

# Fungicide Spraying Programs Reducing Asian Soybean Rust Impact on Soybean Yield Components

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## Abstract

Soybean is one of the leading agricultural commodities, and Brazil is currently the largest producer globally. Despite this, fungal diseases as Asian Soybean Rust (ASR) are among the primary limiters of high crop yields in Brazilian fields. Losses caused by the biotrophic fungus *Phakopsora pachyrhizi* can reach up to 90%, depending on weather conditions, and several components can be affected during soybean growth and reproduction. Here, we assessed fungicide spraying programs to ASR control, aiming to reduce the losses on soybean components. The experiments were performed under field conditions during the harvest season 2014/2015. The evaluated variables were soybean leaf area index, ASR severity, yield components, dry mass grain accumulation, protein, and oil content. The yield components assessed were the number of pods per plant, seeds per pod, seeds per plant, and thousand-grain weight. The disease severity gradient was generated using seven fungicide spraying programs, differing in time, number, and type of fungicides. Three fungicide programs that included applications in the soybean vegetative and reproductive stages were more efficient. These programs resulted in the lower area under the disease progress curve (AUDPC), greater leaf area duration (LAD), and health leaf area duration (HAD) than the untreated soybean. The ASR infection in soybean resulted in reduced LAD and HAD, and as a consequence, interfered negatively with dry matter accumulation, yield components, and grain yield.

**Keywords:** *Glycine max*, *Phakopsora pachyrhizi*, yield, leaf area duration, leaf area index

## 1. Introduction

Soybean [*Glycine max* (L.) Merr.] is the leading agricultural commodity in Brazil, with 38.5 million cultivated hectares in the 2019/2020 season (CONAB, 2021). Currently, Brazil is the largest world soybean producer reaching 135.9 million tons of grains. Despite this, fungal diseases such as Asian Rust often threaten the sustainability of crops in South America. The Asian Soybean Rust (ASR) is caused by the biotrophic fungus *Phakopsora pachyrhizi* Sydow & Sydow, which is considered one of the most destructive diseases attacking the Fabaceae family (Goellner et al., 2020). ASR damages vary according to climatic conditions, and losses can reach up to 90% in the absence of control strategies (Hartman et al., 2015). This disease was first identified in Brazil during the 2000/2001 season and quickly became widespread due to the tropical climate favorable for ASR establishment (Yorinori et al., 2005).

ASR's initial symptoms occur on the abaxial leaf surface, which is characterized by sporulating lesions (uredospores) and frequently associated with chlorosis. The first lesions generally begin in the lower canopy and proceed upward (Kumudini et al., 2008). Severe ASR attack leads to plant tissue yellowing and leaf abscission. The final stage of a soybean rust epidemic is characterized by general foliage yellowing, intense defoliation, and damages to the whole grain (Hartman et al., 2015). The fungus survival relies on the continued production of uredospores on a suitable host. In general, optimal weather conditions that favor the disease establishment range from 10 to 27.5 °C and a minimum dew period of 6 hours (Melching et al., 1989). Continuous leaf wetness caused by dew or rain also favors the development of the disease, considering that rainfall is an essential factor in determining epidemic levels in the field (Del Ponte et al., 2006).

Since its introduction to Brazil, ASR has had a high economic effect on soybean production (Del Ponte et al., 2006). The management of ASR has brought changes to the Brazilian agricultural system, such as adopting public policies, including a soybean-free period in the off-season and a sowing limit in some states (Godoy et al., 2016). Currently, the primary control of *P. pachyrhizi* is performed using fungicides (Gabardo et al., 2020). Spraying carried out preventively or immediately after detecting the first disease symptoms showed the most efficient yield results (Mueller et al., 2009). In addition, this practice can provide an extended healthy leaf area duration (HAD) during the grain fill that directly influences the yield components and accumulates a dry grain weight (Gabardo et al., 2020).

Most studies have used disease severity through the progress curve disease or the area under the disease progress curve (AUDPC) to estimate yield losses (Mukherjee et al., 2010; Alves et al., 2017). However, the relationship of AUDPC with yield is often insufficient once crop yield is related to radiation absorbed throughout the growing season, and AUDPC is not directly related to this parameter (Jesus Junior et al., 2003). Therefore, the healthy leaf area duration (leaf area index and its integration over time) was proposed to estimate the impact of ASR lesions on the canopy's ability to intercept and absorb radiation (Waggoner & Berger, 1987), as well as the growth stage at which leaf defoliation occurs (Kumudini et al., 2008).

