

# Fungicide mixtures to control Asian soybean rust control in regions of Brazil<sup>1</sup>

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**ABSTRACT** - High soybean grain yield is based on adequate crop management, mainly on disease prevention and control, especially Asian soybean rust (ASR), which causes great crop damage. This study aimed to evaluate the ASR chemical control with different fungicide mixtures in three soybean-producing areas in Brazil during two consecutive crop seasons. Four fungicide treatments [T1: benzovindiflupyr + picoxystrobin, T2: benzovindiflupyr + azoxystrobin, T3: fluxapyroxad + pyraclostrobin, and T4: inpyrfluxam + tebuconazole] were evaluated with five replications. Phytotoxicity damage, ASR disease severity, plant defoliation, 1,000-grain weight, and grain yield were evaluated. The results were relatively consistent among all regions and between the crop seasons, indicating that the treatments have similar responses when applied in diverse conditions. The T3 and T4 treatments presented higher phytotoxicity, lower disease severity, lower plant defoliation, and higher 1,000-grain weight and grain yield. The presence and damage caused by ASR tend to increase after the second soybean crop season in the same area, suggesting a loss of efficiency of the evaluated fungicides.

**Key words:** *Phakopsora pachyrhizi*. Fluxapyroxad. Inpyrfluxam. Triazole. Strobilurin.

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DOI: 10.5935/1806-6690.20250039

Editor-in-Chief: Eng. Agrônomo. Manoel Barbosa Filho - manoel.filho@ufc.br

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Received for publication 02/03/2023; approved on 21/11/2023

<sup>1</sup>Paper extracted from the thesis dissertation of the Postgraduate Program in Plant Production at Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro/IFTM, Uberaba Campus, Uberaba, MG, Brazil

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## INTRODUCTION

Brazil is the world's largest soybean producer and has faced critical challenges regarding crop management and yield efficiency. During the soybean cycle, plant diseases affect yield and the sustainability of crop production. Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi*, is the most severe foliar disease due to its rapid air transmission and acute symptoms. Premature soybean defoliation, pod malformation, reduction in grain mass, and losses of up to 90% in yield are regularly reported (Lemes; Gavassoni, 2015; Godoy *et al.*, 2016; Pelin *et al.*, 2020; Meira *et al.*, 2020).

Using fungicides (chemical control) is still the most effective form of ASR management, which should be implemented together with other strategies for effective disease control (Dorighello *et al.*, 2020; Nascimento *et al.*, 2022). The effectiveness of a fungicide depends on the plant's phenological stage, leaf architecture, fungus aggressiveness, damage potential, intervals, and frequency of fungicide applications (Viegas Neto *et al.*, 2021). Currently, there are about 160 fungicides registered for ASR control in Brazil (MAPA, 2022). Most of these fungicides are restricted to triazole, strobilurin, and carboxamide groups. The continuous use of these fungicide groups can induce fungal mutation and the selection of resistant populations (Belufi *et al.*, 2015). Additionally, these fungicides can cause phytotoxicity complications in the crops (Zuntini *et al.*, 2019).

The carboxamide group is one of the most recently developed and acts by blocking the energy supply to fungus cells; however, studies with *P. pachyrhizi* have already indicated decreased fungus sensitivity to this group (Schmitz *et al.*, 2014; Godoy *et al.*, 2016). Godoy *et al.* (2019) and Pelin *et al.* (2020) observed that applying products with different mechanisms of action reduces disease severity, which can be a viable strategy to preserve the efficacy of the fungicides. The evaluation of fungicide efficiency is essential to monitor the resistance of phytopathogen populations in soybean-producing areas. Thus, this study aimed to assess the chemical control of Asian soybean rust with different mixtures of fungicides in three important soybean-producing regions in Brazil.

## MATERIALS AND METHODS

### Experimental areas

The studies were implemented in three experimental areas in Brazil, under a no-tillage system, in two consecutive crop seasons (2017/2018 and 2018/2019).

Soybean crops were cultivated in the early summer and corn in the late summer.

An experimental area was in Uberlândia (Minas Gerais state), at 18°54'28.4" S, 48°18'3.085" W, and 866 meters above sea level. Another experimental area was in Rio Verde (Goiás state), at 17°47'03.8" S, 51°00'39.1" W, and 731 meters above sea level. The third experimental area was in Santa Maria (Rio Grande do Sul state), at 29°43'39.6" S, 53°33'41.3" W, and 153 meters above sea level.

