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# Effects of sowing date and fungicide application schemes on soybean rust severity and grain yield

# Efeito de fungicidas e da semeadura no controle da ferrugem asiática da soja

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ABSTRACT - Asian soybean rust (ASR), caused by the fungus Phakopsora pachyrhizi, is one of the most significant diseases affecting soybean (Glycine max) crops, where the growers usually use scheduled fungicide applications to control ASR. This study aimed to evaluate the effects of different sowing times and fungicide application schemes on ASR severity and grain yield. The study was conducted at the Phytus Institute's experimental farm in Planaltina, DF, Brazil, during the 2014-2015 season, using the soybean cultivar M 6952 IPRO (Intacta). An experimental design with the following sowing dates were done: November 16, November 28, December 11, and December 18 in 2014; and January 2 and January 6 in 2015. The fungicide application schemes were as follow: (T1) no fungicide; (T2) scheduled application with the first application at 15 to 25 days after emergence (DAE); (T3) application based on soybean phenological stages; (T4) scheduled application based on DAE with seven-day delay relative to T2; and (T5) application based on phenological stage with a seven-day delay relative to T3. The following fungicides were applied: trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + cyproconazole (32 g ha<sup>-1</sup>). Early sowing at the beginning of the rainy season, combined with protective fungicide applications based on soybean phenological stages, resulted in lower ASR severity and higher grain yield. Late sowing, no fungicide application, or delayed fungicide applications increased ASR severity and reduced grain yield.

RESUMO - A ferrugem asiática da soja (FAS) (Phakopsora pachyrhizi) é considerada a principal doença da soja, sendo seu controle feito com aplicação periódica de fungicidas. O objetivo deste trabalho foi avaliar diferentes épocas de plantio e momentos de aplicação de fungicida de proteção para o controle da ferrugem asiática, propondo ao fim, um calendário de aplicação do fungicida de proteção e recomendação de época de plantio da soja cv M 6952 IPRO (Intacta). O experimento foi conduzido em Planaltina, DF, Brasil, com seis épocas de semeadura (Novembro de 2014 a Janeiro de 2015). Os tratamentos com fungicidas foram aplicados quatro vezes em cada bloco incluindo (1) Sem fungicida; (2) Aplicação programada com 15 a 25 dias após a emergência (DAE); (3) Aplicação na fase fenológica; (4) Aplicação agendada com atraso de 7 dias em relação ao tratamento-2; e (5) Aplicação do estádio fenológico com atraso de 7 dias em relação ao tratamento-3. Foram utilizados os fungicidas Trifloxistrobina (60 g ha<sup>-1</sup>) + Protioconazol  $(70 \text{ g ha}^{-1})$  e Trifloxistrobina  $(60 \text{ g ha}^{-1})$  + Ciproconazol  $(70 \text{ g ha}^{-1})$ . Os tratamentos de semeadura tardia, sem fungicidas e a aplicação tardia de fungicida aumentaram a severidade da FAS e reduziram a produtividade. A semeadura antecipada no início do período chuvoso e a aplicação de fungicidas protetores de acordo com o estádio fenológico da planta diminuíram a severidade da doença e aumentaram a produtividade.

Palavras-chave: Glycine max. Triazole. Strobilurin. Phakopsora

Keywords: *Glycine max.* Triazole. Strobilurin. *Phakopsora pachyrhizi*. Sowing time.

**Conflict of interest:** The authors declare no conflict of interest related to the publication of this manuscript.



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### INTRODUCTION

Brazil is the world's largest producer of soybean (*Glycine max*), with a grain production of approximately 134 million Mg during the 2021–2022 season (FAOSTAT, 2024). Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi*, is one of the most severe diseases affecting soybean production in Brazil. This disease causes early defoliation of plants, prevents complete grain formation, and results in yield reductions ranging from 10% to 90%. The cost of controlling ASR during the 2018–2019 crop season reached US\$ 2.8 billion (CONSÓRCIO ANTIFERRUGEM, 2018).

pachyrhizi. Época de semeadura.