It is well known that *P. pachyrhizi* can infect soybean crops from early development stages (Del Ponte et al., 2006). High defoliation levels were observed at the soybean R2 stage, reducing leaf area index and yield in a study performed by Haile et al. (1998). These authors confirmed that losses were directly related to the reduction in the healthy leaf area. Another study reported that 100% of defoliation on soybean at the R6 stage caused a 40% yield loss and 20% when the defoliation occurred three weeks later (Board et al., 2010). A recent study performed by Gabardo et al. (2020) showed that the highest disease severity (> 80%) was found when the first ASR symptoms were observed at 53 days after the soybean emergence (R2 stage). Besides this, the authors showed that the maximum severity dropped by half when the first symptoms were observed later, emphasizing the importance of adopting the public policies and early disease detection strategies to extend the health leaf area duration.

Several studies have demonstrated the impact of ASR on different soybean yield components. However, there is still a lack of information about fungicide spraying strategies regarding soybean leaf area duration (LAD) and HAD and the effects on crop yield qualitative and quantitative components. In this way, the present study aimed to assess seven fungicide spraying programs' effects on reducing the ASR effects on the yield components, crop grain yield, dry mass grain accumulation, protein, and oilseed content.

## 2. Method

### 2.1 Field Site and Soybean Management

Field experiments were performed at the University of Passo Fundo Research Station in Passo Fundo, Rio Grande do Sul State, Brazil (28°13'S, 52°23'W, altitude 695 m) in 2014/2015 crop season. The soil where the experiments were carried out is a Dystrophic Red Latosol (Santos et al., 2018). The meteorological data were obtained from the Brazilian Agricultural Research Corporation (Embrapa Trigo) Research Station, located 1.2 km from the experiments. The sum of rainfall events and temperature observed during the study is shown in Figure 1.

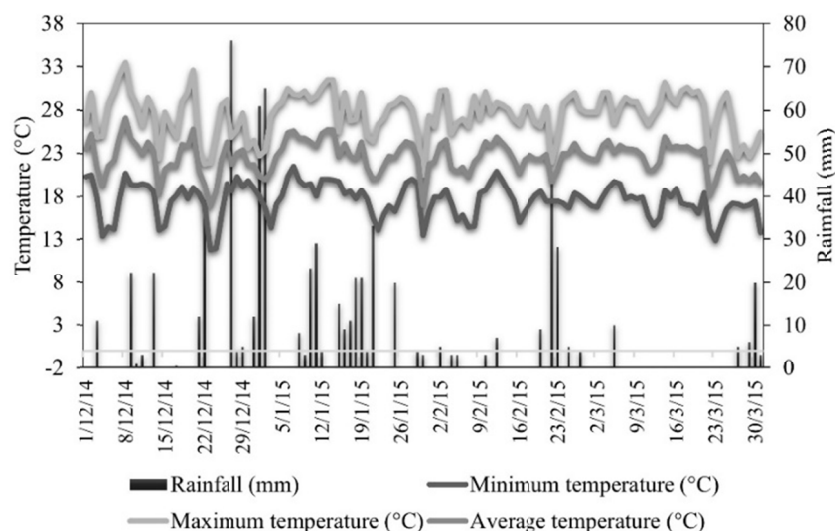


Figure 1. Biweekly rainfall (mm) indicated by bars and air temperature (°C) indicated by lines in the 2014/2015 crop season during soybean experiments in Passo Fundo, Rio Grande do Sul State, Brazil

The field area was covered with wheat crop remains from the 2014 harvest and corn from the 2013/2014 season. The weed management was conducted with glyphosate-potassium salt (1,500 g a.i. ha<sup>-1</sup>, Zapp Qi 620, Syngenta) and 2,4-D (670 g a.e. ha<sup>-1</sup>, DMA, Dow Agrosience) seven days before soybean sowing. One day before sowing, paraquat+diuron (400+200 g a.i. ha<sup>-1</sup>, Gramocil, Syngenta) was applied to control the remaining weeds. The soybean seeds were treated with abamectin (50 g a.i. 100 kg<sup>-1</sup>, Avicta, Syngenta), thiamethoxam (70 g a.i. 100 kg<sup>-1</sup>, Cruiser 350FS, Syngenta), fludioxonil (2.5 g a.i. 100 kg<sup>-1</sup> plus mephenoxam (2 g a.i. 100 kg<sup>-1</sup> plus thiabendazole (15 g a.i. 100 kg<sup>-1</sup>, Maxim Advanced, Syngenta). The soybean cultivar was Syn 13561 IPRO RR2 (maturity group 5.6, early cycle, indeterminate growth habit, and leaf area index 5.1), with density adjusted to 300,000 plants ha<sup>-1</sup> (±5%) and 0.45 m between row spacing. Fertilization was conducted according to soil analysis and soybean technical recommendation (15 kg ha<sup>-1</sup> of N, 90 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, and 45 kg ha<sup>-1</sup> of K<sub>2</sub>O) in-furrow at sowing.

