-1</sup>); <sup>5</sup>Tebuconazole 43.00 % (250 cc ha<sup>-1</sup>); <sup>6</sup>Cyproconazole 15.00 % + Difenconazole 25.00 % (300 cc ha<sup>-1</sup>); <sup>7</sup>Benzovindiflupyr 15.00 % + Azoxystrobin 30.00 % (200 g ha<sup>-1</sup>); <sup>8</sup>Benzovindiflupyr 7.50 % + Prothioconazole 15.00 % (450 cc ha<sup>-1</sup>); <sup>9</sup>Bixafen 12.50 % + Prothioconazole 17.50 % + Trifloxystrobin 15.00 % (500 cc ha<sup>-1</sup>); <sup>10</sup>Fluxapyroxad 5.00 % + Epoxiconazole 5.00 % + Pyraclostrobin 8.10 % (800 cc ha<sup>-1</sup>).

<sup>y</sup>The first application of this treatment was carried out twenty-five days after the emergency.

application was performed with 2 L ha<sup>-1</sup> of ammonium glufosinate 40 % (IRATO 40®, Agrofertil S. A., Paraguay). The experiments were established on November 5, 7, and 8, 2021, in La Paloma, Katueté, and Nueva Esperanza, respectively. In all three locations, seeds of the variety M 5947 IPRO, with indeterminate growth and maturity group 5.9, were used. The seeds were treated with 200 mL per 100 kg of seeds with Rizoliq® TOP (Rizobacter, Argentina), which includes a concentration of 1 x 10<sup>10</sup> colony-forming units per mL of *Bradyrhizobium japonicum*, 150 mL for every 100 kg of seeds of chlorantraniliprole 62.5 % (Dermacor®, Corteva Agriscience Paraguay S. A.), and 100 mL per 100 kg of seeds of ipconazole 2.5 % + metalaxyl 2 % (Rancona® Dimension, Corteva Agriscience Paraguay S. A.). As a base fertilizer, 250 kg ha<sup>-1</sup> of the 04-30-10 formulation (Bunge Clasic Mix 04-30-10, Bunge Paraguay S. A., Paraguay) was applied, and a top-dressing of 50 kg ha<sup>-1</sup> of KCl (Mosaic Fertilizantes, Brazil) was applied thirty days after emergence. Planting was carried out with a 20-row seeder (model 1745, John Deere, Kurosu & Cia. S. A., Paraguay) powered by a 150 hp tractor (model 6150J, John Deere, Kurosu & Cia. S. A., Paraguay). The planting density was 222,222 seeds per hectare (10 seeds per linear meter with 0.45 m row spacing). Control of bugs

and caterpillars was carried out with insecticides as needed.

### Fungicide application

Fungicide applications were carried out at four different times: 35, 50, 65, and 80 days after emergence (approximately in phenological stages V5-V7, R1, R3, and R5 [Fehr et al. 1971]). FP2, however, had the first application twenty-five days after emergence (approximately in V3), following the “T0 spray” criterion (*aplicación cero* in Spanish). “T0 spray” is a very widespread practice in recent years aimed at reducing the inoculum of pathogens causing late-season soybean diseases in Paraguay and leaf spots on wheat (Enciso-Maldonado et al., 2021; Van den Berg et al., 2016).

A pressurized backpack sprayer with CO<sub>2</sub> was used. It had a flow rate of 120 L ha<sup>-1</sup> and six conical nozzles, model M053, spaced at 50 cm, operating at a pressure of 60 PSI.

### Variables evaluated

The severity of the diseases was evaluated ten days after each application, within the useful

plot, comparing ten trefoils of the middle stratum of the crop with the diagrammatic scales of ASR (Godoy et al., 2006), TS (Soares et al., 2009) and CLB (Ivancovich and Lavilla, 2016). The area under the disease progression curve (AUDPC) for each disease (Shaner and Finney, 1977) and the control efficacy (CE) of fungicides were calculated with the formula

$$CE = [(Control\ infection - Infection\ Treatment) / Control\ infection] * 100.$$

When the crop reached the phenological stage of harvest maturity (R8), manual harvesting was performed from the useful plot of each experimental unit. Yield data was acquired by threshing the plants using a motorized thresher (Vencedora B 350, AgroCenter, Ciudad del Este, Paraguay). The weight was determined on an electronic scale (AJ150, Mettler Toledo, Columbus, Ohio, United States), and the value was divided by the harvested area and then extrapolated to kg ha<sup>-1</sup>. Subsequently, the yield was adjusted to 13 % moisture.