Fungicide application is the primary method for controlling ASR (NASCIMENTO et al., 2018; NETTO et al., 2020; NUNES, MARTINS, DEL PONTE, 2018; SCOLIN, CANTERI, GODOY, 2023). Determining the optimal time for fungicide application is crucial for efficient ASR control (GODOY et al., 2009; MUELLER et al., 2009; NASCIMENTO et al., 2018). Therefore, field disease monitoring is essential for effective ASR control. Delays in ASR control after infection can result in significant crop yield losses (MUELLER et al., 2009). Commercial products containing two (trifloxystrobin + prothioconazole) or three



(azoxystrobin + cyproconazole + mancozeb) active ingredients from different chemical groups (triazole, strobilurin, carboxamide, and dithiocarbamate) are frequently recommended to control this disease (BARCELOS et al., 2023; BARRO et al., 2021; VIEGAS NETO et al., 2021). Thus, these chemical products should be used appropriately, avoiding early applications and repeated use of the same product to prevent fungal resistance to fungicides (NASCIMENTO et al., 2018; NETTO et al., 2020; NUNES, MARTINS, DEL PONTE, 2018; SCOLIN, CANTERI, GODOY, 2023).

According to Godoy et al. (2009), sequential fungicide applications based on plant phenology (R2 and R5.1) can reduce ASR severity and increase grain yield. However, regional differences in Brazil prevent the adoption of a national standard for managing the disease (GODOY et al., 2009). Therefore, fungicide use planning should consider regional and risk factors monitored throughout the year. However, producers in Brazil, as well as in other parts of the world, have adopted time-based application programs due to difficulties in locally identifying the disease and the potential for damage under management failure scenarios (GODOY et al., 2009). In addition, soybean sowing time is known to affect disease severity, grain yield, and potentially the effectiveness of fungicide applications (ÁVILA et al., 2003; OLIGINI et al., 2021). Soybean growing seasons with high rainfall are more prone to greater rust severity, requiring additional fungicide applications (DEL PONTE et al., 2006; NASCIMENTO et al., 2018; NASCIMENTO et al., 2022).

In this context, determining the optimal time for fungicide application to control ASR is one of the challenges in soybean production. The objective of this study was to identify the optimal time for fungicide application against ASR and to compare fungicide application schemes based on days after emergence with those based on soybean phenological stages.

#### MATERIAL AND METHODS

The experiment was conducted at the Phytus Institute's experimental station in Planaltina, DF, Brazil ( $15^{\circ}35$ 'S,  $47^{\circ}$  42'W, at an altitude of 1,175 m), from November 2014 to June 2015 (Figures 1A and 1B). Soybean seeds were sown on November 16 (1), November 28 (2), December 11 (3), and December 18 (4) in 2014, and on January 2 (5) and January 6 (6) in 2015 (Figure 1A).



**Figure 1**. (A) Sowing dates (SD): (1) November 16, 2014; (2) November 28, 2014; (3) December 11, 2014; (4) December 18, 2014; (5) January 2, 2015; and (6) January 6, 2015; seedling emergence dates (SE), harvest date (HD), Asian soybean rust detection date (RD), and fungicide application schemes (indicated by dotted short arrows); (B) Daily weather data: minimum and maximum temperatures ((Tmin and Tmax; °C), relative air humidity (Hum, %), and cumulative rainfall (mm) recorded at the experimental area. Planaltina, Brasília, DF, Brazil, 2014–2015.



