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## Asian soybean rust management based on leaf area index and multisite fungicide<sup>1</sup>

Manejo da ferrugem asiática da soja baseado no índice de área foliar e fungicida multissítio

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### HIGHLIGHTS:

*Based on the leaf area index (LAI), a new approach was proposed to start the fungicide applications to control Asian soybean rust. Fungicide application started at 3.6 LAI proved to be the best option to control Asian soybean rust. An increase in carrier volume does not benefit the spray deposition on soybean leaves.*

**ABSTRACT:** Soybean foliar diseases negatively affect leaf area index (LAI) and compromise the main structure for photosynthesis and grain yield. The objective of this study was to propose a new methodology to determine the commencement of fungicide application based on the soybean LAI, spray volumes, and the inclusion of a multisite fungicide to control Asian soybean rust (ASR). The study was implemented in a randomized block design at two locations, with four replicates in a factorial scheme plus one additional treatment ( $4 \times 2 \times 2 + 1$ ) - four soybean LAI (0.9, 1.8, 3.6, and 6.1) indicating the initiation stages of fungicide applications, two spray volumes (70 and 120 L ha<sup>-1</sup>), with or without multisite fungicide (mancozeb), and a control treatment without fungicide. The spray deposition on plants did not differ between the treatments for 1.8 and 3.6 LAI. Treatments including the multisite fungicide for LAI between 3.6 and 6.1 for both spray volumes presented superior ASR control results. The highest soybean yields were observed when fungicide application started at 3.6 LAI for both spray volumes. The best correlation between disease control and grain yield occurred when the applications started at 3.6 LAI with 70 L ha<sup>-1</sup> spray volume and the multisite fungicide.

**Key words:** *Glycine max*, *Phakopsora pachyrhizi*, application timing, spray volume, plant protection

**RESUMO:** As doenças foliares da soja afetam o índice de área foliar (IAF) e a estrutura principal para a fotossíntese e a produtividade. O objetivo deste estudo foi propor uma nova metodologia para determinar o início das aplicações de fungicidas, com base no IAF, na taxa de aplicação e na inclusão de um fungicida multissítio, para controlar a ferrugem asiática da soja (FAS). O estudo foi implementado no delineamento de blocos casualizados e em dois locais, com quatro repetições em um esquema fatorial triplo mais um tratamento adicional ( $4 \times 2 \times 2 + 1$ ), sendo 4 IAF da soja (0,9, 1,8, 3,6 e 6,1) indicadas para iniciar as aplicações de fungicida; dois volumes de pulverização (70 e 120 L ha<sup>-1</sup>); com ou sem fungicida multissítio (mancozeb), e tratamento sem fungicidas. A deposição de calda sobre as plantas não diferiu entre os manejos estudados para o IAF de 1,8 e 3,6. Os tratamentos incluindo fungicida multissítio em IAF de soja entre 3,6 e 6,1, em ambos os volumes, apresentaram resultados de controle superior. Os maiores rendimentos foram observados quando a aplicação de fungicida começou em 3,6 de IAF para os dois volumes. A melhor relação entre controle de doenças e produtividade ocorreu quando as aplicações começaram em 3,6 de IAF com 70 L ha<sup>-1</sup> e fungicida multissítio.

**Palavras-chave:** *Glycine max*, *Phakopsora pachyrhizi*, tempo de aplicação, volume de pulverização, proteção de plantas

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## INTRODUCTION

Soybean is one of Brazil's most important cultivated crops (Silva et al., 2020; Umburanas et al., 2022). The maximum yield of a plant is determined by its ability to intercept solar radiation using the leaves and convert it to dry matter (Zanon et al., 2015). Foliar diseases, such as Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi*, directly affect the leaf area and can result in over 80% loss (Rosa et al., 2015; Godoy et al., 2016; Meira et al., 2020).

Information on the effectiveness of each fungicide, season, plant stage, architecture, intervals, and number of applications is essential for the control of ASR, considering the aggressiveness and potential of damages and losses (Müller et al., 2018; Bandara et al., 2020; Viegas Neto et al., 2021). However, low sensitivity of *P. pachyrhizi* to fungicides, such as demethylation inhibitors (IDM, triazoles), external quinone inhibitors (IQe, strobilurins), and inhibitors of succinate dehydrogenase (ISDH, carboxamides), has already been reported in Brazil (Klosowski et al., 2016; Simões et al., 2018).

Currently, ASR management is based on multisite fungicides that affect different aspects of phytopathogen metabolism. A calendar-based program with two or three applications is widely used and provides adequate yield protection; however, this may result in unnecessary fungicide application (Garcia et al., 2020). One possibility to reduce this problem is to use information about crop development. However, there is a dearth of information in the existing literature on the use of leaf area index for the management of soybean fungicides. Even with fungicides of good efficacy, the application technology is a limiting factor for efficient control (Cunha et al., 2016).