The reduction in productivity (RP) of all evaluated treatments was calculated with respect to the treatment that achieved the highest yield. To do this, the following formula was applied:  $RP (\%) = [1 - (Yield\ obtained\ from\ the\ best\ treatment / Observed\ yield)] * 100.$

### Statistical analyses

The effect of location, treatments, and the interaction between both factors on AUDPC and performance was studied. For this, an analysis of variance (ANOVA) was carried out following the instructions described in the SAS software (version 9.4) for a completely randomized design with a factorial arrangement. To compare the means of the treatments, the Tukey test was applied at 5 % probability.

## RESULTS AND DISCUSSIONS

The predominant diseases during the experimental period were TS and CLB in all three study locations. However, ASR occurred only in Nueva Esperanza and Katueté. In Nueva Esperanza, TS, ASR, and CLB reached severity values in the controls of 20 %, 14 %, and 12 %, respectively, while with FP, the severity ranged between 13-14 %, 3-4 %, and 5-6 %, respectively. In the controls of Katueté, TS, ASR, and CLB reached severity values of 22 %, 5 %, and 14 %, respectively, while with FP, the severity was between 10-14 %, 1 %, and 4-7 %, respectively. Finally, in La Paloma, the controls reached 34 % and 14 %, and the FP reduced severity between 15-20 % and 7-9 %, respectively, for TS and CLB.

Fungicides programs, Location and the interaction between FP x Location was significant for all evaluated variables (Table 2). FPs showed a marked decrease in AUDPC for TS, ASR, and CLB with fungicide application, indicating effective disease suppression. For TS, CE varied between FPs, with FP2, FP6 and FP13 exhibiting the highest CE values (61-64 %), while for ASR there was not much difference between the FPs evaluated, showing CE values between 81 % and 84 %. On the other hand, FP2 and FP6 showed the highest CE for CLB, between 66 % and 69 %, respectively (Table 3). Defoliation was significantly reduced in all fungicide-treated plots compared to the untreated control (FP0), with the lowest levels observed in FP2 and FP13 (66 % and 70 %, respectively) (Table 3). Yield outcomes were enhanced under fungicide treatment, with FP13 yielding the highest (4615 kg ha<sup>-1</sup>), followed by PF2, PF3, PF4, PF5, and PF6, all of which achieved yields ranging from 4286 to 4365 kg ha<sup>-1</sup> without significant statistical variation (Table 3), illustrating the positive correlation between fungicide application and crop productivity. Notably, productivity loss

**Table 2:** Summary of probability values for AUDPC (Area Under the Disease Progress Curve) of Target spot (TS), Asian soybean rust (ASR), and Cercospora leaf blight (CLB), as well as soybean defoliation (Def) and yield, according to fungicide programs (FP), location, interaction between both factors, and blocks in the 2021/2022 season

Factor	AUDPC			Def	Yield
	TS	ASR	CLB		
FP	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Location	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
FP x Local	< 0.0001	< 0.0001	0.0008	0.0029	< 0.0001
Block	0.1770	0.5864	0.3021	0.2392	0.9567
CV (%)	9.6	26.0	32.7	4.1	5.8

**Table 3.** Effect of fungicide programs (FP) on Area Under the Disease Progress Curve (AUDPC) values and fungicide control efficacy (CE) on Target spot (TS), Asian soybean rust (ASR), and Cercospora leaf blight (CLB), soybean defoliation (Def), yield and reduction in productivity (RP) in the three study locations (Nueva Esperanza, Katueté, and La Paloma), season 2021/2022