The soybean cultivar used was the M 6952 IPRO, which features Intacta technology, belongs to the maturation group 6.9, has very early maturation, indeterminate growth, and is resistant to desiccation (MACHADO et al., 2018). The seeds were sown at a density of 200,000 plants ha<sup>-1</sup>, with 10 plants m<sup>-1</sup> and a spacing of 0.5 m between rows

(EMBRAPA, 2013). Soil fertilizers were applied at 200 kg ha<sup>-1</sup> of the 05-20-20 N-P-K formulation. The soybean harvesting dates corresponding to each sowing time were March 28 (1), April 4 (2), April 11 (3), April 18 (4), April 25 (5), and April 25 (6), 2015 (Figures 1A and 1B; Table 1).

 Table 1. Sowing date, seedling emergence date, harvest date, and fungicide application timing and dates (APL) based on days after seedling emergence and phenological stages of the soybean plants of the cultivar M 6952 IPRO Intacta.

	Seedling emergence date	Harvest date	Days after seedling emergence				
Sowing date							
U			1 <sup>st</sup> APL	2 <sup>nd</sup> APL	3 <sup>rd</sup> APL		
(1) $16/N_{ev}/14$	24/Nov/14	29/Man/15	25 20/Dec/14	45 8/Jan/15	15 DA2 22/Jan		
(1) 10/NOV/14	24/INOV/14	20/1v1ar/13	+7 29/Dec/14	+7 15/Jan/15	22 DA2 9/Feb		
(2) $28/N_{\rm OV}/14$	$0/D_{22}/14$	1/Apr/15	23 31/Dec/14	43 20/Jan/15	15 DA2 5/Feb		
(2) 20/100/14	9/Dec/14	4/Api/13	+7 7/Jan/15	+7 27/Jan/15	22 DA2 18/Feb		
(3) $11/Dec/14$	16/Dec/1/	11/Apr/15	21 5/Jan/15	41 24/Jan/15	15 DA2 9/Feb		
(3) 11/Dec/14	10/Dec/14		+7 12/Jan/15	+7 31/Jan/15	22 DA2 22/Feb		
(4) $18/Dec/14$	22/Dec/14	$18/\Lambda pr/15$	19 9/Jan/15	34 24/Jan/15	15 DA2 9/Feb		
(4) 10/Dec/14	22/Dec/14	10/Api/15	+7 17/Jan/15	+7 31/Jan/15	22 DA2 22/Feb		
(5) 2/Jan/15	6/Ian/15	$25/\Lambda nr/15$	17 22/Jan/15	32 6/Feb/15	15 DA2 20/Feb		
	0/3411/15	25/Api/15	+7 24/Jan/15	+7 13/Feb/15	22 DA2 7/Mar		
(6) 6/Ian/15	14/Jan/15	25/Apr/15	15 29/Jan/15	30 13/Feb/15	15 DA2 27/Feb		
(0) 0/341/15			+7 5/Feb/15	+7 20/Feb/15	22 DA2 14/Mar		
			Soybean phenological stage*				
			1 <sup>st</sup> APL	2 <sup>nd</sup> APL	3 <sup>rd</sup> APL		
(1) 16/Nov/14	24/Nov/14	28/Mar/15	V6 23/Dec/14	R1 5/Jan/15	15 DA2 19/Jan		
			+7 30/Dec/14	+7 12/Jan/15	22 DA2 3/Feb		
(2) $28/N_{OV}/14$	0/Dec/14	$1/\Lambda nr/15$	V6 5/Jan/15	R1 10/Jan/15	15 DA2 24/Jan		
(2) 28/Nov/14	9/Dec/14	4/Apt/13	+7 12/Jan/15	+7 17/Jan/15	22 DA2 9/Feb		
(3) $11/Dec/14$	16/Dec/14	$11/\Lambda pr/15$	V5 14/Jan/15	R1 22/Jan/15	15 DA2 6/Feb		
(3) 11/Dec/14	10/100/14	11/Api/15	+7 21/Jan/15	+7 29/Jan/15	22 DA2 20/Feb		
(4) $18/Dec/14$	22/Dec/14	18/Apr/15	V5 22/Jan/15	R1 2/Feb/15	15 DA2 17/Feb		
(4) 10/Dec/14			+7 29/Jan/15	+7 9/Feb/15	22 DA2 3/Mar		
$(5) 2/I_{ap}/15$	6/Ian/15	25/Apr/15	V4 2/Feb/15	VN 5/Feb/15	15 DA2 20/Feb		
( <i>J</i> ) 2/Jan 1J	0/3411/15		+7 9/Feb/15	+7 18/Feb/15	22 DA2 12/Mar		
(6) 6/Ian/15	$1/I_{an}/15$	$25/\Lambda pr/15$	V4 9/Feb/15	VN 5/Mar/15	15 DA2 5/Mar		
(0) 0/Jan/15	14/Jaii/13	23/Api/13	+7 18/Feb/15	+7 25/Feb/15	22 DA2 19/Mar		

