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Impact of Fungicide Application Timing Based on Soybean Rust Prediction Model on Application Technology and Disease Control

Matheus Mereb Negrisoli [1,*], Flávio Nunes da Silva [1], Raphael Mereb Negrisoli [1], Lucas da Silva Lopes [1], Francisco de Sales Souza Júnior [1], Bianca Rezende de Freitas [2], Edivaldo Domingues Velini [1] and Carlos Gilberto Raetano [1]

[1] Department of Plant Protection, School of Agriculture, Sao Paulo State University, 3780 Avenida Universitária, Botucatu 01049-010, SP, Brazil

[2] Department of Crop Science, School of Agriculture, Sao Paulo State University, 3780 Avenida Universitária, Botucatu 01049-010, SP, Brazil

* Correspondence: matheusmnegrisoli@gmail.com (M.M.N); Tel.: +55-18-998150693

Abstract: The application of remote sensing techniques and prediction models for soybean rust (SBR) monitoring may result in different fungicide application timings, control efficacy, and spraying performance. This study aimed to evaluate the applicability of a prediction model as a threshold for disease control decision-making and to identify the effect of different application timings on SBR control as well as on the spraying technology. There were two experimental trials that were conducted in a 2 × 4 factorial scheme: 2 cultivars (susceptible and partially resistant to SBR); and four application timings (conventional chemical control at a calendarized system basis; based on the prediction model; at the appearance of the first visible symptoms; and control without fungicide application). Spray deposit and coverage at each application timing were evaluated in the lower and upper region of the soybean canopy through quantitative analysis of a tracer and water-sensitive papers. The prediction model was calculated based on leaf reflectance data that were collected by remote sensing. Application timings impacted the application technology as well as control efficacy. Calendarized system applications were conducted earlier, promoting different spray performances. Spraying at moments when the leaf area index was higher obtained poorer distribution. None of the treatments were capable of achieving high spray penetration into the canopy. The partially resistant cultivar was effective in holding disease progress during the crop season, whereas all treatments with chemical control resulted in less disease impact. The use of the prediction model was effective and promising to be integrated into disease management programs.

Keywords: *Phakopsora pachyrhizi*; integrated disease management; spraying technology; remote sensing

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1. Introduction

Soybean rust (SBR) causes significant crop yield losses throughout the world [1,2]. The disease is caused by *Phakopsora pachyrhizi* Sydow and is controlled mostly by fungicide application at pre-determined scheduled timings of the soybean growth stages, usually regardless of disease incidence and pressure level [1,3]. Due to constant fungicide spraying over the seasons and several times at the same season, a large number of fungi populations that are resistant to different chemical groups of fungicides have also been reported [4,5], significantly reducing fungicide efficacy over time [6].

Most soybean cultivation is conducted in extensive field areas, which hardens disease monitoring conductance. Nowadays, specialists monitor SBR in the field through extensive scouting that is based on disease symptom recognition. However, monitoring is usually absent, and farmers choose to spray on a calendarized system basis (i.e., at a pre-determined period) as a guarantee of crop yield. Besides input losses, these practices can lead to fungicide resistance selection pressure, poor spraying quality at periods that are not appropriate to achieve the best spray distribution, as well as possible wrong timing, which also leads to lower efficacy [1,6–9]. Other techniques are being applied to the integrated disease management (IDM) of the soybean–*P. pachyrhizi* pathosystem, such as resistant or partially resistant cultivars [10,11], planting date restrictions [1,2], and biological control [12]. Nonetheless, disease monitoring is considered the basis of any control method that is applied.

One of the alternatives to aid in disease control decision-making is the use of remote sensing technologies to facilitate data acquisition in wider fields as well as the obtainment of reliable information. It means that these technologies can precisely detect the disease remotely and at a faster pace since it allows the monitoring of wider fields depending on the sensor and where it is based [13–15]. Studies have reported the possibility of identifying SBR by remote sensing techniques [16–18], as well as for other diseases and crops [15,19]. Moreover, researchers have been applying all of this information in data modeling so that remote sensing could serve as the background information in the construction of disease prediction models as decision support systems [14,20]. These models are expected to improve the application timing, control effectiveness, as well as spraying performance regarding its uniform distribution in the crop.

Fungicide application timing strongly influences disease control, and it may vary depending on the fungicide mode of action and translocation capacity [8,21]. Besides reaching the target at the moment of most fungicide susceptibility, the application timing may influence the application technology in terms of product distribution, coverage, and penetration into the canopy [9,22]. Different timings also represent variations in soybean canopy density, especially due to leaf area index (LAI) and vegetation density [23] which play a major role as a barrier to the spray reaching the interior of the canopy. Most fungicide applications target the lower region of the soybean canopy since this is where SBR starts its development [1]. Therefore, applications at moments of higher LAI tend to have reduced penetration capacity and worse spray distribution [24].