The soils in the Uberlândia and Rio Verde areas are classified as Latossolo Vermelho Distrófico with medium and clayey textures, respectively. The soil in Santa Maria is an Argissolo Bruno-Acinzentado Aluminico with a clayey texture (Santos *et al.*, 2018).

The climate of Uberlândia, according to the updated Köppen and Geiger classification (Beck *et al.*, 2018), is Aw-type, with an average annual temperature and rainfall of 21.5 °C and 1,479 mm. In Rio Verde (GO), the climate is classified as Aw-type, with heavy rains in summer and average annual temperature and rainfall of 23.3 °C and 1,663 mm. In Santa Maria (RS), the climate is classified as Cfa-type, presenting an average annual temperature and rainfall of 19.3 °C and 1,688 mm. Additionally, air temperature, relative humidity, and precipitation volume in the experimental areas were recorded in a weather station (model Vue GSM Vivo) every 60 minutes.

### Experimental design

The experiments were set in a randomized block design arranged in a split-plot-in-time scheme. Four treatments were evaluated: T1: benzovindiflupyr + picoxystrobin, T2: benzovindiflupyr + azoxystrobin, T3: fluxapyroxad + pyraclostrobin, and T4: inpyrfluxam + tebuconazole, with five replications. The information about the fungicides, doses, and the soybean stage in which they were applied is shown in Table 1.

### Soybean crop management

Soybean sown co-occurred in all experimental areas and crop seasons on 11/01/2017 and 11/16/2018. The seed emergence occurred five and six days after sown for the 2017/2018 and 2018/2019 crop seasons, respectively. The seeds were inoculated with 960 grams per 100 kilograms of soybean seeds with a peat inoculant [Adhere@60 (*Bradyrhizobium elkanii*)] to ensure sufficient biological nitrogen fixation.

Soybean plants were arranged in six rows, five meters long, with planting rows spaced by 0.5 m. The useful plot (the area where evaluations were performed) was the four central rows, except the initial and ending 0.5 m in each row, representing 8 m<sup>2</sup>. Only the two central lines were considered within the useful plot for harvesting.

**Table 1** - Treatments including the commercial products - fungicide (CP), active ingredient (AI), concentration (CC), and the soybean stage (AS) in which they were applied to control Asian soybean rust

Treatment	AS*	CP	AI	CC	CP L ha <sup>-1</sup>	IA
T1	V4	Rivax	carbendazim	250	1	0.25
			tebuconazole	125		0.125
	R1	Vessarya	benzovindyflupyr	50	0.6	0.03
			picoxystrobin	100		0.06
	R1 + 14	Vessarya	benzovindyflupyr	50	0.6	0.03
			picoxystrobin	100		0.06
	R5	Aproach Prima	picoxystrobin	200	0.5	0.10
			cyproconazole	80		0.04
T2	V4	Rivax	carbendazim	250	1	0.25
			tebuconazole	125		0.125
	R1	Elatus	azoxystrobin	300	0.2	0.06
			benzovindyflupyr	150		0.03
	R1 + 14	Elatus	azoxystrobin	300	0.2	0.06
			benzovindyflupyr	150		0.03
	R5	Aproach Prima	picoxystrobin	200	0.5	0.10
			cyproconazole	80		0.04
T3	V4	Rivax	carbendazim	250	1	0.25
			tebuconazole	125		0.125
	R1	Orkestra	pyraclostrobin	333	0.4	0.11
			fluxapyroxad	167		0.05
	R1 + 14	Orkestra	pyraclostrobin	333	0.4	0.11
			fluxapyroxad	167		0.05
	R5	Aproach Prima	picoxystrobin	200	0.5	0.10
			cyproconazole	80		0.04
T4	V4	Rivax	carbendazim	250	1	0.25
			tebuconazole	125		0.125
	R1	Excalia Max	inpyrfluxam	60	0.5	0.03
			tebuconazole	200		0.10
	R1 + 14	Excalia Max	inpyrfluxam	60	0.5	0.03
			tebuconazole	200		0.10
	R5	Aproach Prima	picoxystrobin	200	0.5	0.10
			cyproconazole	80		0.04