The objective of this study was to propose another methodology to determine the commencement of fungicide application based on the soybean leaf area index (LAI), spray volumes, and the inclusion of a multisite fungicide to control ASR.

## MATERIAL AND METHODS

Field experiments were conducted in two areas in the municipality of Uberlândia, Minas Gerais State, Brazil. Area 1 was located at the Capim Branco Farm of the Universidade Federal de Uberlândia (UFU) (18° 52' S and 48° 20' W, altitude of 805 m), and area 2 was located at the MR Agropesquisa experimental station (18° 50' S and 48° 06' W, altitude of 887 m). The climate of the region is classified as Aw (tropical savanna, wet) according to Köppen's classification (Beck et al., 2018), which presents hot, rainy summers and cold, dry winters.

The soybean cultivar Monsoy 7739 IPRO was sown in both areas. This cultivar has great regional use. Mechanical sowing occurred in the last third of the spring season (2017).

In area 1, sowing occurred on November 29 and in area 2, on December 11. Maize was the previous crop sown in these areas. A conventional soil tillage system aimed at complete weed control was implemented in these areas. The seeds were sown with the sowing lines spaced 0.5 m apart, resulting in 13 established plants per linear meter.

The experimental area was 18 m<sup>2</sup> (6 × 3 m) with six rows (0.5 m between sowing lines). The useful plot - the area from where all the data except for the soybean LAI were collected - consisted of the four central sowing lines excluding 0.5 m from each end.

The experimental design was in randomized blocks, with four replicates in the factorial scheme 4 × 2 × 2 + 1, consisting of four LAI to start fungicide applications (0.9, 1.8, 3.6, and 6.1); two spray volumes (70 and 120 L ha<sup>-1</sup>); presence or absence of multisite fungicide (mancozeb, 750 g kg<sup>-1</sup>, water-dispersible granules, 1,125 g a.i. ha<sup>-1</sup>) added to the spray solution containing the site-specific fungicides, plus an additional treatment (control) without fungicide application. LAI is the ratio of the leaf area to the area of soil occupied by crop plants (Fang et al., 2019; Parker, 2020).

Three fungicides were applied at 14-day intervals. The site-specific fungicides that were used are listed in Table 1. In the first fungicide application, the soybean methylated oil adjuvant was used (0.25% V/V); in the second application, mineral oil (0.5% V/V) was used as the adjuvant; and no adjuvant was added to the spray solution in the third fungicide application.

A CO<sub>2</sub> pressurized backpack spray equipped with a bar containing six nozzles spaced 0.5 m apart, with flat spray tips (Magnojet 110015 BD), adjusted to a constant pressure of 250 kPa (2.5 bar) and a fine droplet spectrum according to the manufacturer, was used. The average speed of work for the spray volume of 70 L ha<sup>-1</sup> was 8.5 km h<sup>-1</sup>, while for 120 L ha<sup>-1</sup> the average speed was approximately 5 km h<sup>-1</sup>. The speed of the application was checked using a stopwatch to maintain a uniform application.

Meteorological conditions were monitored during the applications using a digital thermo-hygro-anemometer (Luxmeter LM model 8000, AKSO, São Leopoldo, Brazil). The applications were carried out early in the morning (6-9 a.m.). No precipitation was observed during and 24 h after the application. In area 1, during the fungicide application, the air temperature ranged from 24.7 to 29.8 °C, the relative humidity of the air ranged from 53.2 to 84.3%, and the wind speed ranged from 2.3 to 6.8 km h<sup>-1</sup>. In area 2, the air temperature ranged from 24.3 to 29.8 °C, the relative humidity of the air ranged from 59.3 to 75.4%, and the wind speed ranged from 2.1 to 5.7 km h<sup>-1</sup>.

Ten plants were collected at random in late summer (2018), within the borders of each experimental site, and the soybean LAI was measured using a leaf area meter (LI 3100C, LI-COR<sup>\*</sup>

**Table 1.** Site-specific fungicides used to control Asian soybean rust (*Phakopsora pachyrhizi*) on soybean

Active ingredient*	Formulation	Concentration (g a.i. L <sup>-1</sup> )	Dose (L ha <sup>-1</sup> )	Application
Prothioconazol + trifloxistrobina	SC	175 + 150	0.4	First
Epoxyconazole + fluxpyroxade + piraclostrobin	EC	50 + 50 + 81	0.8	Second
Picoxystrobin + benzovindiflupir	EC	100 + 50	0.6	Third

\* Each application also included a multisite fungicide (mancozeb, 1,125 g a.i. ha<sup>-1</sup>). a.i.: Active ingredient. SC: suspension concentrate; EC: emulsifiable concentrate. All fungicides have local registration for use on soybean crops

Biosciences, Lincoln, USA) in the laboratory on the same day. This evaluation was performed outside the useful plot to avoid interfering with the other assessments within the useful plot.