The post-emergent herbicides application was performed using glyphosate-potassium salt (750 g a.i. ha<sup>-1</sup>) 20 days after sowing (DAS) and glyphosate-potassium salt (750 g a.i. ha<sup>-1</sup>) at 35 DAS. Insect control (Coleoptera and Hemiptera) was performed using thiamethoxam plus lambda-cyhalothrin (35.25 g a.i. + 26.5 g a.i. ha<sup>-1</sup>) at V8, R5, and R6 soybean stages following the official recommendations.

## 2.2 Experimental Design and Fungicide Treatments

The ASR severity gradient was generated using seven fungicide spraying programs, differing in number, application timing, and fungicide applied. The plots consisted of seven rows (6 m long) spaced 0.45 m (18.9 m<sup>2</sup>). The experiment consisted of eight fungicides spraying programs (treatments) arranged in a randomized complete block design with three replications. The evaluations were performed using the three central rows. Destructive evaluations were conducted in another paired plot. The treatments were (i) ASR untreated, (ii) ASR maximum control, and (iii) ASR standard control. Treatments from (iv) to (viii) constitute ASR intermediate control treatments (Table 1). The damage caused by *P. pachyrhizi* was estimated through the ASR maximum control treatment (ii), where the ASR was managed for maximum effectiveness. Fungicide applications were performed at 45, 65, 86, and 96 DAS. All treatments were applied using a CO<sub>2</sub> pressurized backpack sprayer (Teejet® XR-110015), spaced 50 cm apart, calibrated to deliver 150 L ha<sup>-1</sup>. The detailed fungicide programs are presented in Table 1.

Table 1. Fungicide spraying programs used to control Asian Soybean Rust (ASR) during four application timing in soybean Syn 13561. Passo Fundo, Rio Grande do Sul State, Brazil, 2014/2015

ASR fungicide treatments <sup>1</sup>	Application timing <sup>2</sup>			
	45 DAS (V7/V8)	65 DAS (R2)	86 DAS (R2+21 d)	96 DAS (R2 + 31 d)
(i) untreated	no fungicide	no fungicide	no fungicide	no fungicide
(ii) maximum control	AC+DP	AB+DP	AB+DP	AB+DP
(iii) standard control	AC+DP	AB	AB	AC
(iv) intermediate-1	AC+DP	AB	AB	no fungicide
(v) intermediate-2	AC+DP	AB	no fungicide	no fungicide
(vi) intermediate-3	AC+DP	no fungicide	no fungicide	no fungicide
(vii) intermediate-4	no fungicide	AB	AB	AC
(viii) intermediate-5	no fungicide	no fungicide	AB	AC

Note. <sup>1</sup> Fungicide and doses applied in each treatment: AC: azoxystrobin+cyproconazole (60 g a.i. ha<sup>-1</sup>+24 g a.i. ha<sup>-1</sup>, PrioriXtra, Syngenta; AB: azoxystrobin+benzovindiflupir (60 g a.i. ha<sup>-1</sup> + 30 g a.i. ha<sup>-1</sup>, Elatus, Syngenta; and DP: difenoconazole + propiconazole (37.5 g a.i. ha<sup>-1</sup> + 37.5 g a.i. ha<sup>-1</sup>, ScoreFlexi, Syngenta. Paraffinic mineral oil (42.8%, Nimbus, Syngenta) was added to the fungicides azoxystrobin+cyproconazole and azoxystrobin+benzovindiflupir at 0.6 L ha<sup>-1</sup>.

<sup>2</sup> Fungicide application timing is presented as days after soybean sowing (DAS).

### 2.3 Soybean Components and Variables Assessed

Five uniform soybean plants per plot (from R5.1 to R7 stage) were used to determine the leaf area index (LAI). Leaf area was quantified through a leaf area electronic integrator (LI-3100C, LI-COR BIOSCIENCE), and the LAI was determined by the Equation (1), where (a) is the leaf area in m<sup>2</sup> and (N) the number of plants per m<sup>2</sup>.

$$LAI = a \times N \quad (1)$$

Leaf area duration (LAD) was calculated by Equation (2), where, LAI<sub>i</sub> and LAI<sub>i+1</sub> are two evaluations performed in times t<sub>i</sub> and t<sub>i + 1</sub> and (i) is the number per plant.

$$LAD = \sum \{[(LAI_i + LAI_{i+1})/2] \times (t_i - t_{i+1})\} \quad (2)$$

The ASR severity was estimated from the first application using a diagrammatic scale proposed by Godoy et al. (2006). Disease severity evaluations were integrated as the AUDPC, according to Equation (3) (Campbell and Madden, 1990), where: y<sub>1</sub> and y<sub>2</sub> are the leaf severity in time t<sub>1</sub> and t<sub>2</sub>. The AUDPC for ASR was obtained by the relation between AUDPC and the evaluation period (43 days).

