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Different Active Ingredients in the Control of Target Leaf Spot and Asian Soybean Rust Southern Maranhense

Diferentes Ingredientes Ativos no Controle de Mancha Alvo e Ferrugem Asiática da Soja no Sul Maranhense

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Abstract

Soybean (*Glycine max* L. Merrill) has its yield affected by several fungal diseases, which are difficult to control. Therefore, chemical control programs are alternatives to reduce the progress and damage caused by diseases. Thus, the objective of this study was to evaluate the performance of different active ingredients in the control of target leaf spot and asian rust. A randomized block design was adopted, with 6 treatments: T1 = control; T2 = A: Prothioconazole + Trifloxytrobin, B: Impirfluxam + Prothioconazole; T3 = A: Impirfluxam + Prothioconazole, B: Prothioconazole + Trifloxytrobin, T4 =

A: Fluxapyroxad + Prothioconazole, B: Pyraclostrobin + Fluxapyroxad + Epoxiconazole, T5 = A: Impirfluxam + Tebuconazole, B: Impirfluxam + Tebuconazole, T6 = A: Azoxitrobin + Mancozeb + Prothioconzal, B: Azoxitrobin + Mancozeb + Prothioconzal, all plots received applications C and D: Trifloxytrobin + Cyproconazole + Mancozeb. The severity, area under the disease progress curve, control efficiency, defoliation, thousand-grain weight and productivity were evaluated. The data were subjected to analysis of variance and means compared by Tukey's test at 5% probability and canonical correlation analysis both through the Software RBio. Plants subjected to fungicide applications showed lower rates of disease progression and defoliation and higher rates of severity control, thousand-grain weight and productivity when compared to the control, with emphasis on the performance of treatments 3 and 5.

Keywords: Plant Health. Chemical Management. Leaf Spots. *Corynespora cassiicola. Phakopsora pachyrhizi.*

Resumo

A soja a (*Glycine max* L. Merrill) tem o rendimento afetado por diversas doenças fúngicas, que apresentam difícil controle, assim os programas de controle químico constituem alternativas para redução do progresso e danos causados pelas doenças. Dessa forma, objetivou-se avaliar o desempenho de diferentes ingredientes ativos no controle de mancha alvo e ferrugem asiática. Foi adotado o delineamento em blocos cazualizados, com seis tratamentos: T1 = testemunha; T2 = A: Protioconazol + Trifloxitrobina, B: Impirfluxam + Protioconazol,; T3 = A: Impirfluxam + Protioconazol, B: Protioconazol + Trifloxitrobina, T4 = A: Fluxapiroxade + Protioconazol, B: Piraclostrobina + Fluxapiroxade + Epoxiconazol, T5 = A: Impirfluxam + Tebuconazol, B: Impirfluxam + Tebuconazol, T6 = A: Azoxitrobina + Mancozeb + Protioconzal, B: Azoxitrobina + Mancozeb + Protioconzal, todas as parcelas receberam aplicações C e D: Trifloxitrobina + Ciproconazol + Mancozeb. Foram avaliadas a severidade, área abaixo da curva de progresso da doença, eficiência de controle, desfolha, peso de mil grãos e produtividade. Os dados foram submetidos a análise de variância e médias comparadas pelo teste de Tukey a 5% de probabilidade e análise de correlações canônicas ambos através do Software RBio. As plantas submetidas as aplicações de fungicidas apresentaram menores índices de progresso das doenças e desfolha e maiores índices de controle da severidade, peso de mil grãos e produtividade quando comparadas a testemunha, com destaque para o desempenho dos tratamentos 3 e 5.

Palavras-chave: Fitossanidade. Manejo Químico. Doenças Foliares. *Corynespora cassiicola. Phakopsora pachyrhizi.*

1 Introduction

Soybean (*Glycine max* (L.) Merr.) is an herbaceous plant belonging to the Leguminosae family, widely cultivated across Brazil and one of the main crops worldwide. It contains approximately 20% oil and 40% protein, being used for both human and animal consumption, as well as biodiesel production (Rocha *et al*., 2018). Brazil is the world's largest soybean producer, with an estimated production of 154.6174 million tons in the 2022/23 season, thus playing an important socioeconomic role (CONAB, 2023).