The mean leaf area of the 10 plants collected was multiplied by the number of plants per square meter to determine the LAI (Weiss et al., 2004).

A tracer dye (food blue coloring, Food, Drug, and Cosmetic Blue No. 1 bright blue) was added ( $0.5 \text{ kg ha}^{-1}$ ) to the spray solution to evaluate spray deposition on plants. The tracer dye on the leaves was detected using absorbance spectrophotometry (photoelectric photometer tapes with a tungsten-halogen lamp). Tracer dye quantification was performed by measuring the absorbance at 630 nm, which is the detection range of the tracer dye used (as described by Palladini et al. (2005)).

After the first application in each LAI was studied, 10 leaflets each were collected from the upper and lower third of the soybean plant canopy. The leaves were placed in plastic bags to maintain the moisture content. The bags were immediately placed in a thermal plastic insulated box ( $80 \times 40 \text{ cm}$ ). Tracer dye extraction was performed in the laboratory on the same day by adding 0.1 L of distilled water to each bag. After stirring for 10 s, the solution was placed in plastic cups and left to rest for 24 h in the dark, for later absorbance readings. The leaflet areas used for tracer dye extraction were also measured (LI 3100C, LI-COR Biosciences, Lincoln, USA). Absorbance data were transformed using a calibration curve obtained from a standard solution to determine the tracer dye mass in the corresponding leaf area ( $\mu\text{g cm}^{-2}$ ).

The severity of ASR was estimated using six ratings throughout the crop cycle. Ten soybean leaflets were randomly collected from each plot and were rated according to the scale proposed by Godoy et al. (2006). Disease severity data were integrated to obtain the area under the disease progression curve (AUDPC) according to the formula proposed by Campbell & Madden (1990). The effectiveness (E) of the fungicide treatments compared with the control treatment was verified using the AUDPC values obtained using the formula proposed by Abbott (1925). By the end of the soybean crop cycle, soybean grain yield was estimated from the useful plot area (the four central sowing lines). The results were extrapolated to kilograms per hectare and corrected for moisture content of 13%.

The statistical data analysis included the evaluation of the analysis of variance (ANOVA) presumptions tested by the Shapiro-Wilk and Levene tests for normality of the residues and homogeneity of variances, respectively. After attending to these presumptions, the data were subjected to ANOVA ( $p \leq 0.05$ ). The mean values were compared using Tukey's test ( $p \leq 0.05$ ). The control was compared with other fungicide treatments using Dunnett's test ( $p \leq 0.05$ ). The soybean LAI, used as a reference to start fungicide applications, was studied using polynomial regressions.

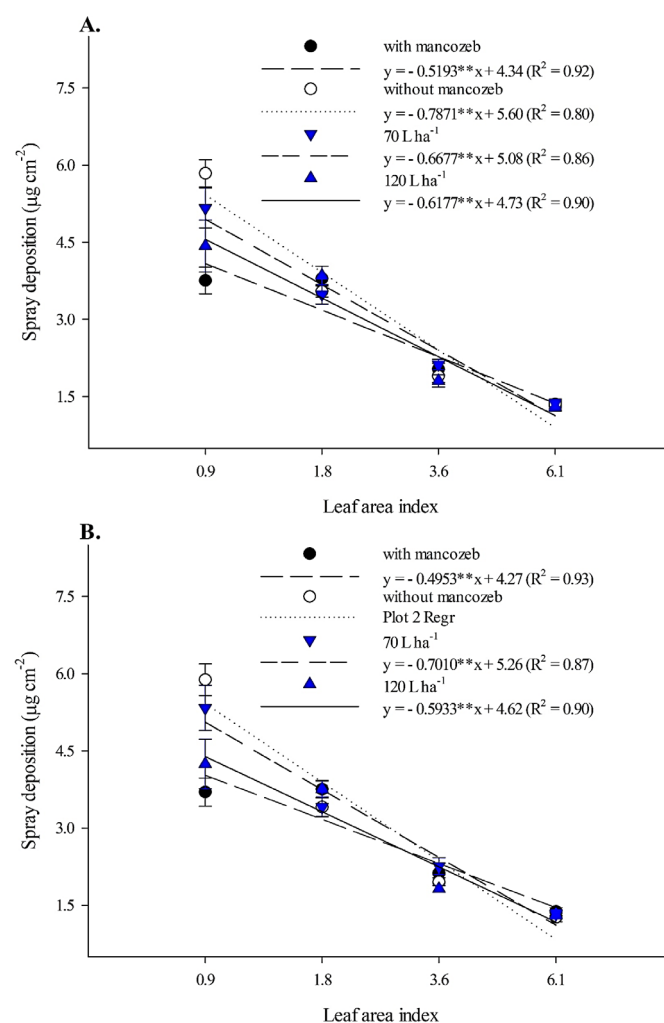
## RESULTS AND DISCUSSION

Spray deposition on the lower and upper third in both areas was not affected by the triple interaction (leaf area

index  $\times$  spray volume  $\times$  multisite fungicide (mancozeb)). The double interactions of LAI with spray volume or mancozeb were significant ( $p < 0.05$ ) in both areas, but no interaction was observed between spray volume and mancozeb ( $p > 0.05$ ). Significant interactions were studied using polynomial regression and tables of means.