$$AUDPC = \sum \{[(y_1 + y_2)/2] \times (t_2 - t_1)\} \quad (3)$$

The efficiency of the fungicide treatments (E) was determined through the Abbott formula (4) (Abbott, 1925), where, (T) is the AUDPC obtained in the untreated treatment and (t) related to the other treatments.

$$E = (T - t) \times 100/T \quad (4)$$

The health leaf area index (HLAI) was calculated using Equation (5), where (y) is the ASR severity (%).

$$HLAI = HLA \times (1 - y) \quad (5)$$

The leaf area duration (HAD) was calculated through Equation (6), where HLA<sub>i</sub> and HLA<sub>i+1</sub> are two evaluations executed at times t<sub>i</sub> and t<sub>i+1</sub>.

$$HAD = \sum \{[(HLA_i + HLA_{i+1})/2] \times (t_i - t_{i+1})\} \quad (6)$$

Dry mass grain accumulation was measured from R5.1 to R7 stage using the same plants collected to determine the leaf area. The pods were sampled and dried in a drying oven at 65 °C. The grains were separated from the pods to determine the grain mass using a precision scale (Marte, AD500). Ten uniform plants per plot were collected at the R8 stage to assess the yield components: number of pods, number of grains per pod, and grain mass. The three central plant rows were harvested the pods were threshing. The seeds were weighed, and the humidity was determined with specific equipment (Dickey Jhon, Multi-Grain) and standardized to 13%. A thousand seeds mass was determined using a volume sampling per plot. The oil and protein content were measured in 1 kg of seeds using the methods AOCS Ac 3-44 (AOCS, 1993) and AACC 46 (AACC International, 2010) performed by the BSBIOS laboratory (Passo Fundo, Brazil).

### 2.4 Data Analysis

Data were analyzed using the SASM-AGRI<sup>®</sup> program (Canteri et al., 2001). The data obtained from the leaf area, ASR severity, defoliation rate, yield components, yield, and dry mass grain accumulation, were submitted to analysis of variance (ANOVA), and the means compared by the Scott Knott test ( $p \leq 0.05$ ). The LAD and HAD data were submitted to linear regression, and the dry mass grain accumulation data were submitted to non-linear regression by the logistic model using the InfoStat<sup>®</sup> software.

### 3. Results

Weather conditions during the experiments were favorable for soybean development and to the progress of ASR as well. The air temperature average was 23 °C, and the biweekly rainfall average was 60 mm (Figure 1). Three fungicide programs that included applications in the soybean vegetative and reproductive stage (maximum, standard, and intermediate-1 control) were more efficient and showed lower rAUDPC and greater LAD and HAD than the untreated soybean (Table 2). The ARS positive control showed the lowest rAUDPC. Leaf area duration and HAD in the intermediate-1 and -2 showed similar results to the maximum and standard control. A single application of azoxystrobin+benzovindiflupir at 65 DAS (standard control) showed similar control of ASR that the two applications used in the maximum control treatment. Fungicide programs without applications in the vegetative stage or late application provided high rAUDPC or low ASR control. The LAD and HAD were reduced when the fungicide application was performed at 45 DAS or later (65 or 86 DAS).

Table 2. The relative area under the disease progress curve (rAUDPC) ( $\pm$ SD) and control effectiveness of Asian Soybean Rust (ASR), leaf area duration (LAD), and healthy leaf area duration (HAD) in soybean Syn 13561 IPRO submitted to fungicide spraying programs. Passo Fundo, Rio Grande do Sul State, Brazil, 2014/2015

ASR fungicide treatments <sup>1</sup>	rAUDPC	Control effectiveness (%)	LAD	HAD
(i) untreated	20.4 ( $\pm$ 1.0) a	-	144 ( $\pm$ 4.0) c	128 ( $\pm$ 4.0) b
(ii) maximum control	2.1 ( $\pm$ 0.4) g	89.8	177 ( $\pm$ 4.0) a	174 ( $\pm$ 3.0) a
(iii) standard control	3.1 ( $\pm$ 0.2) f	85.0	166 ( $\pm$ 7.0) a	162 ( $\pm$ 6.0) a
(iv) intermediate-1	4.9 ( $\pm$ 0.5) e	76.0	176 ( $\pm$ 9.0) a	169 ( $\pm$ 9.0) a
(v) intermediate-2	6.3 ( $\pm$ 0.6) d	69.2	176 ( $\pm$ 10.0) b	168 ( $\pm$ 9.0) a
(vi) intermediate-3	17.4 ( $\pm$ 0.4) b	14.8	156 ( $\pm$ 1.0) c	141 ( $\pm$ 1.0) b
(vii) intermediate-4	4.0 ( $\pm$ 0.2) f	80.8	169 ( $\pm$ 9.0) b	164 ( $\pm$ 9.0) a
(viii) intermediate-5	10.7 ( $\pm$ 1.5) c	47.8	145 ( $\pm$ 9.0) c	135 ( $\pm$ 7.0) b
Coefficient of variation (%)	8.0	-	4.5	4.4