Soybean production in the state of Maranhão began in the second half of the 1980s. Its expansion occurred rapidly, spreading from the south to the east of the state. In the 2022/23 season, soybean cultivation in Maranhão exceeded 1 million hectares, reaching a production of 3.9 million tons

(CONAB, 2023; Lemos, 2015).

This crop has high productive potential; however, its yield can be affected by diseases caused by various pathogens such as fungi, bacteria, nematodes, and viruses. Among the many diseases, target spot caused by the necrotrophic fungus Corynespora cassiicola and Asian soybean rust caused by the biotrophic fungus Phakopsora pachyrhizi are particularly noteworthy (Godoy *et al*., 2023; Cerutti; Muller; Brustolin, 2021).

Target spot (*C. cassiicola*) has gained prominence in the Cerrado region of Maranhão. Under favorable conditions, the pathogen causes leaf lesions that begin as brown specks with a yellow halo, resembling a target. Control alternatives for target spot in soybeans include the use of resistant cultivars and fungicide application (Ribeiro *et al*., 2019). Moreover, the increase in occurrence and severity in Brazil may be related to large monoculture areas, no-till farming, the use of susceptible cultivars, and changes in climate patterns, such as frequent rainfall during vegetative growth stages (Avozani; Reis; Tonin, 2014).

Asian soybean rust, caused by the biotrophic fungus *P. pachyrhizi*, is considered one of the most harmful diseases for soybean crops, with the potential to reduce productivity by up to 90% (Cerutti; Muller; Brustolin, 2021). It can occur at any phenological stage of the soybean crop, leading to early defoliation, which in turn affects grain filling and quality (Godoy *et al*., 2023). Control strategies include planting at the recommended time, complying with the sanitary break, using resistant cultivars, and applying fungicides (Hoffmann *et al*., 2019).

Fungicides registered for disease control have different modes of action, being classified as Quinone outside Inhibitors (QoI – strobilurins), Succinate Dehydrogenase Inhibitors (SDHI – carboxamides), and Demethylation Inhibitors (DMI – triazoles), which penetrate plant tissue, as well as multi-site fungicides that act on various metabolic points of the pathogen (Chechi *et al*., 2021). However, repeated applications of products with the same active ingredient, high adaptability of the fungus, and deviations from recommended doses contribute to the selection of resistant populations (Souza *et al*., 2022).

Given the above, the objective of this study was to evaluate the performance of different active ingredients in the control of target spot and Asian soybean rust.

2 Material and Methods

2.1 Experimental conditions and plant material

The experiment was conducted in the field, in the municipality of Fortaleza dos Nogueiras (MA), located at a latitude of 7º 05’ 20.5’’ S, longitude of 45º 56’ 45.8’’ W, and an average altitude of 585 meters. The experimental area has soil classified as dystrophic Dark Red Latosol with a clayey texture and a tropical climate with dry winters and rainy summers (Aw), according to the Köppen-Geiger climate classification (1928).

Sowing was carried out under a no-tillage system, with a row spacing of 0.50 meters, a stand of 17 plants/m, and a seeding depth of 3.5 cm. The experimental area consisted of plots measuring 7 meters in length and 3 meters in width, totaling 21 m² each.

For nutrient supply, fertilization equivalent to 209 kg ha^{-1} of MAP and 190 kg ha^{-1} of KCl (potassium chloride) was used, along with seed inoculation using 200 mL per kilogram of seeds of the product *Masterfix®*, based on *Bradyrhizobium elkanii* and *Bradyrhizobium japonicum* (5×10^9 CFU/mL), in addition to foliar sprays of micronutrient complexes at phenological stages V3, V6, and R1.

The area used for the experiment had a low weed population, and only one pre-emergence application of the herbicide Dual Gold (s-metolachlor) was necessary under the "plant-and-spray" method to prevent weed competition with the crop of interest.