DA2 = days after the second fungicide application. Sowing dates: November 16, November 28, December 11, and December 18 in 2014; and January 2 and January 6 in 2015. Fungicide application schemes: (T1) no fungicide; (T2) scheduled application with the first application at 15 to 25 days after emergence (DAE); (T3) application based on soybean phenological stages; (T4) scheduled application based on DAE with sevenday delay relative to T2; and (T5) application based on phenological stage (SPS) with a seven-day delay relative to T3. Fungicides applied: trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + cyproconazole (32 g ha<sup>-1</sup>). \*Phenological stages: V4, V5, and V6 = 4, 5, and 6 nodes on the primary stem with fully developed leaves, respectively; R1 = beginning of flowering (at least one flower on the main stem); R2 = Full flowering (flowers on any one of the top two nodes); R4 = Full pod formation (2-cm long pods on one of the top four nodes); R5 = beginning of grain formation (3 mm-long seeds on one of the top four nodes) (RITCHIE et al., 1977).

Soybean seeds were sown between each plot and grown without any fungicide application to minimize interference among plots. Thus, each treatment was exposed to the same inoculum source influence as the control treatment, which received no fungicide application.

The fungicide application schemes were as follows: (T1) no fungicide (water only); (T2) scheduled application with the first application at 15 to 25 days after emergence (DAE); (T3) application based on soybean phenological stages (RITCHIE et al., 1977); (T4) scheduled application based on DAE with a seven-day delay relative to T2; and (T5) application based on phenological stage with a seven-day delay relative to T3 (Table 1).

The following fungicides were applied alternately:

trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + cyproconazole (32 g ha<sup>-1</sup>), along with an oil-based adjuvant (soy methylated) at 0.25% (v v<sup>-1</sup>) (AGROFIT, 2024; EMBRAPA, 2013; MACHADO et al., 2018) (Table 1). The applications were performed using a  $CO_2$ -pressurized sprayer (30 psi) with four spray nozzles featuring double-range tips (TJ60 110.02), spaced 0.5 m apart, and applying a fungicide suspension spray volume of 150 L ha<sup>-1</sup>.

Five leaflet samples were collected weekly from the middle part of five randomly selected plants in each plot to quantify rust severity percentage, using the diagrammatic scale proposed by Godoy et al. (2006). This scale uses leaflets with varying severity levels to establish severity limits,



including maximum, minimum, and intermediate levels, based on Weber-Fechner's stimulus-response law. The disease severity evaluation results were used to calculate the Area Under the Disease Progress Curve (AUDPC) as described by Campbell and Madden (1990), as follows:

$$AUDPC = \sum [((Y_{i+1} + Y_i) \times 0.5) \times (T_{i+1} - T_i)]$$

where  $Y_i$  = disease severity at evaluation i (i = 1, ..., n);  $Y_{i+1}$  = disease severity at evaluation i+1;  $T_i$  = initial evaluation time (i);  $T_{i+1}$  = time of the subsequent evaluation (i+1); and n = number of evaluations.