Note. Means followed by the same letter in the column do not differ by the Scott-Knott test ( $p \leq 0.05$ ).

Thousand-grain weight, number of pods per plant, and seeds per plant were affected by the ASR in the intermediate-3 and -5 treatments (Table 3). The other treatments were similar to the positive control (maximum control). The thousand-grain weight reduced 30g in the intermediate-3 treatment regarding the positive control and did not differ from the untreated control. The late fungicide applications reduced 10% in the number of seeds per plant, whereas the number of seeds per pod did not show a significant difference ( $p \leq 0.05$ ).

Table 3. Pods per plant, seeds per pod, seeds per plant, and thousand-grain weight ( $\pm$ SD) in soybean Syn 13561 IPRO submitted to fungicide spraying programs during the 2014/2015 season. Passo Fundo, Rio Grande do Sul State, Brazil

ASR fungicide treatments	Pods per plant	Seeds per pod	Seeds per plant	Thousand-grain weight (g)
(i) untreated	35 ( $\pm$ 2) b	2.2 ( $\pm$ 0.1) n.s.	76 ( $\pm$ 2.0) b	136 ( $\pm$ 2.0) c
(ii) maximum control	40 ( $\pm$ 2) a	2.4 ( $\pm$ 0.1)	91 ( $\pm$ 3.0) a	167 ( $\pm$ 1.0) a
(iii) standard control	40 ( $\pm$ 1) a	2.3 ( $\pm$ 0.1)	92 ( $\pm$ 5.0) a	165 ( $\pm$ 5.0) a
(iv) intermediate-1	39 ( $\pm$ 3) a	2.3 ( $\pm$ 0.1)	91 ( $\pm$ 5.0) a	169 ( $\pm$ 1.0) a
(v) intermediate-2	38 ( $\pm$ 2) a	2.4 ( $\pm$ 0.1)	90 ( $\pm$ 3.0) a	161 ( $\pm$ 2.0) a
(vi) intermediate-3	35 ( $\pm$ 1) b	2.3 ( $\pm$ 0.1)	82 ( $\pm$ 5.0) b	137 ( $\pm$ 1.0) c
(vii) intermediate-4	40 ( $\pm$ 2) a	2.3 ( $\pm$ 0.2)	91 ( $\pm$ 6.0) a	165 ( $\pm$ 1.0) a
(viii) intermediate-5	37 ( $\pm$ 1) b	2.3 ( $\pm$ 0.1)	85 ( $\pm$ 3.0) b	151 ( $\pm$ 4.0) b
Coefficient of variation (%)	4.5	4.4	5.1	1.7

Note. Means followed by the same letter in the column do not differ by the Scott-Knott test ( $p \leq 0.05$ ).

The grain yield and the oil content were similar in the maximum, standard, and intermediate-1 and -2 treatments, while the protein content did not show a significant difference ( $p \leq 0.05$ ) (Table 4).

Table 4. Grain yield ( $\pm$ SD) and protein and oil content in soybean Syn 13561 IPRO seeds after fungicide treatments for Asian Soybean Rust (ASR) during harvest season 2014/2015. Passo Fundo, Rio Grande do Sul State, Brazil

ASR fungicide treatments	Yield (kg.ha <sup>-1</sup> )	Protein (%)	Oil (%)
(i) untreated	2,869 ( $\pm$ 234) c	32.7 ( $\pm$ 0.2) n.s.	19.6 ( $\pm$ 0.12) c
(ii) maximum control	4,388 ( $\pm$ 168) a	32.5 ( $\pm$ 0.1)	20.9 ( $\pm$ 0.10) a
(iii) standard control	4,141 ( $\pm$ 275) a	33.4 ( $\pm$ 0.1)	20.7 ( $\pm$ 0.12) a
(iv) intermediate-1	4,051 ( $\pm$ 357) a	33.0 ( $\pm$ 0.1)	20.8 ( $\pm$ 0.21) a
(v) intermediate-2	3,752 ( $\pm$ 372) a	32.5 ( $\pm$ 0.2)	20.9 ( $\pm$ 0.21) a
(vi) intermediate-3	3,110 ( $\pm$ 226) c	32.7 ( $\pm$ 0.6)	20.2 ( $\pm$ 0.31) b
(vii) intermediate-4	3,824 ( $\pm$ 306) a	32.4 ( $\pm$ 0.2)	21.3 ( $\pm$ 0.65) a
(viii) intermediate-5	3,537 ( $\pm$ 120) b	32.3 ( $\pm$ 0.1)	21.2 ( $\pm$ 0.38) a
Coefficient of variation (%)	7.7	1.3	1.4

Note. Means followed by the same letter in the column do not differ by the Scott-Knott test ( $p \leq 0.05$ ).