FP <sup>s</sup>	TS		ASR		CLB		Def (%)	Yield (kg ha <sup>-1</sup> )	RP (%)
	AUDPC	CE (%)	AUDPC	CE (%)	AUDPC	CE (%)			
FP0	1284 d <sup>x</sup>	0	91 b	0	398 b	0	83 d	3631 a	27
FP1	605 bc	53	16 a	82	136 a	66	72 bc	4257 bcd	8
FP2	468 a	64	15 a	84	106 a	73	66 a	4286 bcde	8
FP3	541 abc	58	15 a	84	135 a	66	70 b	4361 cde	6
FP4	613 c	52	16 a	82	147 a	63	73 bc	4292 cde	8
FP5	537 abc	58	17 a	81	143 a	64	72 bc	4337 cde	6
FP6	480 a	63	16 a	82	122 a	69	69 ab	4365 cde	6
FP7	537 abc	58	16 a	82	134 a	66	72 bc	4498 de	3
FP8	591 bc	54	16 a	82	134 a	66	72 bc	4243 bcd	9
FP9	589 bc	54	15 a	84	146 a	63	72 bc	3954 ab	17
FP10	611 bc	52	15 a	84	155 a	61	75 c	4210 bcd	10
FP11	540 abc	58	15 a	84	150 a	62	73 bc	4220 bcd	9
FP12	531 abc	59	14 a	85	139 a	65	73 bc	4092 bc	13
FP13	505 a	61	17 a	81	133 a	67	70 b	4615 e	0

<sup>s</sup> FP: Fungicides programs

was minimized in fungicide-treated plots, with FP13 maintaining the highest productivity against disease threats, while the RP of the other FPs was between 3 % and 17 %, and the untreated control (FP0) showed a RP of 27 % (Table 3). However, upon analyzing the FPs by location, it was found that in Nueva Esperanza, FP12 yielded the highest, with the RP for other FPs varying from 0.3 % to 5.4 % and reaching 11.9 % in the untreated control (Table 5). In Katueté, FP7 led to the maximum yield, whereas other FPs showed a yield decrease ranging from 0.4 % to 19.1 %, compared to a 28.1 % reduction in the control (Table 6). In La Paloma, FP13 exhibited the highest yield, with other FPs experiencing a yield decline between 5 % and 32.8 %, and the untreated control showing up to a 45.1 % reduction in productivity (Table 7).

The effect of the FP x Location interaction indicates that the variables evaluated differ in their response depending on the location; therefore, a fungicide program could be the most efficient to reduce the disease in one location, while not in another. For example, FP11, FP12, and FP13 were the most efficient in reducing the AUDPC of TS with a CE between 61-62 % in Nueva Esperanza (Table 5), while in Katueté, FP2, FP6 and FP13 were the most efficient with an CE between 65-68 % (Table 6), and in La Paloma, FP6 stood out the most with a CE of 62 % (Table 7). Regarding ASR, the effect was similar in Nueva Esperanza and Katueté; the AUDPC of the controls differed from those of the treatments based on FP, where all of them had the same effect in reducing the AUDPC of ASR and

maintaining the CE of fungicides between 82 % and 86 % in Nueva Esperanza, and between 74 % and 80 % in Katueté. Similarly, in each location the FPs had the same effect in reducing the AUDPC of CLB compared to the control.

The percentage of defoliation observed in the study varied significantly across the three locations (Table 2), with Nueva Esperanza recording the highest rate at 86 %, followed by Katueté at 70 %, and La Paloma exhibiting the lowest rate at 61 % (Table 4). This gradient in defoliation suggests that local environmental or management factors might have played a crucial role in the extent of leaf loss due to disease pressures. Defoliation directly impacts the photosynthetic capacity of soybean plants and, consequently, can significantly affect yield (Maidana-Ojeda et al., 2021). La Paloma, with the lowest percentage of defoliation of 61 %, also had the highest yield, indicating a possible correlation between lower defoliation levels and higher productivity. The absence of ASR in this location could have contributed to the reduced defoliation, underscoring the importance of disease management and local environmental conditions in mitigating leaf loss, and enhancing yield.

The Location factor significantly influenced soybean yields, as evidenced by the yield discrepancies among La Paloma, Katueté, and Nueva Esperanza. Despite similar edaphoclimatic conditions and consistent phytosanitary management across these locations, yield variation was notable. La Paloma achieved the

**Table 4.** Effect of Location on Area Under the Disease Progress Curve (AUDPC) values on Target spot (TS), Asian soybean rust (ASR), and Cercospora leaf blight (CLB), soybean defoliation (Def), and yield in Nueva Esperanza (NE), Katueté (K), and La Paloma (LP) (Canindeyú, Paraguay), season 2021/2022

Location	AUDPC			Def (%)	Yield (kg ha <sup>-1</sup> )
	TS	ASR	CLB		
NE	180 a <sup>x</sup>	36 b	45 a	86 c	3739 a
K	653 b	6 a	203 b	70 b	4330 b
LP	974 c	NA <sup>y</sup>	221 b	61 a	4651 c

<sup>x</sup> Mean ranks with the same letter are not significantly different according to the Scott-Knott test with probability of  $\alpha = 0.05$ .