For pest control, the following products were applied: Belt (flubendiamide) for caterpillar control, Sperto (acetamiprid + bifenthrin) for stink bugs, and Epingle 100 (pyriproxyfen) targeting whitefly infestations.

Seeds of the cultivar BMX DOMINIO IPRO were used, along with fungicides containing different active ingredients.

The treatments, containing the active ingredients (Table 1), were applied using a CO_2-pressurized backpack sprayer with a flow rate of 150 L ha^{-1} over a 3-meter spray boom. Applications were made every 14 days, starting when the crop reached the V8 phenological stage (pre-row closure).

Table 1 - Treatments applied to the soybean crop, cultivar BMX DOMINIO IPRO, with respective active ingredients, concentrations, and doses

Treatment	Application	Active Ingredient	Concentration (g/L or g/kg)	Dose a.i./ha (g/L or g)
T1	Control	-	-	-
	A	Prothioconazole + Trifloxystrobin	175 + 150	87.5 + 75
	B	Imifluxam + Prothioconazole	120 + 240	42 + 84
T2	C	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	C	Mancozeb	800	1.200
	D	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	D	Mancozeb	800	1.200
	A	Imifluxam + Prothioconazole	120 + 240	42 + 84
	B	Prothioconazole + Trifloxystrobin	175 + 150	87.5 + 75
T3	C	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	C	Mancozeb	800	1.200

	D	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	D	Mancozeb	800	1.200
	A	Fluxapyroxad + Prothioconazole	200 + 280	60 + 84
	B	Pyraclostrobin + Fluxapyroxad + Epoxiconazole	81 + 50 + 50	64.8 + 40 + 40
T4	C	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	C	Mancozeb	800	1.200
	D	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	D	Mancozeb	800	1.200
	A	Imifluxam + Tebuconazole	60 + 200	30 + 100
	B	Imifluxam + Tebuconazole	60 + 200	30 + 100
T5	C	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	C	Mancozeb	800	1.200
	D	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	D	Mancozeb	800	1.200
	A	Azoxystrobin + Mancozeb + Prothioconazole	37.5 + 525 + 37.5	75 + 1.050 + 75
	B	Azoxystrobin + Mancozeb + Prothioconazole	37.5 + 525 + 37.5	75 + 1.050 + 75
T6	C	Trifloxystrobin + Cyproconazole	375 + 160	75 + 32
	C	Mancozeb	800	1.200
	D	Trifloxytrobin + Cyproconazole	375 + 160	75 + 32
	D	Mancozeb	800	1.200

A = 1st application; B = 2nd application; C = 3rd application; D = 4th application.
Source: research data

The leaves were analyzed based on the diagrammatic scale proposed by Godoy, Koga, and Canteri (2006) for assessing the progression of Asian soybean rust, and the scale proposed by Soares, Godoy, and Oliveira (2009) for target spot. Evaluations were conducted seven days after the first, second, and third applications, and at 7, 14, and 21 days after the last application.The percentage of disease control was calculated using the formula proposed by Abbott (1925): Control Efficiency (%) = 100 – (y/St) × 100,where:y = Mean severity of the treatment program; St = Severity of the untreated control. The Area Under the Disease Progress Curve (AUDPC) was determined using the formula proposed by Campbell and Madden (1990):$AUDPC = \sum\left[\frac{y_i+y_{i+1}}{2}\right] * (t_{i+1} - t_1)$. Where: yiy_iyi = disease severity at the iii-th evaluation, tit_iti = time (in days) at the iii-th evaluation, nnn = total number of evaluations.

Defoliation was estimated at the R6 growth stage by visually comparing the plants to the diagrammatic scale proposed by Hirano *et al*. (2010). Grain yield (kg/ha) was determined by weighing the samples to calculate the yield per hectare. The thousand grain weight (TGW) was measured using a precision scale, according to Brazil (2009).