A randomized block experimental design with four replications was used in a split-plot arrangement, with each block corresponding to a sowing date. Each replication consisted of a five-row plot (5.0 m long, 2.5 m wide; 12.5 m<sup>2</sup>). Evaluations were conducted using plants from the three central rows, excluding 50 cm from each end (6 m<sup>2</sup>). The obtained data were subjected to analysis of variance (ANOVA, F test,  $p \le 5\%$ ) and significant different means were compared using Tukey's test ( $p \le 5\%$ ) (BARBOSA; MALDONADO JUNIOR., 2015).

#### **RESULTS AND DISCUSSION**

Seedlings from seeds sown on November 16 emerged on November 24 (Table 1), and the first rust pustules were detected on March 4 (Figure 1A; Table 3), corresponding to the phenological stage R8.2, near harvest (Table 1). The results showed that the later the sowing date, the earlier the phenological stage at which the first rust pustules were detected (Figure 1A; Table 3). The first rust pustules were found at stage R6 (March 6) for the sowing on November 28; at R5.5 (March 7) for the sowing on December 11; at R5.4 (March 14) for the sowing on December 18; at R5.3 (March 14) for the sowing on January 2; and at R5.2 (March 21) for the sowing on January 6 (Figure 1A; Table 3). Therefore, the first rust pustules were detected at the reproductive soybean stage for each sowing date (Figure 1A; Table 3).

The analyses of variance for disease severity (%), AUDPC, and soybean grain yield (kg ha<sup>-1</sup>) revealed significant differences among the fungicide treatments trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + cyproconazole (32 g ha<sup>-1</sup> and sowing dates (Table 2). Rust severity was significantly affected by the interactions among treatments, sowing dates, and rust severity (Table 2; Figures 1A and 1B; Table 4). However, grain yield was not significantly influenced by these interactions (Table 2). Plants from the earliest sowing (November 16) developed the first pustules at R8.2, after more than 50% defoliation had occurred; therefore, no further rust severity evaluation was performed. Consequently, no AUDPC was calculated for this sowing date. Rust likely had minimal influence on grain yield at this sowing date (Table 3).

Selecting the correct soybean sowing date is crucial for producing healthy plants and seeds and for achieving higher grain yields (OLIGINI et al., 2021). In areas within the Cerrado biome in Brazil, early soybean sowing, between October and mid-November, typically results in fewer plant health problems (ÁVILA et al., 2003). This strategy may reduce the need for fungicide applications by exploiting unfavorable weather conditions for the disease (DEL PONTE et al., 2006). Furthermore, combining practices from different strategies may enhance the efficacy of disease control (NEGRISOLI et al., 2022). Combining sowing dates with scheduled or phenological-based fungicide schemes can reduce both disease severity and fungicide applications (MUELLER et al., 2009; NEGRISOLI et al., 2022).

**Table 2**. Analyses of variance for the effect of fungicide application schemes (FAS), sowing dates (SD), and rust severity (RS) on the area under the disease progress curve (AUDPC) and yield of soybean plants of the M 6952 IPRO Intacta.

Course of variation	Disease severity		AUDPC		Grain yield	
Source of variation	DF	MS	DF	MS	DF	MS
Block	3	62.05*	3	2706.70*	3	64847.26 NS
FAS <sup>1</sup>	4	3888.41**	4	507046.47**	4	8109909.33**
Error	12	12.99	12	758.94	12	110215.80
SD	4	3505.69**	4	491606.18**	5	6068213.68**
FAS X SD	16	235.42**	16	35733.91**	20	269138.22 NS
Error	60	25.20	60	1483.78	75	234208.47
RS <sup>2</sup>	2	60244.54**	-	-	-	-
FAS x RS	8	587.10**	-	-	-	-
SD x RS	8	1531.30**	-	-	-	-
FAS x SD x RS	32	194.43**	-	-	-	-
Error	150	24.48	-	-	-	-