<sup>y</sup> No applicable (NA) = not determined due to lack of disease symptoms development.

**Table 5.** Effect of Fungicide Programs (FP) on Area Under the Disease Progress Curve (AUDPC) values and fungicide control efficacy (CE) on Target spot (TS), Asian soybean rust (ASR), and Cercospora leaf blight (CLB), soybean defoliation (Def), yield, and reduction in productivity (RP) in Nueva Esperanza (Canindeyú, Paraguay), season 2021/2022

FP	TS		ASR		CLB		Def (%)	Yield (kg ha <sup>-1</sup> )	RP (%)
	AUDPC	CE (%)	AUDPC	CE (%)	AUDPC	CE (%)			
FP0	379 b <sup>x</sup>	0	159 b	0	141 b	0	94 b	3436 a	11.9
FP1	177 a	53	25 a	84	38 a	73	85 a	3752 ab	2.5
FP2	160 a	58	26 a	84	33 a	76	83 a	3833 b	0.3
FP3	177 a	53	26 a	84	40 a	72	86 a	3747 ab	2.6
FP4	175 a	54	28 a	83	41 a	71	86 a	3647 ab	5.4
FP5	171 a	55	29 a	82	37 a	74	86 a	3733 ab	3.0
FP6	167 a	56	26 a	83	36 a	74	85 a	3792 ab	1.4
FP7	171 a	55	29 a	82	40 a	72	86 a	3701 ab	3.9
FP8	174 a	54	28 a	82	38 a	73	85 a	3707 ab	3.7
FP9	161 a	57	27 a	83	35 a	76	86 a	3779 ab	1.7
FP10	160 a	58	27 a	83	39 a	72	85 a	3742 ab	2.8
FP11	149 a	61	25 a	84	37 a	74	86 a	3803 b	1.1
FP12	146 a	62	23 a	86	35 a	76	85 a	3845 b	0.0
FP13	148 a	61	28 a	82	38 a	73	84 a	3834 b	0.3

<sup>x</sup> Mean ranks with the same letter are not significantly different according to the Scott-Knott test with probability of  $\alpha = 0.05$ .

**Table 6.** Effect of Fungicide Programs (FP) on Area Under the Disease Progress Curve (AUDPC) values and fungicide control efficacy (CE) on Target spot (TS), Asian soybean rust (ASR), and Cercospora leaf blight (CLB), soybean defoliation (Def), yield, and reduction in productivity (RP) in Katueté (Canindeyú, Paraguay), season 2021/2022

FP	TS		ASR		CLB		Def (%)	Yield (kg ha <sup>-1</sup> )	RP (%)
	AUDPC	CE (%)	AUDPC	CE (%)	AUDPC	CE (%)			
FP0	1450 d <sup>x</sup>	0	23 b	0	521 b	0	76 e	3758 a	28.1
FP1	564 abc	61	6 a	74	180 a	66	61 bcd	4208 ab	14.4
FP2	460 a	68	4 a	85	135 a	74	52 a	4533 ab	6.2
FP3	598 abc	59	5 a	80	174 a	67	56 ab	4314 ab	11.6
FP4	710 c	51	5 a	78	181 a	65	62 bcd	4342 ab	10.8
FP5	632 bc	56	5 a	80	174 a	67	59 abc	4165 ab	15.6
FP6	511 ab	65	5 a	80	149 a	71	56 abc	4794 b	0.4
FP7	602 abc	58	4 a	85	169 a	68	62 bcd	4813 b	0.0
FP8	642 bc	56	4 a	85	184 a	65	64 cd	4373 ab	10.1
FP9	620 bc	57	4 a	85	187 a	64	59 abcd	4039 ab	19.1
FP10	682 c	53	4 a	83	196 a	62	67 d	4262 ab	12.9
FP11	581 abc	60	5 a	80	199 a	62	62 bcd	4102 ab	17.3
FP12	582 abc	60	5 a	80	185 a	65	64 cd	4267 ab	12.8
FP13	504 ab	65	5 a	80	210 a	60	60 abcd	4644 b	3.6

<sup>x</sup> Mean ranks with the same letter are not significantly different according to the Scott-Knott test with probability of  $\alpha = 0.05$ .