The fungicide active ingredient doses were those recommended by manufacturers. Spray adjuvants (commercial name and dose): T1 = no adjuvant; T2 = Ochima (phosphate ester) 0.5 L ha<sup>-1</sup>; T3 = Assist (hydrocarbon) 0.5 L ha<sup>-1</sup>, and T4 = Agris (hydrocarbon) 0.5 L ha<sup>-1</sup>. \* = soybean stages defined according to Fehr *et al.* (1971): V4 = four nodes on the main stem beginning with the unifoliate node; R1 = one flower at any node; R1 + 14 = fourteen days after the first flower at any node; R5 = grains beginning to develop (can be felt when the pod is squeezed) at one of the four uppermost nodes with a fully expanded leaf

The seed treatment was performed with Derosal<sup>®</sup> Plus (fungicides: carbendazim and thiram) and Cropstar<sup>®</sup> (insecticides: imidacloprid and thiodicarb) at doses of 200 and 500 mL per 100 kg of soybean seeds, respectively. Crop maintenance applications are described in Table 2.

**Table 2** - Commercial product (CP), active ingredient (AI), concentration (CC), and soybean stage that the treatments were applied during the soybean crop cycle for all treatments

AS*	CP	AI	CC	CPL ha <sup>-1</sup>	IA
Pre-sowing	Crucial	glyphosate (k.isopropylamine)	640	2	1280
	ZethaMaxx	imazethapyr flumioxazin	212 + 100	0.5	0.106 + 0.05
V3	Crucial	glyphosate (k.isopropylamine)	640.0	2	1280
	Kraken	clethodim	240.0	1	0.24
V4	Rivax	carbendazim tebuconazole	250 + 125	1	0.25 + 0.125
	Prêmio	chloranthraniliprole	200	0.05	0.01
V6	Prêmio	chloranthraniliprole	200	0.05	0.01
R3	Epingle	pyriproxyfen	100	0.5	0.05
	Carnadine	acetamiprid	200	0.3	0.06
R4	Epingle	pyriproxyfen	100	0.5	0.05
	Carnadine	acetamiprid	200	0.3	0.06
R5.2	Approach Prima	picoxystrobin	200	0.5	0.1 + 0.04
		cyproconazole	80		
R5.4	Platinum Neo	thiamethoxan	141	0.3	0.042 + 0.03
		lambda cyhalothrin	106		
R6	Platinum Neo	thiamethoxan	141	0.3	0.042 + 0.03
		lambda cyhalothrin	106		
R7	Nuquat	paraquat dichloride	200	2	0.4

\* = soybean stages defined according to Fehr *et al.* (1971): V3 = three nodes on the main stem beginning with the unifoliate node; V4 = four nodes on the main stem beginning with the unifoliate node; V6 = six nodes on the main stem beginning with the unifoliate node; R3 = pod 0.5 cm long at one of the four uppermost nodes with a fully expanded leaf; R4 = pod 2 cm long at one of the four uppermost nodes with a fully expanded leaf; R5.2 (adapted) = grains beginning to develop (11 to 25% seed fill) at one of the four uppermost nodes with a fully expanded leaf; R5.4 (adapted) = grains beginning to develop (51 to 75% seed fill) at one of the four uppermost nodes with a fully expanded leaf; R6 = pod containing full-size green grains at one of the four uppermost nodes with a fully expanded leaf; R7 = pods yellowing; 50% of leaves yellow. Physiological maturity

The soybean cultivar used in both crop seasons (11/01/2017 and 11/16/2018) and the three areas (Uberlândia, Rio Verde, and Santa Maria) was BMX Ponta (relative maturity group: 6.9), susceptible to ASR, and sown at a population of 300.000 plants per hectare (15 viable plants per meter). Soybean fertilization in all crop seasons and experimental areas was performed based on soil analysis and crop needs. Crop management was the same as those conducted in commercial crop fields.

The application of Rivax® (1 L ha<sup>-1</sup>) at the V4 soybean phenological stage was intended to prevent end-of-cycle diseases, such as anthracnose (*Colletotrichum truncatum*) and brown spot (*Septoria glycines*). The application of Approach Prima® (0.5 L ha<sup>-1</sup>) at the R5.2 stage was intended to improve fungicide crop protection and avoid early defoliation, which may compromise the results.

A CO<sub>2</sub>-pressurized backpack-sprayer was used to perform the application of the treatments. The spray volume was calibrated to 150 L ha<sup>-1</sup> in a 6-nozzle boom.

Teejet nozzles (model TT110:02 VP Mesh 50), median drop, and 1 m s<sup>-1</sup> application speed were used.