Spray deposition in the lower third of the soybean canopy in both areas decreased linearly with increasing soybean LAI (Figure 1). Higher LAI compromised the efficacy of the fungicides in the lower third of the plant canopy, regardless of the presence of the multisite fungicide (mancozeb) or the spray volume studied.

The spray deposition according to spray management was equal ( $p > 0.05$ ) for the two areas (Table 2). In both areas (Figures 1A and B), the increase in the spray volume in the studied range ( $70$  and  $120 \text{ L ha}^{-1}$ ) did not result ( $p > 0.05$ ) in increased spray deposition in the lower third for any of the soybean LAI studied ( $0.9$ ,  $1.8$ ,  $3.6$ , and  $6.1$ ) (Table 2). However, Roehrig et al. (2018) reported that reductions in spray volume conversely affect spray deposition on the leaves of the lower third of the soybean plant canopy. Typically, farmers use  $50$ – $150 \text{ L ha}^{-1}$ .



Vertical bars indicate standard errors. \*\*: significant at  $p \leq 0.05$

**Figure 1.** Spray deposition ( $\mu\text{g cm}^{-2}$ ) in the lower third of soybean canopy as a function of leaf area index for two spray volumes, in the presence and absence of mancozeb in (A) area 1 (CV: 15.86%), and (B) area 2 (CV: 15.64%)

The presence of mancozeb also did not influence ( $p > 0.05$ ) spray deposition in the lower third, except for the 0.9 LAI, which was lower than that of the other treatments (Table 2). The differences observed among the treatments occurred for applications starting at 0.9 LAI, when the crop presented no physical impediments to the applications, due to their low stage of development, ensuring good leaf coverage in the lower third for all treatments.

A quadratic regression model was significant for the upper third of all treatments studied in both areas, except when mancozeb was applied because of the low coefficient of determination ( $R^2 < 0.60$ ), and indicated that the lowest spray depositions were observed around the LAI of 3.6 (Figure 2).

Changes in the tendency of spray deposition are shown in Figures 2A and B. The decrease in spray deposition from 0.9 to 3.6 LAI was due to the increasing cover caused by the development of new leaves as the plant grew. The higher spray deposition observed in 6.1 LAI was due to changes in the leaf collection region caused by the fall of older soybean leaves from the lower third. This natural senescence forced the collection of leaves more exposed to the spray solution during application.

In the 6.1 LAI, the spray deposition of the lower spray volume (70 L h<sup>-1</sup>) without mancozeb was higher for both areas (Figures 2A and B), than the spray deposition of the higher volume (120 L h<sup>-1</sup>) with mancozeb. The higher deposition of the lower spray volume (70 L h<sup>-1</sup>) could be caused by the tracer concentration (0.5 kg ha<sup>-1</sup>), which was higher at this volume than at 120 L h<sup>-1</sup>.

The spray deposition on the upper third showed differences between the studied treatments when fungicide application started at 0.9 and 6.1 soybean LAI (Table 3).