DF = Degrees of freedom; MS = Mean square. <sup>1</sup>Treatments including a control without fungicide application. <sup>2</sup>Three rust severity evaluations based on the percentage of leaves with symptoms. AUDPC was not calculated for SD-1, in which rust appeared in R 8.2. SD: November 16, November 28, December 11, and December 18 in 2014; and January 2 and January 6 in 2015. FAS: (T1) no fungicide; (T2) scheduled application with the first application at 15 to 25 days after emergence (DAE); (T3) application based on soybean phenological stages; (T4) scheduled application based on DAE with seven-day delay relative to T2; and (T5) application based on phenological stage (SPS) with a seven-day delay relative to T3. Fungicides applied: trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + cyproconazole (32 g ha<sup>-1</sup>).



Sowing date	Rust detection date	PS <sup>3</sup>	kg ha⁻¹	AUDPC
(1)16/Nov/14	4/Mar/15	R 8.2	4318.7 b <sup>4</sup>	(-) <sup>5</sup>
(2)28/Nov/14	4/Mar/15	R6	4785.3 a	287.6 d
(3)11/Dec/14	7/Mar/15	R 5.5	4122.9 b	321.2 d
(4)18/Dec/14	14/Mar/15	R 5.4	3985.4 b	496.2 b
(5)2/Jan/15	14/Mar/15	R 5.3	3915.0 b	673.2 a
(6)6/Jan/15	21/Mar/15	R 5.2	3113.5 c	378.2 c
	Rust detection date			
SAF	SD-1 - SD-2 - SD-3 - SD-4 - SD-5 - SD-6	-	kg ha <sup>-1</sup>	AUDPC <sup>2</sup>
(1) Control <sup>1</sup>	4/Mar - 4/Mar - 7/Mar - 14/Mar - 14/Mar - 21/3	-	3740.2b	683.5 a
(2) DAE	4/Mar - 6/Mar - 18/Mar - 14/Mar - 21/Mar - 25/Mar	-	4140.3 a	349.1 c
(3) SPS	- / 6/Mar - 14/Mar - 14/Mar - 18/Mar - 25/Mar	-	4227.2 a	289.5 d
(4) DAE + 7 d	4/Mar - 7/Mar - 11/Mar - 14/Mar - 21/Mar - 25/Mar	-	4040.9 ab	489.6 b
(5) SPS + 7 d	4/Mar - 6/Mar - 11/Mar - 14/Mar - 21/Mar - 25/3	-	4052.2 a	344.5 c

**Table 3.** Influence of sowing dates (SD) and schedules of applications of fungicides (SAF) on the yield (kg ha<sup>-1</sup>) of soybean plants of the M 6952 IPRO Intacta; and on the area under the disease progress curve (AUDPC).

Means followed by same letter in the columns are not significantly different from each other by the Tukey's test ( $p \le 0.05$ ). <sup>1</sup>Treatment without fungicide application. <sup>2</sup>AUDPC was not calculated for SD-1, in which rust appeared in R 8.2. Sowing dates: November 16, November 28, December 11, and December 18 in 2014; and January 2 and January 6 in 2015. Fungicide application schemes: (T1) no fungicide; (T2) scheduled application with the first application at 15 to 25 days after emergence (DAE); (T3) application based on soybean phenological stages; (T4) scheduled application based on DAE with seven-day delay relative to T2; and (T5) application based on phenological stage (SPS) with a seven-day delay relative to T3. Fungicides applied: trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + cyproconazole (32 g ha<sup>-1</sup>).

Table 4. Effects of sowing date and fungicide application schemes (FAS) on rust severity (%) for the soybean cultivar M 6952 IPRO Intacta.