The LAI and HLAI were affected according to the ASR progress. Figure 2 shows the decreasing of these parameters 50 days after the soybean R2 stage (65 DAS).

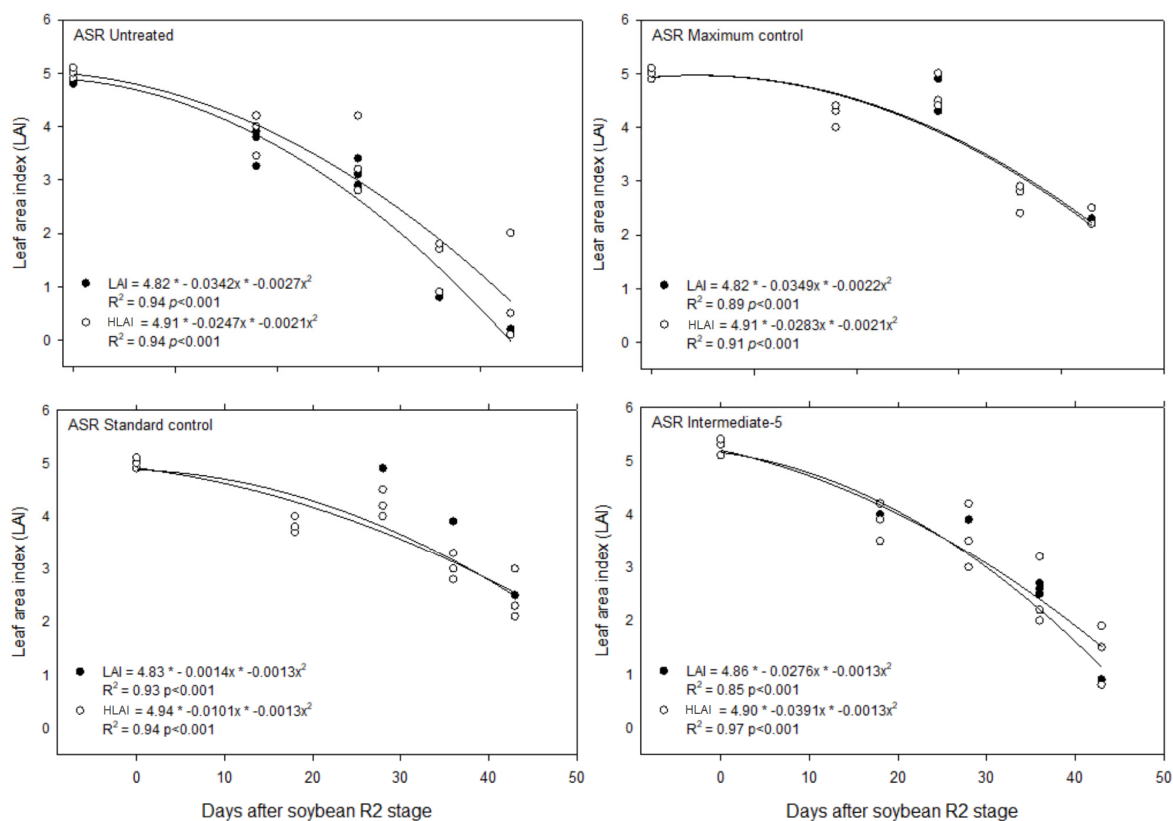


Figure 2. Leaf area index (LAI) and healthy leaf area index (HLAI) 50 days after soybean R2 stage (65 DAS) in response to fungicide spraying programs for Asian Soybean Rust (ASR). Passo Fundo, Rio Grande do Sul State, Brazil, 2014/2015

Leaf area index and HLAJ showed a positive correlation in response to four fungicide programs for ASR ( $p \leq 0.001$ ). The lowest LAI was found when the soybean did not receive treatment to ASR and when the fungicides were applied after the R2 stage (ASR intermediate-5 treatment). The highest dry matter grain accumulation was shown in the maximum control, followed by the standard control (Figure 3). The LAD and HAD were positively correlated with the soybean grain yield (Figure 4). The highest soybean grain yield matched the greatest LAD and HAD parameters.