### Asian soybean rust occurrence and evaluations

The incidence and dissemination of the Asian soybean rust pathogen (*P. pachyrhizi*) in the experimental areas occurred naturally, and the fungus was identified at the R3 soybean phenological stage. The ASR severity evaluations were performed 7 and 14 days after the application at R1 and R1 + 14 soybean phenological stage, using the diagrammatic scale that assigns percentage scores of ASR infection (Godoy *et al.*, 2006).

Plant defoliation was evaluated 35 days after application at the R6 stage when the pods were fully filled and green leaves were still attached to the plant (Hirano *et al.*, 2010). The efficacy of the phytosanitary product applied was evaluated by the Abbott (1925) formula:  $E (\%) = (S_c - S_f/S_c) \times 100$ , where E (%) = treatment's efficacy; S<sub>c</sub> = severity in

the control treatment;  $S_f$  = severity in the fungicide treatment. All plant evaluations were performed by the same professional, who was qualified to make these evaluations in both crop seasons, one per area, which at no time had access to the protocol, so did not know where the treatments were located in each experimental unit.

In the pre-harvest desiccation (110 days after germination), Paraquat dichloride (2 L ha<sup>-1</sup>) was applied in all areas and both crop seasons.

Grain yield was estimated from the useful area of each plot. The pods were manually harvested, quantified after threshing, and weighed. After the determination of seed mass, the seed humidity was corrected to 14%; then, the 1,000-grain weight (1000 W) was determined.

The data were initially submitted to the assumptions of analysis of variance (ANOVA) ( $p > 0.01$ ) for normality of residues (Kolmogorov-Smirnov) and homogeneity of variances (Levene). The outliers were

identified through boxplot graphs of the residuals and replaced as lost plots. Then, ANOVA was performed, and when significant differences ( $p < 0.05$ ) were detected, the crop seasons were compared through joint analysis using the Tukey test ( $p < 0.05$ ) (Genes® v.2009.7.0 software). The treatments in each experimental area were also compared using the Tukey test ( $p < 0.05$ ). The R® Core statistical software was used for all analyses performed.

## RESULTS AND DISCUSSION

The soybean phytotoxicity in all areas and crop seasons was generally similar between T3 (fluxapyroxad + pyraclostrobin) and T4 (inpyrfluxam + tebuconazole) treatments and higher than the soybean phytotoxicity observed in T1 (benzovindiflupyr + picoxystrobin) and T2 (benzovindiflupyr + azoxystrobin) treatments (Table 3).

**Table 3** - Soybean phytotoxicity at 7 and 14 days after application (DAA) according to the fungicide applications to control Asian soybean rust in the 2017/2018 and 2018/2019 crop seasons

Treatment	Phytotoxicity (%)			
	2017-2018		2018-2019	
	7 DAA	14 DAA	7 DAA	14 DAA
Uberlândia				
T1	0 Ab	0 Ab	0 Ab	0 Ab
T2	0 Ab	0 Ab	0 Ab	0 Ab
T3	5.6 Aa	5.3 Aa	3.9 Ba	1.6 Ba
T4	5.0 Aa	5.0 Aa	4.6 Aa	2.3 Ba
CV (%)	43.8	65	12.4	8.0
Rio Verde				
T1	0 Ac	0 Ac	0 Ab	0 Ab
T2	0 Ac	0 Ac	0 Ab	0 Ab
T3	4.3 Bb	5.0 Ab	5.6 Aa	3.9 Ba
T4	10.0 Aa	7.2 Aa	6.3 Ba	4.6 Ba
CV (%)	15.3	17.1	8.1	6.9
Santa Maria				
T1	0 Ab	0 Ab	0 Ac	0 Ab
T2	0 Ab	0 Ab	0 Ac	0 Ab
T3	1.6 Ba	1.6 Ba	5.3 Ab	6.3 Aa
T4	1.3 Ba	1.3 Ba	7.6 Aa	7.0 Aa
CV (%)	58.0	29.6	9.1	8.1

Means followed by equal uppercase letters in the lines and lowercase letters in the columns do not differ by the Tukey test ( $p \leq 0.05$ ). T1: benzovindiflupyr + picoxystrobin, T2: benzovindiflupyr + azoxystrobin, T3: fluxapyroxad + pyraclostrobin, and T4: inpyrfluxam + tebuconazole. CV (%) = coefficient of variation

In Uberlândia, the T3 and T4 treatments presented a similar phytotoxicity index ranging from 5.0% to 5.6% in the 2017/2018 crop season. The phytotoxicity index decreased in the 2018/2019 crop season, ranging from 1.6% to 4.6%. The phytotoxicity was consistently lower 14 days after fungicide application (Figure 1A).