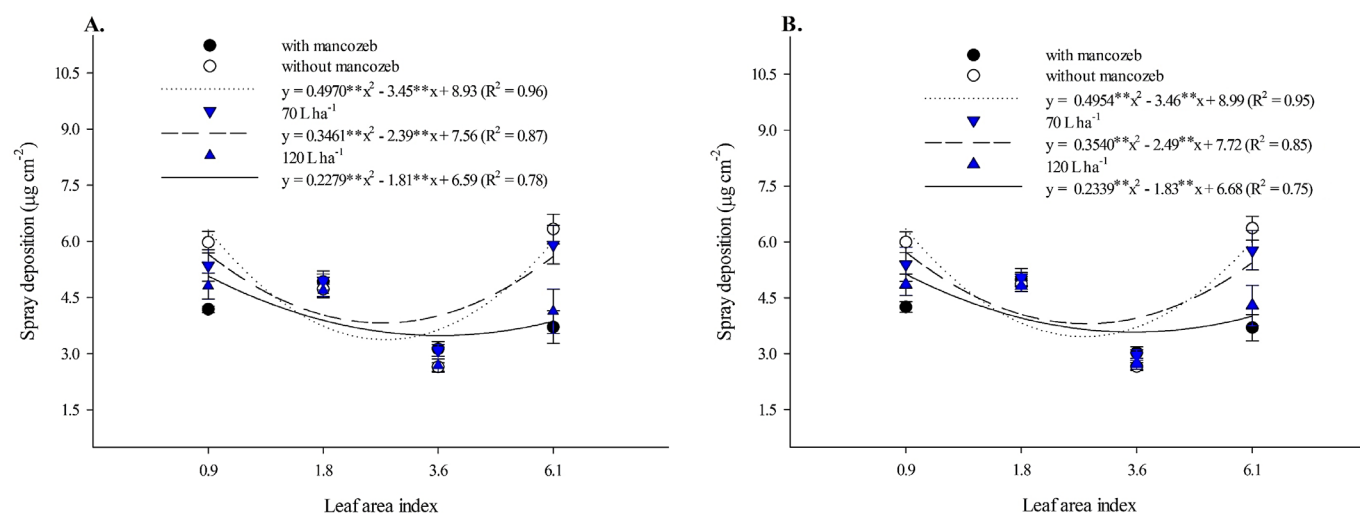
The LAI affects spray deposition on the plant canopy and should be considered when choosing the appropriate spray technology (Chechi et al., 2018; Viegas Neto et al., 2021). Cunha et al. (2018) indicated that a spray volume of 80 L ha<sup>-1</sup> provided, in general, more extensive spray solution retention on soybean leaves than 150 L ha<sup>-1</sup>. In this study, the lowest spray volume applied also presented superior deposition at 0.9 LAI for the lower (Table 2) and upper (Table 3) thirds of the plant canopy, and at the 6.1 LAI only for the upper third.

The ASR presented different degrees of severity between the areas, as expressed by AUDPC (Figure 3). In area 1, there was less disease pressure, no occurrence in the previous year or in

**Table 2.** Spray deposition ( $\mu\text{g cm}^{-2}$ ) in the lower third of the soybean canopy in each studied LAI, with two spray volumes, in the presence and absence of mancozeb

LAI	Area 1				Area 2											
	wM	w/oM	70 L ha <sup>-1</sup>	120 L ha <sup>-1</sup>	wM	w/oM	70 L ha <sup>-1</sup>	120 L ha <sup>-1</sup>								
0.9	3.76	b	5.84	a	5.17	a	4.43	b	3.70	b	5.88	a	5.34	a	4.24	b
1.8	3.79	a	3.54	a	3.36	a	3.72	a	3.75	a	3.40	a	3.41	a	3.75	a
3.6	2.03	a	1.90	a	2.13	a	1.81	a	2.12	a	1.96	a	2.26	a	1.82	a
6.1	1.33	a	1.36	a	1.38	a	1.31	a	1.38	a	1.26	a	1.33	a	1.31	a

Averages followed by different lowercase letters differ (Tukey's test,  $p \leq 0.05$ ) in the spray volume for the same area. wM: with mancozeb; w/oM: without mancozeb



Vertical bars indicate standard errors. \*\*: significant at  $p \leq 0.05$

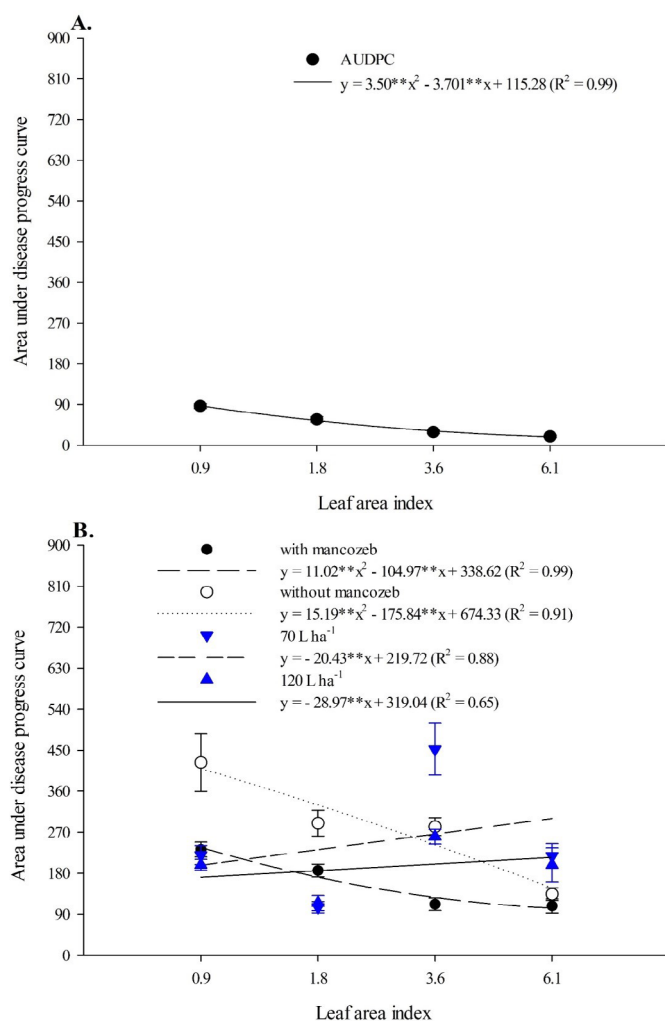
**Figure 2.** Spray deposition ( $\mu\text{g cm}^{-2}$ ) in the upper third of soybean canopy as a function of leaf area index for two spray volumes, in the presence and absence of mancozeb in (A) area 1 (CV: 13.58%), and (B) area 2 (CV: 11.28%)