FAS		Rust Severity (percentage of leaves with symptoms)							
	November 2014		December 2014		January 2015		Mean		
	16	28	11	18	2	6			
Control <sup>1</sup>	<b>-</b> <sup>2</sup>	37.0 a AB	24.1 a C	35.7 a B	42.4 a A	33.9 a B	34.6 a		
DAE	-	15.4 b BC	10.3 b C	18.4 bc B	29.1 c A	13.5 bc BC	17.3 c		
SPS	-	4.4 c C	8.5 b BC	13.4 c B	32.8 bc A	11.7 c B	14.2 d		
DAE + 7 d	-	10.0 b C	21.8 a B	30.4 a A	35.2 b A	18.2 b B	23.1 b		
SPS + 7 d	-	12.1 b CD	10.1 b D	20.6 b B	28.6 c A	17.9 b BC	17.9 c		
Mean	-	15.8 D <sup>4</sup>	14.9 D	23.7 B	33.6 A	19.0 C	-		

Means followed by same lowercase letter in the columns or uppercase letter in the rows are not significantly different from each other by the Tukey's test ( $p \le 0.05$ ). <sup>1</sup>Treatment without fungicide application. <sup>2</sup>Soybean rust appeared in R 8.2. Sowing dates: November 16, November 28, December 11, and December 18 in 2014; and January 2 and January 6 in 2015. FAS: (T1) no fungicide; (T2) scheduled application with the first application at 15 to 25 days after emergence (DAE); (T3) application based on soybean phenological stages; (T4) scheduled application based on DAE with seven-day delay relative to T2; and (T5) application based on phenological stage (SPS) with a seven-day delay relative to T3. Fungicides applied: trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + cyproconazole (32 g ha<sup>-1</sup>).

Fungicide application is among the most effective methods for controlling Asian soybean rust, provided the timing is optimized (REIS, 2013; REIS, CARREGAL, ZANATTA, 2019). Fungicide formulations containing active ingredients from different chemical groups (e.g., triazole and strobilurin), such as the combinations used in the present study (trifloxystrobin at 60 g ha<sup>-1</sup> + prothioconazole at 70 g ha<sup>-1</sup> and trifloxystrobin at 75 g ha<sup>-1</sup> + cyproconazole at

32 g ha<sup>-1</sup>), are more effective and less likely to promote the selection of fungicide-resistant fungal isolates compared to formulations with a single active ingredient (VIEGAS NETO et al., 2021). According to Negrisoli et al. (2022), determining the timing of fungicide applications by monitoring disease onset ensures more effective control, and early preventive applications can improve control efficacy compared to scheduled schemes based on days after soybean seedling





emergence. Therefore, alternatives to scheduled fungicide applications should be explored to either reduce the number of applications or improve the efficacy of a fixed application schedule. Such alternatives include fungicide applications based on soybean phenological stage (NASCIMENTO et al., 2022), as demonstrate by the results of the present study.

Sowing on November 28 (Table 3) resulted in the highest grain yield (~4785 kg ha<sup>-1</sup>). Sowing on November 16 (~4319 kg ha<sup>-1</sup>) did not result in a yield significantly different from that of November 28. The sowings on December 11, December 18, and January 2 resulted in no significant differences in yield, producing approximately 4123, 3985, and 3915 kg ha<sup>-1</sup>, respectively. Sowing on January 6 resulted in the lowest grain yield (~3114 kg ha<sup>-1</sup>).

The highest grain yield and the lowest disease severity were observed in the treatment with fungicide applications based on plant phenology (yield: ~4227 kg ha<sup>-1</sup>; AUDPC: ~290). The grain yields of treatments based on DAE (days after seed emergence), DAE + 7 days, and phenology + 7 days were not significantly different from the treatment based on plant phenology, yielding approximately 4227, 4041, and 4052 kg ha<sup>-1</sup>, respectively. The control treatment (no fungicide) exhibited the lowest grain yield (~3740 kg ha<sup>-1</sup>), the highest disease severity (Table 3; Figure 2), and early defoliation (data not shown) compared to the other treatments. In contrast, the treatment based on phenology exhibited the lowest severity (Table 2) and later defoliation (data not shown).