According to Müller et al. [8], effective monitoring along with fungicide application as soon as the disease is identified in the field is a key factor to mitigate excessive and unnecessary application. However, since most systemic fungicides that are used for SBR control have both curative and preventive action modes, it is unknown how the application timing will affect control efficacy. The hypothesis is that SBR monitoring through prediction models that are based on remote sensing can help identify the first appearance of the disease in the field, and, thereby, improve application timing accuracy. Moreover, different application timings will potentially influence product distribution across soybean canopy regions.

The goal of this study was to evaluate the applicability of prediction models as the threshold for disease control decision-making and to identify the effect of different application timings on soybean rust control as well as on the spraying technology. This study is a continuation of the research that was conducted by Negrisoli et al. [18], using a prediction model to determine the fungicide application timing and how it differs from conventional methods.

2. Materials and Methods

There were two experiment replications that were carried out in the field over the 2020/2021 crop season, in different experimental areas of Botucatu, SP, Brazil (Field 1: 22°48′48″ S and 48°25′37″ W; Field 2: 22°49′38″ S and 48°25′40″ W) (Figure 1). In both experimental fields, the no-tillage system was adopted, and all sowing operations, phytosanitary management, and evaluations were carried out homogeneously.

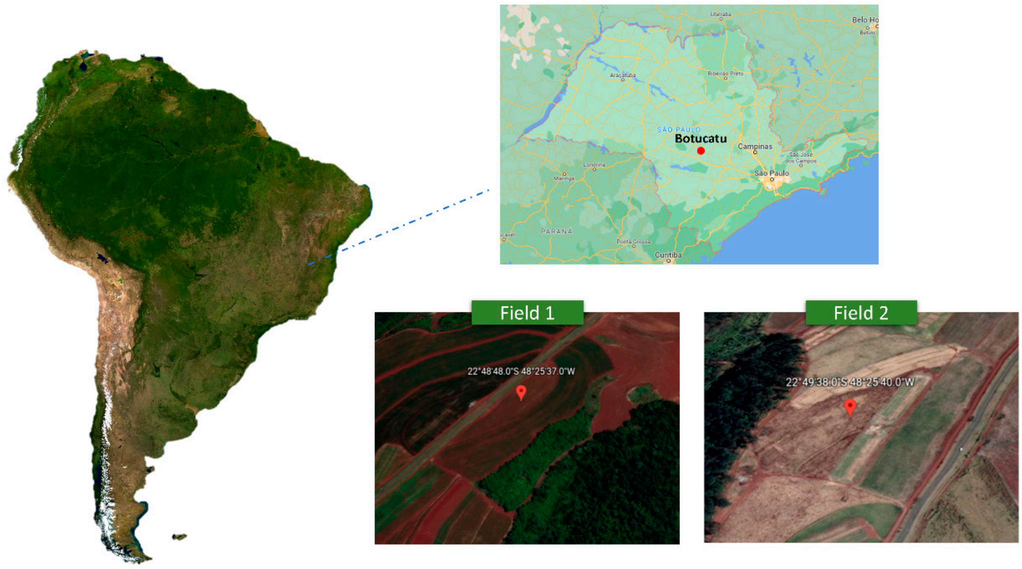

Figure 1. Study area map of both field trial replications.

The trials were sown on 8 December 2020, spaced at 0.45 m between rows, and with a population of 196,528 plants ha^{-1} in Field 1 and 211,806 plants ha^{-1} in Field 2. The crop was fertilized with 250 kg ha^{-1} of the commercial formula 02-20-20 (NPK) homogeneously throughout the experimental area.

The experiments were carried out in a randomized complete block design and the treatments were distributed in a 2 × 4 factorial scheme: 2 cultivars (Brevant DS6217 IPRO, susceptible to SBR; TMG 7063 IPRO, partially resistant to SBR); and 4 application timings that were based on different parameters as decision-making of control: conventional chemical control, spraying at a scheduled basis at the soybean reproductive stage R1 and R1 + 15 days; application timing (A1) defined based on the disease prediction model and A1 + 15 days; application timing (A2) at the appearance of the first visible symptoms and A2 + 15 days; and control treatments without fungicide application (Table 1), and all in four repetitions. The descriptions of each cultivar are shown in Table 2.

Table 1. Description of the treatments according to the soybean cultivar and application timing.

Treatments	Cultivar	First Application Timing	Second Application Timing
1	DS6217 IPRO (Susceptible)	-	-
2	TMG 7063 (Partially resistant)	-	-
3	DS6217 IPRO (Susceptible)	Calend *	Calend + 15
4	TMG 7063 (Partially resistant)	Calend	Calend + 15
5	DS6217 IPRO (Susceptible)	Model	Model + 15
6	TMG 7063 (Partially resistant)	Model	Model + 15
7	DS6217 IPRO (Susceptible)	Sympt	Sympt + 15
8	TMG 7063 (Partially resistant)	Sympt	Sympt + 15

* Calend: calendarized application at reproductive stage R1; Model: spraying according to SBR prediction model; Sympt: spraying at the first symptoms observations; "+15": second application conducted at 15 days after the first application; "-": no application (control treatments).