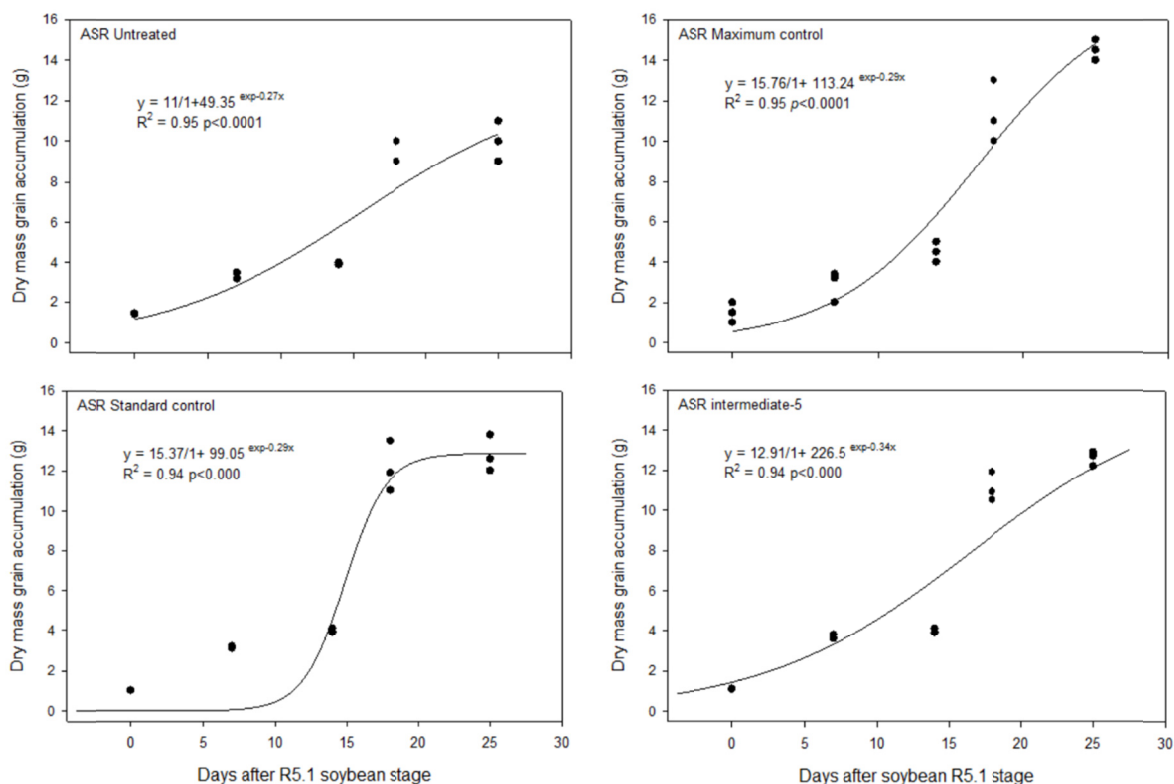


Figure 3. Dry mass grain accumulation per plant (g) 25 days after the R5.1 stage in response to fungicide spraying programs for Asian Soybean Rust (ASR) during the 2014/2015 season. A non-linear regression logistic model was used to adjust curves. Passo Fundo, Rio Grande do Sul State, Brazil

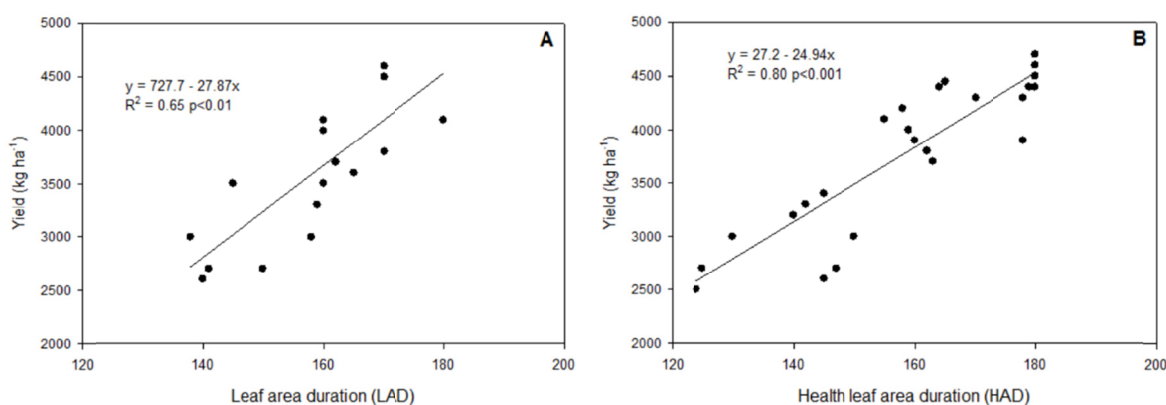


Figure 4. Relationship between soybean grain yield (kg ha<sup>-1</sup>) and A) Leaf area duration (LAD) and B) Health leaf area duration (HAD) during the crop season 2014/2015. Passo Fundo, Rio Grande do Sul State, Brazil