**Figure 2**. Soybean rust severity (%) progression as a function of (A) fungicide application schemes: (T1) no fungicide; (T2) scheduled application with the first application at 15 to 25 days after emergence (DAE); (T3) application based on soybean phenological stages; (T4) scheduled application based on DAE with seven-day delay relative to T2; and (T5) application based on phenological stage (SPS) with a seven-day delay relative to T3. Fungicides applied: trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + cyproconazole (32 g ha<sup>-1</sup>); (B) sowing dates (SD), and (C) accumulated rainfall (mm); and (D) accumulated rainfall (mm) between each sowing to harvesting period (Nov 16 to Mar 28; Nov 28 to Apr 4, Dec 11 to Apr 11, Dec 18 to Apr 18, Jan 2 to Apr 25, and Jan 6 to Apr 25). (A) and (B): HSD = Tukey's Honest Significant Difference test ( $p \le 0.05$ ); (C) \*\* = Regression analyses significance ( $p \le 0.01$ )].

Soybean plants from seeds sown on January 2 (fifth sowing date) exhibited the highest disease severity (~34%) compared to other sowing dates (Table 4). Sowing on December 18 (fourth sowing date) differed significantly from sowing on January 2, as well as from sowings on December 11 and January 6 (third and sixth sowing dates, respectively). Plants from the sowing on November 28 exhibited the lowest disease severity, with no significant differences between the sowing times on December 11 and January 6. Higher rainfall volumes at the end of crop cycle, combined with the early onset of the disease, resulted in the highest severity observed in plots with late sowing (Figure 2).

Early sowing of short cycle cultivars, such as M 6952 IPRO is an effective control strategy that minimizes favorable weather conditions for disease development on susceptible plant tissues (MACHADO et al., 2018). This approach likely reduces inoculum pressure, enhances the efficacy of chemical fungicides, and increases grain yield. Del Ponte et al. (2006) found a significant positive correlation between higher rainfall levels and increased severity of Asian soybean rust, a



relationship also observed in the presented study ( $R^2 = 0.93$ ; p < 0.01).

Preventive fungicide applications effectively reduce disease severity and mitigate potential crop yield losses (REIS, 2013; REIS, CARREGAL, ZANATTA, 2019). Godoy and Canteri (2004) conducted greenhouse experiments and reported longer residual period and improved performance of fungicides applied preventively. Additionally, Mueller et al. (2009) emphasized the importance of fungicide application timing in controlling soybean rust, reporting that proper cultural practices and sowing times are essential to prevent yield losses without excessive fungicide applications. Moreover, Godoy et al. (2009) reported that sequential fungicide applications based on plant phenology reduced Asian rust severity and increased grain yield.

A trend of increased grain yield gains linked to fungicide applications timed according to plant phenology. Additionally, a meta-analysis in the United States and Canada indicated that properly timed fungicide applications are profitable when foliar diseases reduce green leaf area (KANDEL et al., 2021). In contrast, these authors found that unnecessary fungicide applications in soybean crops are less likely to be economically beneficial.

## CONCLUSION

Fungicide applications based on plant phenology, using trifloxystrobin (60 g ha<sup>-1</sup>) + prothioconazole (70 g ha<sup>-1</sup>) and trifloxystrobin (75 g ha<sup>-1</sup>) + (cyproconazole 32 g ha<sup>-1</sup>), significantly increased the soybean grain yield (~4227 kg ha<sup>-1</sup>; ~13% increase) while reducing rust severity (~58% reduction). Plants sown on November 28 achieved the highest grain yield (~4785 kg ha<sup>-1</sup>) and the lowest disease severity (~57% less). In contrast, seeds sown on January 6 resulted in the lowest grain yield (~1620 kg ha<sup>-1</sup> less). Delaying fungicide applications by seven days increased rust severity and reduced grain yield (~ 180 kg ha<sup>-1</sup> reduction). The absence of fungicide applications led to a significant grain yield reduction (~ 480 kg ha<sup>-1</sup>).

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