In Rio Verde, the phytotoxicity was consistently higher in the T4 treatment, which ranged between 7.2% and 10.0% in the 2017/2018 crop season. This phytotoxicity range was lower in the 2018/2019 crop season (from 4.6% to 7.3%) but higher compared to T1 and T2 treatments (Figure 1B).

In Santa Maria, the lowest phytotoxicity was observed in the 2017/2018 crop season, which ranged

between 1.3% (T4) and 1.6% (T3). These indexes are within the commercially acceptable limit, as they do not significantly affect soybean grain yield. In the 2018/2019 crop season, phytotoxicity increased significantly, ranging from 5.3% to 7.6% (Figure 1C).

The main ASR symptoms observed in all experimental areas were visible discoloration and deformation in some leaves and plants. Still, the severity rates are within the economically acceptable loss limits, causing low defoliation rates. According to Nascimento *et al.* (2020), 30% and 15% of plant defoliation in the vegetative and reproductive periods, respectively, did not reduce yield.

**Figure 1** - Phytotoxicity index observed in soybean leaf in Uberlândia (A), Rio Verde (B), and Santa Maria (C) in the 2018/2019 crop season

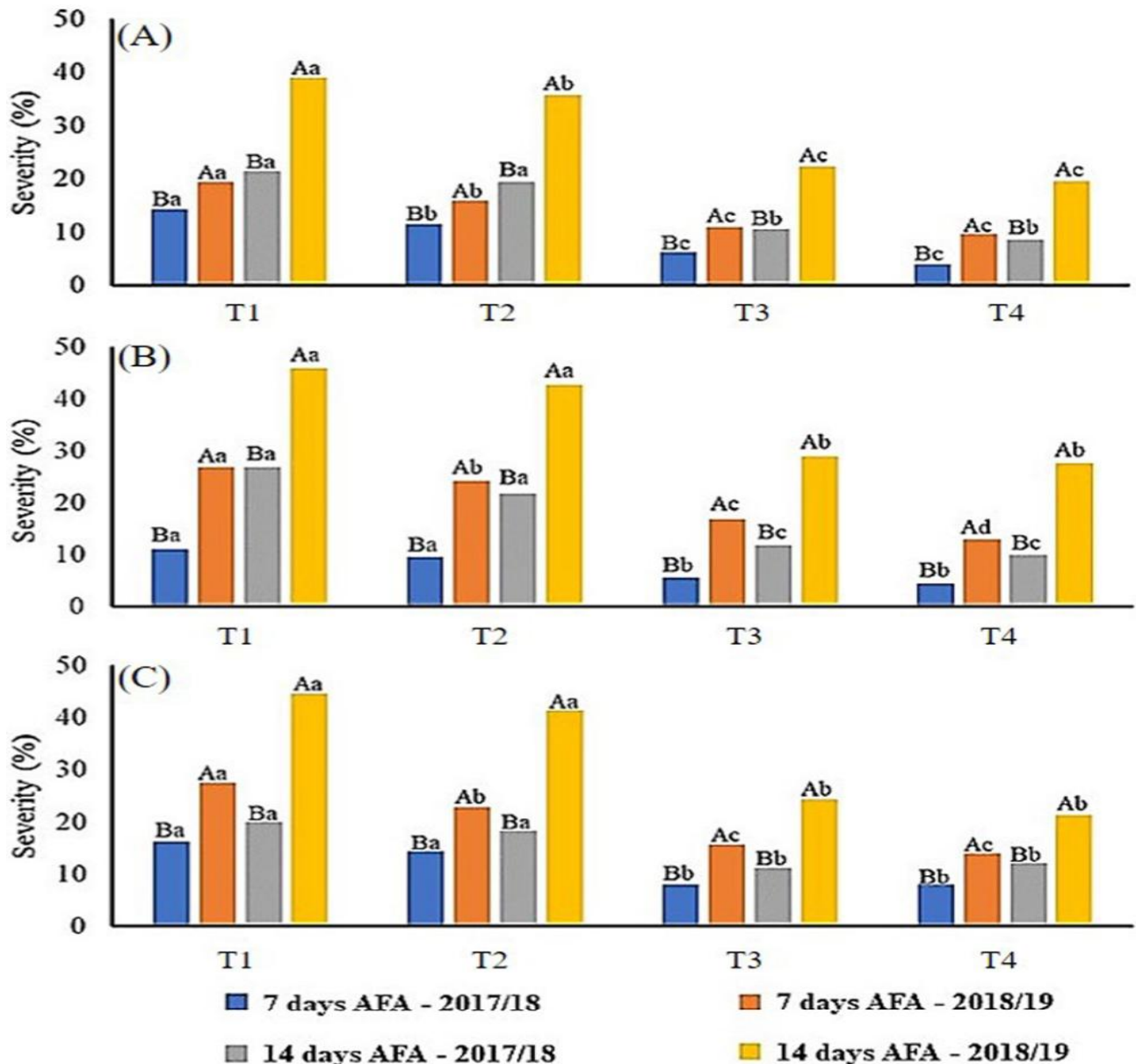


In the T4 treatment, the phytotoxicity can be attributed to tebuconazole, similar to what Zuntini *et al.* (2019) reported for experiments where tebuconazole was applied. The authors also developed their study in the Rio Verde area. The phytotoxicity caused by fluxapyroxad and tebuconazole in T4 and T5 occurs due to the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) formation. The

formation of oxidative species in plant metabolism was also reported by Mohamed and Akladios (2017) in cotton plants.

The ASR severity observed for all three areas consistently presented low indexes for T3 and T4 treatments compared to T1 and T2 treatments, which showed high indexes (Figure 2A).

**Figure 2** - Asian soybean rust severity in Uberlândia, MG (A), Rio Verde, GO (B), and Santa Maria, RS (C) in the 2017/2018 and 2018/2019 crop seasons



Means followed by equal uppercase letters for the crop seasons and lowercase letters for the treatments do not differ by the Tukey test (p ≤ 0.05). T1: benzovindiflupyr + picoxystrobin, T2: benzovindiflupyr + azoxystrobin, T3: fluxapyroxad + pyraclostrobin, and T4: inpyrfluxam + tebuconazole. AFA: after fungicide application