**Table 7.** Effect of Fungicide Programs (FP) on Area Under the Disease Progress Curve (AUDPC) values and fungicide control efficacy (CE) on Target spot (TS), Asian soybean rust (ASR), and *Cercospora* leaf blight (CLB), soybean defoliation (Def), yield, and reduction in productivity (RP) in La Paloma (Canindeyú, Paraguay), season 2021/2022

FP	TS		CLB		Def (%)	Yield (kg ha <sup>-1</sup> )	RP (%)
	AUDPC	CE (%)	AUDPC	CE (%)			
FP0	2022 e <sup>x</sup>	0	532 b	0	80 c	3699 a	45.1
FP1	1073 d	47	191 a	64	69 ab	4812 def	11.6
FP2	784 ab	61	151a	72	64 a	4491 bcd	19.5
FP3	846 abc	58	191 a	64	70 ab	5021 def	6.9
FP4	953 abcd	53	220 a	59	71 ab	4886 def	9.9
FP5	810 abc	60	217 a	59	70 ab	5113 ef	5.0
FP6	761 a	62	180 a	66	68 ab	4508 bcde	19.1
FP7	839 abc	58	192 a	64	70 ab	4980 def	7.8
FP8	957 abcd	53	217 a	59	68 ab	4649 bcde	15.5
FP9	985 bcd	51	211 a	60	70 ab	4043 ab	32.8
FP10	991 cd	51	230 a	57	72 b	4627 bcde	16.0
FP11	890 abcd	56	215 a	60	72 b	4756 cdef	12.9
FP12	866 abc	57	199 a	63	70 ab	4162 abc	29.0
FP13	864 abc	57	151 a	72	67 ab	5368 f	0.0

<sup>x</sup> Mean ranks with the same letter are not significantly different according to the Scott-Knott test with probability of  $\alpha = 0.05$ .

highest yield of 4651 kg ha<sup>-1</sup>, followed by Katueté with 4330 kg ha<sup>-1</sup>, and Nueva Esperanza with the lowest at 3739 kg ha<sup>-1</sup>. Given the proximity between these localities (32.11 km from Nueva Esperanza to Katueté, 56.51 km from Nueva Esperanza to La Paloma, and 31.22 km from La Paloma to Katueté) one would expect minimal yield variation if all other factors were constant. However, the absence of ASR and the reduced defoliation in La Paloma may be significant factors contributing to its higher yield. This disease, known for its detrimental effects on soybean crops, can reduce yields substantially (Hartman et al., 2015). The lack of ASR incidence in La Paloma, despite the similar management and environmental conditions, suggests there could be localized factors or microclimatic conditions that deterred the development or spread of the rust in this area.

In Paraguay, ASR is considered the most economically significant disease in soybeans, and its management is primarily carried out with fungicides. However, the situation regarding the sensitivity of *P. pachyrhizi* isolates to the fungicides available in Paraguay is unknown (Enciso-Maldonado et al., 2021). In contrast, in Brazil, annual trials are conducted to monitor the efficacy of fungicide control for various soybean diseases. Through these trials, they have reported the evolution of *P. pachyrhizi* resistance to fungicides under field conditions (Godoy et al., 2016). For example, in the 2022/2023 season, they evaluated pre-mixes of commercial fungicides, pre-mixes of fungicides combined with multi-site fungicides,

and isolated active ingredients in eighteen locations. They observed that the average control efficacy of *P. pachyrhizi* was 57 % for bixafen + prothioconazole, 52 % for benzovindiflupyr + prothioconazole, 49 % for tebuconazole, 44 % for fluxapyroxad + epoxyconazole + pyraclostrobin, 44 % for mancozeb, 41 % for picoxystrobin + cyproconazole, and 28 % for benzovindiflupyr + azoxystrobin (Godoy et al., 2023). Additionally, the report showed variation in response among locations concerning severity, control efficacy, and yield. These findings enhance the comprehension of fungicide impacts in Paraguay, given the lack of prior studies that have extensively examined these effects across different times and locations. Furthermore, evaluating fungicide programs provides a more comprehensive and realistic perspective of efficacy compared to the individual application of fungicides. It explores how the combination of different active ingredients and application at key points in the crop cycle can have significant impact on disease control and, consequently, crop yield. The results showed that the efficacy of fungicide programs also varies by location (Table 4). The response of the disease control efficacy of fungicide programs against ASR, compared to the controls, was similar in La Paloma and Katueté (Table 6, Table 7).