2.2 Experimental design

The experiment was conducted in a randomized block design with six treatments and repetitions. The data obtained were subjected to analysis of variance (ANOVA), and the means were compared using Tukey's test at a 5% significance level. Canonical correlation analysis was also performed, both analyses conducted using the Rbio software, version 140 for Windows (Bhering, 2017).

3 Results and Discussion

For target spot, all treatments resulted in lower disease severity percentages compared to the untreated control, which reached 9% at the end of the evaluations (Table 2). The presence of leaves with target spot symptoms was observed at the R1 phenological stage (beginning of flowering) in plants from the untreated control plots, with 5% severity, indicating greater susceptibility.

Thus, it is inferred that when initial fungicide applications are neglected, there will be greater difficulty in controlling the disease due to the significant early progression of the pathogen in the crop. According to Balardin *et al.* (2017), proper planning of the management program leads to higher levels of disease control.

At the end of the evaluations, the untreated control reached 9% severity, a value higher than that observed in all other fungicide-treated plots. Ribeiro *et al.* (2019) reported similar results when evaluating the performance of protectant and systemic fungicides in controlling target spot in soybean. In their study, all fungicide programs resulted in lower severity values compared to the untreated control, which reached 72.50%.

Table 2 - Severity percentage (SEV), Area Under the Disease Progress Curve (AUDPC), and Control Efficiency (CE) in soybean plants (cv. BMX DOMÍNIO) subjected to different treatments for the control of target spot and Asian soybean rust

Tratamento	SEV (%)		AUDPC		EC (%)	
	TS	ASR	TS	ASR	TS	ASR
T1	9.00b	42.00d	148.75b	280.0d	0.00b	0.00d
T2	4.50a	18.00c	39.66a	96.60bc	66.66a	57.14c
T3	1.75a	3.25a	46.37a	27.47a	80.55a	92.26a
T4	4.00a	18.00c	75.25a	114.10b	55.55a	57.14c
T5	4.00a	9.75b	84.00a	50.22a	55.55a	76.78b
T6	2.00a	18.00c	40.25a	79.10c	77.77a	57.14c
C.V. (%)	36.10	13.91	31.40	10.34	20.88	10.61

Where: TS = target spot; ASR = Asian soybean rust.Means followed by the same lowercase letter in the column do not differ statistically from each other according to Tukey's test at a 5% probability level. C.V. = coefficient of variation.
Source: research data.

Asian soybean rust (ASR) was observed at the R5 phenological stage, with 2% severity in the untreated control plants and 0.6% in the other treatments. In this disease, severity was lower in plants that received fungicide applications compared to the control (Table 2). Treatment 3 resulted in the smallest affected leaf area (3.25%), followed by Treatment 5 (9.75%) and Treatments 2, 4, and 6, all of which showed 18% severity and did not differ statistically from one another. Rust detection occurred at the R5 stage, by which time all application programs had already been completed. Therefore, plants that did not receive chemical treatment showed greater susceptibility to the initial inoculum and, consequently, to the pathogen's progression.

For the untreated control, there was a significant progression in the epidemiological status of the diseases, reaching 42% severity, while Treatments 3 and 5 reached 3.25% and 9.75% severity, respectively—standing out as the most effective programs for rust control. Both treatments included the active ingredient imifluxam, which, like other carboxamides, inhibits the succinate dehydrogenase enzyme, disrupting the connection between mitochondrial complex II and complex III, thereby suppressing fungal energy production. Additionally, this active ingredient presents high fungitoxicity, with an IC_{50} (mg/L × 10^{-4}) of 0.57, indicating high inhibitory activity against pathogens (Kiguchi *et al*., 2021).

It is worth noting that Treatment 2 also included a fungicide based on imifluxam in its application program; however, during application A, the action was left to trifloxystrobin for preventive control of the pathogen. Fochesatto *et al.* (2020), when evaluating the efficiency of different fungicides against yellow spot and leaf rust progression in wheat, concluded that the mixture of trifloxystrobin + prothioconazole resulted in a high level of yellow spot severity, contributing to a disease progression area of 359.6, second only to the untreated control.