#### 4. Discussion

Air temperature and rainfall during the assays favored the ASR progress. In general, optimal weather conditions for the crop favor the establishment and development of *P. pachyrhizi* (Godoy et al., 2016). The optimal temperature for fungus infections ranges from 18 to 25 °C, and continuous leaf wetness caused by dew or rain also favors disease development, once the rainfall is a key factor in determining epidemic levels in the field (Del Ponte et al., 2006).

Asian Rust most commonly develops between the flowering and pod-filling growth stages (Melching et al., 1988). However, we showed ASR occurrence at the end of the vegetative stage in the Syn 13561 IPRO RR2 soybean cultivar. We observed that when the fungicide treatments were performed early (at 45 or 65 DAS), the AUDPC was lower than when the fungicide treatments were performed during the reproductive stage (at 86



DAS or later) (Table 2). These results corroborate a study conducted by Silva Júnior et al. (2009), which recommended sequential fungicide spraying to reduce the ASR severity in Brazilian fields. Also, the LAD and HAD showed the highest values in the treatments with early fungicide applications. Leaf area duration and HAD affect the progress of ASR under conditions favorable for epidemics once these variables are based on the ASR severity and temporal disease progress. However, the LAI, which is used to provide the LAD, varies among growing seasons and soybean maturity groups (Moreira et al., 2015), and other crop components must be considered to assess the ASR effects.

Fungicide application performed only in the vegetative stage (intermediate-3) or at 86 and 96 DAS (intermediate-5) affected the number of pods per plant and, consequently, the number of seeds per plant (Table 3). The number of pods per plant is one of the main yield components affected by ASR in soybean since the photosynthetic plant area is compromised, leading to premature dropping of leaves and consequently preventing the full development of pods and grains (Barros et al., 2008; Gabardo et al., 2020). The number of seeds per pod is controlled by genetic means (Board et al., 1995), explaining the no influence in the numbers of grains per pod due to the present study's ASR occurrence (Table 3).

Another component affected by the ASR progress was the dry mass grain accumulation. The thousand-grain weight was reduced by 18% in the intermediate-3 treatment regarding positive control. The plant biomass production is related to photosynthetically active radiation (PAR) intercepted and absorbed by the leaves and its conversion efficiency into chemical energy (Radin et al., 2003). Dry mass accumulation in soybean increases until the R7 stage (Lazarini et al., 2000). Therefore, it is important to keep the crop healthy until its maturation. Our results showed that the longest HAD promoted greater dry grain mass accumulation and higher grain yield due to the fungicide treatment protection during the reproductive stage. The cultivar Syn 13561 IPRO RR2 shows early maturation, starting grain filling 25 days after the R5.1 stage. The grain fill started slowly up to 10 days and then increased rapidly. In the negative control (ASR untreated), dry mass accumulation did not increase at the final stage due to early defoliation.

The reduction of soybean components—especially the number of pods per plant—caused by the ASR resulted in a decrease in crop yield and the qualitative variables represented by loss in the oil content. High-quality seeds are one of the factors that define soybean cultivation's success, which will generate high vigor plants with superior performance in the field (Gabriel et al., 2018). The greater yield and oil content were obtained using fungicide spraying programs that protected the crop during the entire cycle, starting the fungicide spraying programs in the vegetative stage and applied until the end of the soybean cycle.

For the soybean cultivar Syn 13561 IPRO RR2, the LAD and HAD also responded to fungicide spraying programs. Here, we showed that the grain yield was positively correlated with the LAD and HAD. IN this way, we suggest that the HAD can be proposed as a crop yield indicator in plants affected by ASR since its variations interfere in the dry mass accumulation, yield components, yield, and quality of soybean seeds. However, it is important to consider that many variables could affect the HAD, such as cultivar, soil, weather conditions, fungicides applied, and the plant stage exposed to the disease stress.

Three fungicide programs were more efficient and showed lower rAUDPC and greater LAD and HAD than the untreated soybean. The LAD and HAD reduction caused by the ASR interfered in the dry mass accumulation, yield, and yield components in soybean. We recommended extending the HAD during the reproductive stage to obtain maximum yields. Other studies should be carried out to contemplate different cultivar cycles and plants genetically resistant to ASR.

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