On the other hand, resistance to various fungicide modes of action in *P. pachyrhizi*, *C. cassiicola*, and *Cercospora* spp. has been reported under laboratory conditions (Wang et al., 2023; Mello et al., 2022; Müller et al., 2021; Sautua et



al., 2020; Price III et al., 2015; Xavier et al., 2013). Additionally, the Fungicide Resistance Action Committee (2024a, 2024b, 2024c) categorizes DMIs as moderately risky fungicides, and QoI and SDHI as high-risk fungicides for the development of resistance by pathogens. Therefore, there is a reinforced need to implement anti-resistance strategies or a programmatic approach to evaluate and adjust disease management strategies to counteract the loss of sensitivity and assist farmers in disease control.

The rotation of fungicide modes of action as a programmatic approach or anti-resistance strategy has proven successful in other pathosystems. For example, it has been effective in managing cucurbit downy mildew, reducing the accumulation of fungicide-resistant isolates of *Pseudoperonospora cubensis* (Bagi et al., 2014; D'Arcangelo et al., 2021). Similarly, in the management of powdery mildew in cucurbits (*Podosphaera xanthii*) the addition of chlorothalonil or azoxystrobin to a fungicide program with triadimefon resulted in fewer triadimefon-resistant strains, and when the combination of azoxystrobin and triadimefon/chlorothalonil was used in alternating applications, there were no instances of identified resistant strains (McGrath, 2001). Although this study did not address the detection of resistant strains, it was evident that all fungicide programs succeeded in reducing the intensity of the evaluated diseases while maintaining high crop yields compared to the control. Additionally, the evaluated programs include the incorporation of mancozeb in at least one of the three or four applications during the crop cycle. Due to the complex integration of mancozeb into each fungicide program, it was not possible to analyze its individual effect separately in this study. However, it is widely recognized that incorporating a multi-site fungicide like mancozeb into specific-site fungicide mixtures has proven to enhance disease control efficacy (CE) and increase crop yield in soybean cultivation in Paraguay and Brazil. For example, treatments with specific-site fungicides combined with mancozeb outperformed the use of specific-site fungicides alone in terms of disease control and crop yield, indicating the potential of mancozeb as a valuable tool in fungicide resistance management (Silva et al., 2015; Machado et al., 2022). Also, mancozeb can offer additional benefits in integrated disease management in soybeans, particularly under high disease pressure or where a decline in efficacy of specific-site fungicides due to resistance has been observed (Mendoza-Duarte et al., 2023; Enciso-Maldonado et al., 2019). This also suggests that mancozeb can help extend the lifespan of specific-site fungicides by reducing the

likelihood of resistance development in pathogen populations.

In soybean production, Enciso-Maldonado et al. (2022) assessed the impact of eleven different fungicide programs with various modes of action on the severity of ASR during two planting seasons and at two locations. They observed that the application of fungicide rotation programs, specifically pre-mix rotations, reduced ASR severity and increased soybean grain yield. Furthermore, these researchers considered treatments with up to four applications and noted that there was no significant additional benefit in ASR control or a substantial yield increase with a fourth application. Similarly, Ploper et al. (2015) found that there was no effect on reducing ASR severity or the incidence of foliar diseases, nor on yield, when one or two fungicide applications were made. Both studies align with the findings in this work, where the incorporation of an additional fungicide within the crop cycle did not have a significant influence on disease reduction or crop yield. The number of fungicide applications directly impacts production costs, so various factors should be considered for making applications, such as crop monitoring, the fungicide protection period, and other criteria (Reis et al., 2018).