**Table 3.** Spray deposition ( $\mu\text{g cm}^{-2}$ ) in the upper third of the soybean canopy in each studied LAI, with two spray volumes, in the presence and absence of mancozeb

LAI	Area 1				Area 2											
	wM	w/oM	70 L ha <sup>-1</sup>	120 L ha <sup>-1</sup>	wM	w/oM	70 L ha <sup>-1</sup>	120 L ha <sup>-1</sup>								
0.9	4.19	b	5.98	a	5.36	a	4.81	a	4.25	b	6.00	a	5.40	a	4.85	b
1.8	4.92	a	4.72	a	4.96	a	4.69	a	4.99	a	4.87	a	5.05	a	4.81	a
3.6	3.13	a	2.65	a	3.09	a	2.69	a	3.03	a	2.66	a	2.96	a	2.73	a
6.1	3.71	b	6.33	a	5.91	a	4.13	b	3.70	b	6.37	a	5.78	a	4.29	b

Averages followed by different lowercase letters differ (Tukey's test,  $p \leq 0.05$ ) in the spray volume for the same area. wM: with mancozeb; w/oM: without mancozeb





Vertical bars indicate standard errors. \*\*: significant at  $p \leq 0.05$

**Figure 3.** Area under the disease progress curve (AUDPC) of Asian soybean rust as a function of leaf area index, with two spray volumes, in the presence and absence of mancozeb in (A) area 1 (CV: 35.77%), and (B) area 2 (CV: 20.66%)

areas close to the experiment, and late occurrence compared to area 2. Historically, soybean rust has occurred in area 2.

**Table 4.** Area under the disease progress curve (AUDPC) and effectiveness (E) of Asian soybean rust, with two spray volumes, in the presence and absence of mancozeb, in area 1 and area 2

LAI	Area 1											
	70 L ha <sup>-1</sup>				120 L ha <sup>-1</sup>							
	wM		w/oM		wM		w/oM					
	AUDPC	E (%)	AUDPC	E (%)	AUDPC	E (%)	AUDPC	E (%)				
0.9	73.1 <sup>ns</sup>	52	99.8 <sup>ns</sup>	33	87.5 <sup>ns</sup>	41	85.3 <sup>ns</sup>	43				
1.8	56.9	61	57.3	60	50.3	66	63.9	55				
3.6	25.4	82	32.8	77	32.8	76	25.4	83				
6.1	13.1	92	25.8	83	18.4	88	20.6	87				
Control	152.3*											
LAI	Area 2											
	70 L ha <sup>-1</sup>				120 L ha <sup>-1</sup>							
	wM		w/oM		wM		w/oM					
	AUDPC	E (%)	AUDPC	E (%)	AUDPC	E (%)	AUDPC	E (%)				
0.9	252.0	aA	79	566.1	bB	54	211.8	aA	83	280.0	aA	77
1.8	187.3	aA	85	338.6	bB	72	184.6	aA	85	240.6	aA	80
3.6	102.4	aA	92	277.4	bA	77	121.6	aA	90	287.0	bA	76
6.1	108.5	aA	91	158.4	aA	87	107.6	aA	91	109.4	aA	91
Control	1220.6*											

ns: not significant ( $p > 0.05$ ). Averages followed by different capital letters differ (Tukey's test,  $p \leq 0.05$ ) in spray volume for the same mancozeb management. Averages followed by different lowercase letters differ (Tukey's test,  $p \leq 0.05$ ) in the LAI for the same spray volume management. \* All fungicide treatments differed from the control treatment (Dunnnett's test,  $p \leq 0.05$ ). LAI, leaf area index; wM, with mancozeb; w/oM, without mancozeb

In addition, no differences were detected ( $p > 0.05$ ) between the spray volumes studied and the presence or absence of mancozeb in area 1, as can be observed in the graph in Figure 3A.