The T3 and T4 treatments in Uberlândia for the 2017/2018 crop season presented 10.6% and 8.6% ASR severity levels, respectively (Figure 2A). This effect can be attributed to the great amplitude of fungi control by carboxamides (fluxapyroxad). This effect was highlighted by Freitas *et al.* (2016), where fluxapyroxad presented high efficacy in the control of ASR.

The T1 and T2 treatments presented similar disease severities and the highest rates of diseases. This result is comparable to that observed by Godoy *et al.* (2019), who evaluated different fungicides to control ASR in Brazilian soybean-producing regions. Nascimento *et al.* (2020) also observed a unique application of azoxystrobin + cyproconazole at the R3 soybean phenological stage, and applications at the R2 and R5.1 stages resulted in lower ASR severities and higher grain yields, compared to applications at the R3 and R4 stages. This result was also observed in the present study.

In the 2018/2019 crop season, the severity pattern repeated in Uberlândia; however, the severity rate increased in T3 (22.3%) and T4 (19.6%) treatments compared to the preceding year (Figure 3). This increase can result from a selection of fungicide-resistant ASR populations, which would maintain inoculum potential in the area for longer, mainly if no adequate management is applied to the germinated plants after harvest (Godoy *et al.*, 2006; Müller *et al.*, 2021).

A similar pattern was also detected in Rio Verde, with the lowest ASR severities observed in T4 (12%) and T3 (10%) treatments in the 2017/2018 crop season. The highest ASR severity was observed for T2 (22%) and T1 (27%) treatments, demonstrating their low control efficiency (Figure 2B). In the 2018/2019 crop season, severity rates increased by 29%, 28%, 46%, and 41% in T4, T3, T2, and T1 treatments, respectively. In Santa Maria, T3 and T4 treatments presented the lowest ASR severity rates and T1 and T2 the highest, similar to what was observed in Uberlândia and Rio Verde (Figure 2C).

The evaluation of the treatment's responses in all areas and crop seasons indicates a reduction in the ASR control in the second crop season (2018/2019). This loss of disease control efficacy may be correlated with variations in disease pressure and/or favorable climatic conditions, as Nascimento *et al.* (2020) reported.