Treatments containing Pyraclostrobin, Fluxapyroxad, and Epoxiconazole (T4), as well as Azoxystrobin (T6), showed higher disease severity percentages (18%). These results suggest a reduction in the effectiveness of these molecules over successive seasons due to the development of pathogen resistance to their modes of action, as reported by Souza *et al*. (2020).

Regarding AUDPC (Table 2), for both diseases, the plots that received fungicide applications—regardless of timing or active ingredient—showed a reduced disease progression curve compared to the control. This reduction is attributed to the lower severity percentages observed in the evaluations, highlighting the importance of fungicide applications for disease management in soybean crops.

According to Godoy *et al*. (2016), between two and six fungicide applications should be performed throughout the crop cycle, depending on the initial inoculum pressure and environmental conditions at the cultivation site. These findings align with those reported by Celoto and Celoto (2022), who studied the sensitivity of *C. cassiicola* to chemical products and concluded that molecules such as tebuconazole, epoxiconazole, and pyraclostrobin exerted fungitoxic effects, although no significant differences were observed between them in controlling fungal colonization—similar to the results found in this study. Furthermore, triple-mix applications should be timed for later stages, as they are more effective when acting eradicatively, with a greater leaf area allowing higher absorption of active ingredients (Augustin *et al*., 2014).

For control efficiency (CE) (Table 2), no significant differences were observed between fungicide programs for target spot, with all differing only from the untreated control. However, mean CE values ranged from 55.55% (T5 and T4) to 80.55% (T3). Godoy *et al*. (2023) reported similar results when evaluating fungicide efficacy for target spot control in the 2022/23 season, observing no statistical differences in CE between products such as Mancozeb + Prothioconazole + Azoxystrobin, Prothioconazole + Fluxapyroxad, among others also used in the present study.

For ASR, the control efficiency observed in this study corroborates the findings of Camera *et al.* (2023), who emphasized that most fungicides tested effectively reduced rust severity, although efficiency varied depending on the molecule or formulation used.

Therefore, at least one fungicide application at any crop stage is sufficient to achieve better outcomes for both diseases when compared to the untreated control (Dal Pogetto *et al*., 2012).

However, according to Reis, Reis and Zanatta (2018), control is considered efficient only when the percentage exceeds 80%. In this study, only Treatment 3 achieved a control efficiency above 80% (92.26%) for ASR, differing significantly from the other treatments. Treatment 3 included a fungicide combining a triazole and a strobilurin, similar to the study by Netto (2020), in which a treatment using trifloxystrobin + prothioconazole achieved control levels of 80.4% in the 2016/17 season and 89.7% in 2017/18.

Regarding defoliation (Table 3), at the R6 phenological stage, 100% defoliation was observed in the untreated control. Additionally, Treatment 4, which included fluxapyroxad + prothioconazole in the first application, did not differ statistically from the control. These results contrast with those reported by Ribeiro *et al*. (2019), who found the lowest defoliation rates in treatments using fungicides composed of pyraclostrobin and fluxapyroxad.

Treatments 3 and 5 resulted in the lowest defoliation rates (22.50%), differing statistically from Treatments 2, 4, and 6 (Table 3).

Table 3 - Defoliation, Thousand Grain Weight (TGW), and Yield (YLD) in soybean plants (cv. BMX DOMÍNIO) subjected to different treatments for target spot and Asian soybean rust control

Treatment	**Defoliation (%)**	**TGW (g)**	**Yield (Kg ha⁻¹)**
T1	100.00c	180.00b	3431.56c
T2	60.00b	187.50ab	4004.96b
T3	22.50a	193.75a	4559.57a
T4	75.00bc	193.75a	3996.13b
T5	22.50a	192.50a	4424.11ab
T6	55.00b	196.25a	4093.31ab
C.V. (%)	22.42	2.60	5.66

TGW = Thousand Grain Weight; C.V. = Coefficient of Variation; Means followed by the same letter in the column do not differ statistically by Tukey's test at 5% significance.
Source: Research data.