In area 1, no differences were detected between the spray volume and mancozeb for any of the soybean LAI studied (Table 4). This result is probably related to the lower disease pressure in this area, as seen in the control treatment (no fungicide) (Table 4). The only difference observed in area 1 was between the control and fungicide treatments. Similar differences between the control and fungicide treatments were observed in area 2.

In area 2, disease control was affected by the soybean LAI, the spray volume applied, and the presence or absence of mancozeb fungicide (Table 4). The behavior of each factor (spray volume and mancozeb) is shown in Fig. 3B. The graphs in Figure 3 indicate that the presence of mancozeb was important for improving ASR control, especially when applications started with 3.6 LAI at a spray volume of 70 L ha<sup>-1</sup>. This result is probably related to the improved plant protection provided by multisite fungicides in ASR management.

Lucion et al. (2017) indicated that multisite fungicides improve the effectiveness of systemic fungicides, providing better disease control. These authors also showed that mancozeb was the multisite fungicide that provided the best crop protection and yields. In this study, the 120 L ha<sup>-1</sup> spray volume only improved disease control compared to the 70 L ha<sup>-1</sup> spray volume for early applications (LAI of 0.9 and 1.8) (Table 4).

Nascimento et al. (2018) reported that fungicides are essential for ASR management. However, the decision about the application time depends on certain factors, such as monitoring, disease pressure, and favorable climatic conditions for disease development. Similar results were observed in this study because the disease control efficiency was directly influenced by the inoculum pressure in the area (low disease pressure in area 1) and the commencement of fungicide application. In general, the ideal periods for disease control,

in terms of LAI, were those that also presented the smallest difference between spray deposition on the upper and lower thirds of the soybean plant canopy.

LAI influenced the soybean grain yield in both areas (Figures 4A and B), at both spray volumes, and with and without mancozeb. In all treatments from both areas, the estimated yields occurred when fungicide applications started at 3.6 soybean LAI, suggesting that this stage presented the best relationship between the time of fungicide application and disease control. When the fungicide applications started at 6.1 soybean LAI, the yield losses were evident compared to those at applications starting at 3.6 soybean LAI. This result indicates that spray applications later in the soybean crop season - when ASR has already caused damage - will present reduced fungicide effectiveness and consequently increase the losses in soybean grain yield.

In area 1, the yield at 70 and 120 L ha<sup>-1</sup> spray volumes indicated that inclusion of mancozeb was not significant when the fungicide application started at 0.9 LAI (Figure 4). When the fungicide application included mancozeb and began at 6.1 LAI, the spray volumes significantly affected the yield. At 6.1 LAI, 120 L ha<sup>-1</sup> spray volume resulted in a greater soybean yield. With mancozeb treatment, the grain yield gap between the applied spray volumes became more evident, except when the applications were initiated at 0.9 LAI (Table 5).

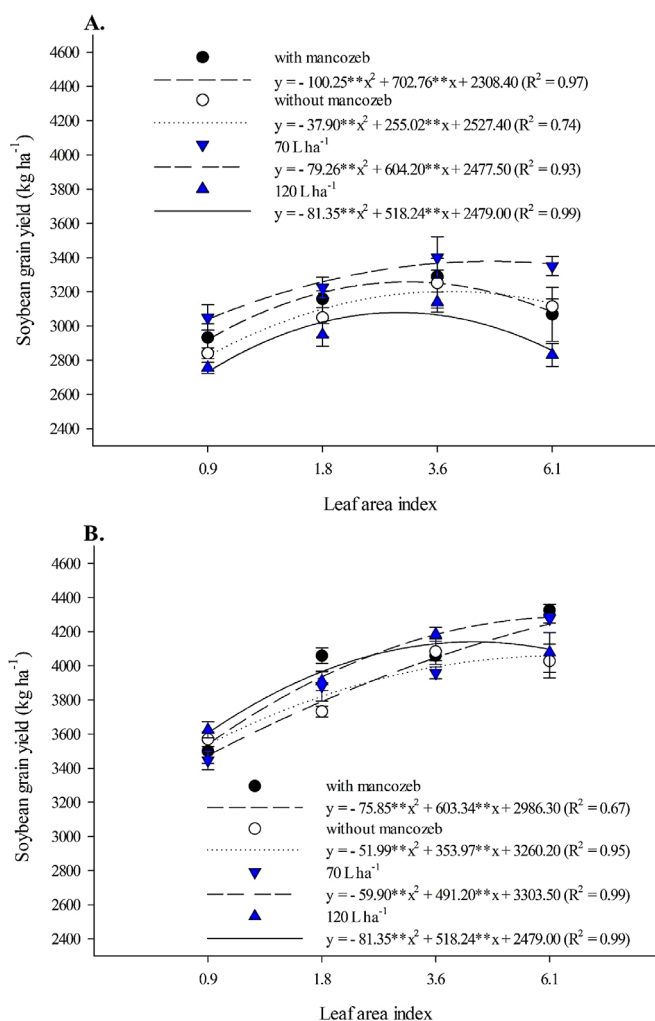
In area 2, unlike in area 1, when the fungicide applications were initiated at 0.9 LAI, and 70 L ha<sup>-1</sup> spray volume, treatment with mancozeb resulted in a lower yield. Miles et al. (2007) reported that mancozeb can be effective in rust control, but the results varied, explaining the data obtained in this study. For 120 L ha<sup>-1</sup> spray volume, only the treatments that began fungicide applications at 0.9 LAI showed no differences in the yield, with mancozeb treatment. In general, 120 L ha<sup>-1</sup> spray volume improved ASR management without the multisite fungicide (mancozeb) (Table 5).