In general, the treatments with higher defoliation rates (T1 and T2), which ranged from 80% to 97%, were the same ones that presented the highest severity rates. Defoliation levels increased in the second crop evaluated (2018/2019) for all treatments and areas (Table 4).

The defoliation rate is widely used in ASR studies since the pathogen causes intense defoliation in high incidence and severity (Doreto *et al.*, 2012; Reis *et al.*, 2019). The loss

of leaves directly reduces the photosynthesis rate and impairs grain filling; thus, a high ASR severity will negatively impact soybean leaf metabolism and the durability of the trifoliate leaves (Fiallos *et al.*, 2011).

Meneghetti *et al.* (2010) observed that the lowest levels of defoliation caused by ASR occurred when the treatments were composed of different active ingredients. The authors demonstrated that mixing two or more active ingredients with diverse mechanisms of action provides more efficient ASR control. According to the authors, the combination of fungicides also increases the fungicide action spectrum and their residual effect and reduces the chances of developing resistant pathogen populations. Viegas Neto *et al.* (2021) also reported improved fungicide control of ASR when protective and systemic were applied in mixtures. These observations were similarly reported in the present study.

**Table 4** - Soybean defoliation rates (%) 35 days after fungicide applications to control Asian soybean rust in the 2017/2018 and 2018/2019 crop seasons

Treatment	Defoliation	
	2017/2018	2018/2019
..... % .....		
Uberlândia		
T1	85 Ba	97 Aa
T2	82 Ba	93 Aa
T3	69 Bb	88 Ab
T4	62 Bc	81 Ac
CV%	4.5	2.4
Rio Verde		
T1	88 Ba	99 Aa
T2	85 Ba	95 Aa
T3	65 Bb	90 Ab
T4	60 Bb	86 Ab
CV (%)	3.0	3.9
Santa Maria		
T1	90 Ba	97 Aa
T2	80 Bb	95 Aa
T3	68 Bc	70 Ab
T4	66 Bc	65 Ac
CV (%)	2.2	1.0

Means followed by equal uppercase letters in the lines and lowercase letters in the columns do not differ by the Tukey test ( $p \leq 0.05$ ). T1: benzovindiflupyr + picoxystrobin, T2: benzovindiflupyr + azoxystrobin, T3: fluxapyroxad + pyraclostrobin, and T4: inpyrfluxam + tebuconazole. CV (%) = coefficient of variation



The lower premature defoliation in T4 and T3 treatments favored the soybean grain yield parameters. Durlí *et al.* (2020) reported the critical role that soybean leaves play in grain yield and that it is essential to maintain the quantity and quality of leaves. In the present study, the grain yield parameters were negatively affected by the presence of Asian soybean rust in all evaluated areas and crop seasons; however, T4 and T3 treatments presented the best results for soybean grain yield due to lower disease severity and defoliation rates (Table 5).

Similar results to those observed in the present study were reported by Mendonça Jr *et al.* (2019). They evaluated different fungicides for ASR control and observed that the association of carboxamides, strobilurin, and triazoles increased soybean grain yield parameters. Souza *et al.* (2020) also evaluated the efficiency of different fungicide formulations. They highlighted that the association of carboxamides and triazoles increased the number of grains per plant and grains per pod and reduced the number of aborted grains. Dalla Lana *et al.* (2015) also concluded that grain yield losses strongly correlated with

ASR disease (between 0.79 and 0.90), where there was high disease pressure (years with severe epidemics).

Godoy *et al.* (2019) evaluated Asian soybean rust control by fungicides in different Brazilian soybean-producing regions. They observed that inpyrfluxam + tebuconazole presented great responses to disease control efficacy and lower defoliation. Barbosa *et al.* (2014) highlight that ASR occurrence is still in the early reproductive phases and increases the formation of empty pods. They reported that the rapid yellowing and leaf fall still impair complete grain formation and filling. This situation was also observed in the present study.

The lower soybean grain yield observed in the second crop season (2018/2019), compared to the previous crop season (2017/2018), is related to factors such as the ASR severity and defoliation in that crop season. Improved ASR aggressiveness can be due to improved fungus resistance to fungicides (selection of resistant populations) and/or a set of favorable climatic conditions, which positively affected the progress index of the ASR epidemic.