The greater the number of lesions on the plant, the more severe the defoliation will be in affected plants (Godoy *et al*., 2006). Similar results were observed by Botega, Sousa and Nozaki (2020), in which soybean plants used in the control plots showed high levels of defoliation (97.25%), leading to significant yield losses.

Treatments 2 and 3, which provided greater control of disease progression, showed higher percentages of remaining leaves. Therefore, in order to avoid yield losses due to the lack of photosynthetic structures, it is essential to implement an effective spray program targeting target spot and Asian soybean rust.

When soybean crops undergo high levels of defoliation during the grain filling stage, they experience substantial losses in productive capacity, since the absence of leaves reduces the source of photoassimilates produced by the photosynthetic activity of the leaves (Hirano *et al*., 2010).

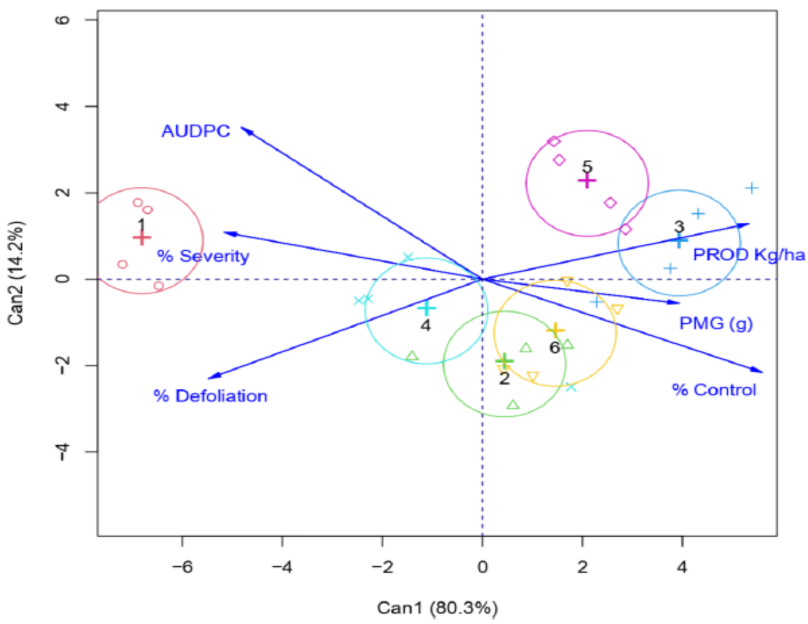
For the thousand grain weight (TGW) variable (Table 3), with the exception of Treatment 2, all other treatments had statistically different means from the control (no application). Thus, it is suggested that the use of fungicides allowed soybean plants to better benefit from the adopted management practices, differing from Carmo *et al*. (2018) and Rambo *et al*. (2004), who suggest that this yield component is not significantly influenced by these factors, attributing it solely to the cultivar's genetic characteristics. Moreover, Basso, Bonaldo and Ruffato (2015) observed beneficial effects of applying fungicides based on pyraclostrobin and fluxapyroxad on the thousand grain weight, which is consistent with the present study.

Regarding yield (Table 3), it was observed that all treatments with fungicide application had higher values than those obtained in Treatment 1 (control), which reached only 3431.56 kg/ha or 57.19 bags/ha. On the other hand, higher yields were observed in plots subjected to different fungicide treatments, with the highest increase of 1,128.01 kg/ha (T3) and the lowest of 564.57 kg/ha (T5). However, there were no statistical differences among them, similar to the results obtained by Ribeiro *et al*. (2016), who evaluated the combination of protective and systemic fungicides for controlling target spot in soybeans, reporting the greatest increase of 1,121.22 kg/ha (T6) and the smallest of 298.58 kg/ha (T2).

The average yield values in Treatments 3 (4,559.5701 kg/ha) and 5 (4,424.1101 kg/ha) are similar to those found by Alves and Juliatti (2018), in which plots that received applications of Trifloxystrobin + Prothioconazole showed the highest yield averages (2,879.630 kg/ha) in the trial.