However, Cunha et al. (2014) evaluated 115-250 L ha<sup>-1</sup> and found no major improvements in ASR control or increased grain yield using larger volumes. Therefore, lower spray volumes are logistically preferred for farm administration because more cropping areas can be covered with one tank load.

**Table 5.** Soybean grain yield (kg ha<sup>-1</sup>) for each LAI of initiation of fungicide applications, with two spray volumes, in the presence and absence of mancozeb in area 1 and area 2

LAI	Area 1							
	70 L ha <sup>-1</sup>				120 L ha <sup>-1</sup>			
	wM		w/oM		wM		w/oM	
0.9	2813.1	aA	2782.2	aA	2901.3	aA	2898.2	aA
1.8	3333.0	aA	2762.1	bB	3409.5	aA	3114.5	bA
3.6	3490.6	aA	3012.5	bB	3567.4	aA	3309.8	bA
6.1	2875.3	aB	2660.1	bA	3226.5	aA	2609.1	bA
Control	2310.4*							
LAI	Area 2							
	70 L ha <sup>-1</sup>				120 L ha <sup>-1</sup>			
	wM		w/oM		wM		w/oM	
0.9	3322.6	bB	3567.5	aA	3677.6	aA	3572.0	aA
1.8	4090.0	aA	3673.0	bA	4028.7	aA	3791.6	bA
3.6	4024.2	aA	3892.8	aB	4275.2	aA	4094.6	bA
6.1	3877.0	aB	3478.0	bA	4075.1	aA	3578.2	bA
Control	2218.3*							

Averages followed by distinct capital letters differ (Tukey's test,  $p \leq 0.05$ ) in spray volume for the same mancozeb treatment. Averages followed by different lowercase letters differ (Tukey's test,  $p \leq 0.05$ ) in the LAI for the same spray volume management. \* all fungicides treatments differed from the control treatment (Dunnett's test,  $p \leq 0.05$ ). LAI: Leaf area index; wM: With mancozeb; w/oM: Without mancozeb



Vertical bars indicate standard errors. \*\*: significant at  $p \leq 0.05$

**Figure 4.** Soybean grain yield as a function of leaf area index, for two spray volumes, in the presence and absence of mancozeb in (A) area 1 (CV: 2.90%), and (B) area 2 (CV: 2.62%)

Preventive fungicide application is strategic to better control foliar diseases, maintain healthy leaf areas, and improve yield (Bandara et al., 2020). However, defining the ideal period, in terms of LAI, for the commencement of fungicide application is critical, yet difficult.

In contrast, Sarto et al. (2013) reported that fungicide application in R2, R4, and R6 soybean reproductive stages did not influence the hundred-grain mass and grain yields. These results indicate that the most appropriate time for fungicide application, in terms of LAI, must consider other factors such as disease pressure, genotype susceptibility, and the occurrence of favorable climatic conditions. According to these authors, the pathogens that occur in soybean crops exhibit different behaviors each year, depending mainly on environmental conditions. This condition makes the definition of application time difficult, as there is no clear relationship among leaf areas, days after emergence, or days after sowing.

The present study achieved the best soybean yields when fungicide application started in the transitional phase between the vegetative and reproductive soybean stages. Improved ASR control and spray deposition on soybean leaves were generally observed in fungicide applications beginning between 3.6 and 6.1 LAI for the multisite fungicide (mancozeb) and for both the spray volumes studied (70 or 120 L ha<sup>-1</sup>).

### CONCLUSIONS

1. An increase in spray volume from 70 to 120 L ha<sup>-1</sup> did not result in greater spray deposition in the lower third of the soybean plant canopy.
2. In most cases, the multisite fungicide mancozeb increased the efficiency of the control of Asian soybean rust.
3. The best control of Asian soybean rust was observed when the fungicide application began between 3.6 and 6.1 leaf area index (LAI), with mancozeb in both spray volumes (70 or 120 L ha<sup>-1</sup>).
4. When the fungicide applications started at 6.1 LAI, the yield losses were evident.

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