**Table 5** - 1,000-grain weight and grain yield of soybean according to the fungicide applications to control Asian soybean rust in the 2017/2018 and 2018/2019 crop seasons

Treatments	1,000-grain weight		Grain yield			
	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19
	----- g -----		----- Mg ha <sup>-1</sup> -----		----- 60-kg bags ha <sup>-1</sup> -----	
Uberlândia						
T1	151 Ab	155 Bb	3.2 Ab	3.1 Bb	53 Ab	52 Ab
T2	161 Ab	166 Bb	3.5 Ab	3.5 Bb	58 Ab	59 Bb
T3	182 Aa	186 Ba	4.1 Aa	4.1 Ba	68 Aa	68 Ba
T4	181 Aa	185 Ba	4.2 Aa	4.2 Ba	70 Aa	70 Ba
CV (%)	2.30	2.35	3.41	3.03	2.90	3.40
Rio Verde						
T1	171 Ab	157 Bb	3.0 Ab	2.8 Ab	50 Ab	47 Ab
T2	170 Ab	158 Bb	3.2 Ab	2.9 Bb	54 Ab	47 Bb
T3	199 Aa	179 Ba	3.5 Aa	3.3 Ba	59 Aa	55 Ba
T4	197 Aa	182 Ba	3.7 Aa	3.4 Ba	62 Aa	57 Ba
CV (%)	3.0	2.80	2.91	3.02	5.10	5.40
Santa Maria						
T1	171 Ab	170 Ab	3.6 Ab	3.2 Bb	61 Ab	54 Ab
T2	179 Ab	178 Ab	3.8 Ab	3.2 Bb	64 Ab	53 Bb
T3	191 Aa	190 Aa	4.5 Aa	3.6 Ba	74 Aa	60 Ba
T4	194 Aa	194 Aa	4.4 Aa	3.6 Ba	73 Aa	60 Ba
CV (%)	5.25	5.23	4.01	3.88	3.90	3.40

Means followed by equal uppercase letters in the lines and lowercase letters in the columns do not differ by the Tukey test ( $p \leq 0.05$ ). T1: benzovindiflupyr + picoxystrobin, T2: benzovindiflupyr + azoxystrobin, T3: fluxapyroxad + pyraclostrobin, and T4: inpyrfluxam + tebuconazole. CV (%) = coefficient of variation

Some studies have reported the resistance of ASR to chemical control. Juliatti *et al.* (2015) monitored isolates of *P. pachyrhizi* and observed some stability of strobilurins with the effective concentration to kill 50% (CI50) of the fungus population around 0.05 to 0.5 ppm in the first year. In the second year of evaluation, ASR sensitivity reduced, requiring a CI50 of 50 to 500 ppm for azoxystrobin and pyraclostrobin, whereas, for picoxystrobin and trifloxystrobin, the CI50 was 4.5 to 45 ppm and 0.5 to 3.5 ppm, respectively.

The high risk of resistance for fungicide groups such as strobilurins and carboxamides requires crop management that preserves the efficacy of the fungicide molecules. Mixing fungicide groups is a strategy to improve control and reduce the risk of disease resistance development. In the present study, the fungicide treatments fluxapyroxad + pyraclostrobin (T3) and inpyrfluxam + tebuconazole (T4) presented more soybean phytotoxicity damage but lower ASR disease severity, less leaf fall, and higher 1,000-grain weight and grain yield. However, even with a mixed fungicide spray, the presence and damage caused by Asian soybean rust tended to increase in the second crop season, suggesting a loss of efficiency of the evaluated treatments.

Actions such as respecting the sanitary break (period of the year where no soybean crop can be cultivated), rotation of chemical groups, use of protective fungicides, and monitoring of climatic conditions improve the effective longevity of fungicide molecules and preserve the options for controlling epidemics. The maintenance of the effectiveness of different fungicide molecules is essential for severe diseases such as ASR (a disease of high dispersal potential), which can effectively spread resistance to specific fungicide molecules over extensive areas in a short period.

## CONCLUSIONS

1. The fluxapyroxad + pyraclostrobin and inpyrfluxam + tebuconazole treatments are more phytotoxic to soybean; however, they provide lower disease severity and defoliation and higher 1,000-grain weight and grain yield;
2. The occurrence and damage of Asian soybean rust tend to increase after the second soybean crop season in the same area. This observation suggests a loss of control efficiency of the evaluated fungicides.

## ACKNOWLEDGMENT

To the Curso de Mestrado Profissional em Produção Vegetal of the Federal Institute of Education, Science and Technology of Triângulo Mineiro

(IFTM), Campus of Uberaba. To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). To the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Nufarm Company.

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