Regarding TS, in Paraguay there have been no previous reports on the effect of fungicides on this disease. Therefore, this study serves as background information. During the 2019/2020 and 2020/2021 seasons, outbreaks of this disease were reported in the Canindeyú department (Enciso-Maldonado and Fernández-Gamarra, 2021a), with yield losses of up to 500 kg ha<sup>-1</sup> in private fungicide efficacy trials (Sidinei Neuhaus and Jonas Vogt, personal communication, December 2020). This aligns with the present results, indicating high levels of this disease in the three study locations. Estimating the severity reduction is complex because the involvement of other observed diseases in the field should be considered. However, it is estimated that yield loss caused by TS in Brazil ranges from 8 % to 42 %, with a severity of 50 %. Additionally, depending on the variety used, yield reduction can vary between 11 % and 42 % (Edwards-Molina et al., 2019).

In a study on farmers' perception of the main phytosanitary problems of soybeans in Paraguay, 7 % of farmers stated that TS is the most important disease in this crop (Caballero-Mairesse et al., 2024). Therefore, this disease deserves more attention when developing a fungicide management plan.

CLB was the second most predominant disease

in this study. Similarly to TS, there are no previous reports on the effect of fungicides for its control in Paraguay. However, Enciso-Maldonado and Fernández-Gamarra (2021b) reported up to 100 % incidence of CLB in productive plots that received three or more fungicide applications in Paraguay. Additionally, 12 % of farmers in Paraguay indicated that CLB is the main phytosanitary problem in soybean cultivation in the country (Caballero-Mairesse et al., 2024). In Brazil, Japan, Taiwan, Uganda, and Zambia, the yield loss caused by this disease ranges between 30 % and 50 % (Hartman et al., 2015), while in Argentina, a reduction in yield of 11 % has been observed (Lavilla and Ivancovich, 2021). It is recommended to control this disease with a first application during the advanced vegetative or early reproductive stages (growth stage R1-R2), when latent fungal biomass increases, and a second application at the beginning of pod filling (growth stage R4) (Hartman et al., 2015). Fungicide applications in Paraguay do not have a single objective; therefore, recommendations would depend on the diseases observed through monitoring (Reis et al., 2018).

This research generates valuable insights for the scientific community, expanding understanding of fungicide efficacy in the Paraguayan context. The identification of predominant diseases, variations in disease severity across locations, and the effectiveness of different fungicide programs emphasize the need for tailored strategies. The comprehensive dataset, encompassing disease severity, control efficacy, defoliation rates, and crop yield, offers a nuanced perspective on fungicide program performance. Beyond its academic impact, the practical utility of this research directly benefits soybean farmers. The data-driven insights, highlighting effective fungicide programs tailored to specific disease pressures in different regions, empower farmers to make informed decisions in disease management. Looking ahead, to enhance the robustness and applicability of this study, future research should focus on establishing a comprehensive system to monitor pathogen-resistance development. This could involve broader geographical representation, longer-term monitoring, and a more thorough assessment of resistance risks and economic factors.

## CONCLUSIONS

In conclusion, this study highlights the significant impact of fungicide programs on the control of TS, ASR, and CLB, with variations observed across different locations. In Nueva Esperanza, all

fungicide programs proved effective in reducing the AUDPC of the evaluated diseases compared to the control. In Katueté, FP2, PF6 and FP13 stood out as particularly effective in reducing the AUDPC of TS, while all fungicide programs demonstrated similar effectiveness against ASR and CLB. La Paloma exhibited notable results, with FP6 being effective in reducing the AUDPC of TS, and all fungicide programs proving equally efficient in reducing the AUDPC of CLB. Additionally, the fungicide programs contributed to a reduction in defoliation, with FP2, FP3, and FP6 showing lower defoliation rates in Katueté. Furthermore, the application of fungicide programs resulted in increased yield across all locations. The best-yielding locations were La Paloma, with the highest yield (4651 kg ha<sup>-1</sup>) and lowest defoliation (61 %), and Katueté, which also showed strong results (4330 kg ha<sup>-1</sup> with 70 % defoliation). Nueva Esperanza had a yield of 3739 kg/ha with 86 % defoliation. This study underscores the importance of fungicide application in maintaining soybean productivity, as evidenced by a significant reduction in yield when fungicides were not applied. Overall, these findings emphasize the need for location-specific considerations when designing fungicide programs for disease management in soybean crops. The present work provides valuable insights for farmers and agronomists to optimize fungicide strategies based on the prevailing disease pressures in different regions.

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