In the canonical correlation analysis (Figure 1), for target spot, canonical axes 1 and 2 explained 94.5% of the variation among the evaluated components. The severity vector reached its maximum magnitude in treatment 1 (control), which also showed the strongest association with AUDPC and defoliation. The yield vector reached its highest magnitude in Treatment 3, but was also strongly associated with Treatment 5. The TGW vector was most associated with treatments 3 and 6, as was control efficiency (CE). Finally, Treatments 2 and 4 showed an intermediate association with the evaluated components, performing better than the control and slightly lower than treatments 3, 5, and 6.

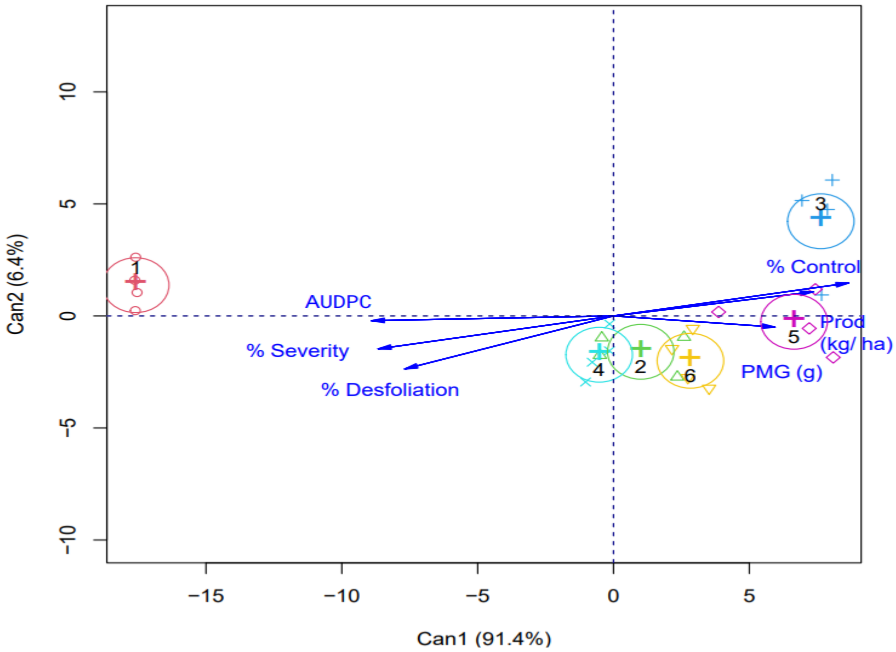
Figure 1 - Canonical variable analysis composed of different treatments (different active ingredients) and the variables: disease severity, area under the disease progress curve (AUDPC), control efficiency (CE), defoliation, thousand grain weight (TGW), and yield (YLD)



Source: Research data.

For FAS in the Canonical Correlation Analysis (Figure 2), canonical axes 1 and 2 explained 97.8% of the variation among the evaluated components. The axes follow the same trend observed in Figure 1. The variables yield, control efficiency, and thousand grain weight show maximum magnitude in Treatment 3, followed by Treatment 5. For the variables severity, area under the disease progress curve, and defoliation, the maximum magnitude is found in treatment 1 (control). Treatments 2, 4, and 6 show an intermediate association with the evaluated components, performing better than the control and slightly below Treatments 3 and 5.

Figure 2 - Canonical correlation analysis composed of different treatments (different active ingredients) and the variables: disease severity (% Severity), area under the disease progress curve (AUDPC), control efficiency (% Control), defoliation (% Defoliation), thousand grain weight (TGW (g)), and yield (YLD (kg/ha))



Source: research data.

4 Conclusion

Fungicide applications ensure lower rates of disease progression and defoliation, and higher levels of severity control, thousand grain weight, and yield, with emphasis on the performance of Treatment 3 (A: Impirfluxam + Prothioconazole, B: Prothioconazole + Trifloxystrobin) and Treatment 5 (A: Impirfluxam + Tebuconazole, B: Impirfluxam + Tebuconazole).

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