Table 2. Description of the cultivars that were adopted in the experimental trials.

Description	Brevant DS6217 IPRO [1]	TMG 7063 [2]
P. pachyrhizi susceptibility	Susceptible	Partially resistant (Inox)
Maturity groups	6.2	6.3
Growth habit	Indeterminate	Indeterminate
Traits	Intacta RR2 PRO®	Intacta RR2 PRO®

Data from Brevant [1] [25] and Tropical Melhoramento Genético [2] [26].

The definition of the application timings “A1” and “A2” were based on weekly disease severity monitoring starting at the vegetative growth stage, V6, in each plot. Firstly, the definition of A1 was conducted using an SBR prediction model that was proposed by Negrisoli et al. [18], based on the leaf reflectance, as the decision support system of control, as described subsequently herein.

2.1. Leaf Reflectance Assessment

This assessment was conducted every five days after the V6 vegetative growth stage, by randomly collecting 5 leaflets per plot from the lower region of the canopy for leaf reflectance analysis. The UV 2700 non-imaging spectrophotometer (Shimadzu, Kyoto, Japan) was used coupled to an integrated base ISR-603: Integrating Sphere Attachment, analyzing the reflectance of each leaflet in the range of 270 to 1000 nm with an interval of 3.0 nm, as described by Negrisoli et al. [18]. Each sample (leaflet) was evaluated separately, generating the reflectance values of each sample in the previously mentioned spectral range interval.

The spectral curves were reduced from 270–1000 nm to 270–900 nm for high noise levels reduction [27] and the Savitzky–Golay filter was applied using 11 central points and a third-degree polynomial [28,29]. The data were used to calculate a list of 19 vegetation indices (Vis) that was representative of the disease effect on the crop and to allow disease severity classification and, therefore, to predict or detect diseased plants among the samples [16,18,30]. Vegetation indices were chosen to be used instead of the full spectra length so that other spectral sensors besides the hyperspectral ones could be used to acquire the data that were required to run this model [18], besides being able to supply valuable information for disease detection and plant stress identification [14].

The Vis were calculated for each sample at every reflectance evaluation date and this database was used to supply the prediction model that was based on the Support Vector Machine (SVM) algorithm, which is programmed to classify into four classes: “healthy”, “low severity”, “moderate severity”, and “high severity”. At the moment when the samples from susceptible (T5) and partially resistant (T6) cultivars that averaged 1 plant per plot were classified at “low severity” (diseased plant), the application was thereafter immediately conducted. All the Vis calculations, evaluation methodologies, and prediction model construction are fully described by [18]. The formulas and list of Vis that were used are also fully described by Negrisoli et al. [18].

For the definition of “A2” application timing, 10 leaflets were collected from the lower region of the canopy in each plot for visual assessment of the symptoms. The samples were taken to a laboratory for better visualization of the fungal structures and symptoms under a stereoscopic microscope. Susceptible and resistant reactions may result in different SBR symptoms. A susceptible reaction results in tan to light-brown lesions (TAN reaction type) and the presence of uredinia throughout the leaf, while in partially resistant cultivars the lesions are characterized by reddish-brown lesions (RB-reaction type) and a lower quantity of uredinia [3,11]. The spraying was conducted at the time when the first SBR symptoms were detected in the plots of susceptible (T7) and partially resistant cultivars (T8).

The standard application timing was conducted at the end of the vegetative growth and the beginning of the reproductive stage (R1) of susceptible (T3) and partially resistant cultivars (T4). This application timing is common throughout the country and was used as the standard timing parameter.

2.2. Fungicide Sprayings

The fungicide Elatus®, Syngenta (azoxystrobin 60 g L^{-1} + benzovindiflupyr 30 g L^{-1}) was used for SBR control at a dose of 0.250 kg ha^{-1}, following the manufacturer’s recommendations. The spraying was carried out with a CO_2 pressurized backpack sprayer with a 2.0-m boom that was equipped with four flat fan spray nozzles (Teejet XR110-02), displacement speed of 5 km h^{-1}, and a working pressure of 200 kPa, providing a spraying volume of 150 L ha^{-1}.

2.3. Quali-Quantitative Analysis of Spraying

The spray deposit capacity as well as the percentage of coverage of each treatment were evaluated in both first and second applications (Table 1). For the quantitative analysis of the deposit, the food dye Brilliant Blue marker (Duas Rodas Industrial LTDA, São Bernardo do Campo, Brazil) was applied immediately before fungicide spraying to avoid the influence of the fungicide formulation on the optical reading of the marker by spectrophotometry. The Brilliant Blue marker dye was solubilized in distilled water at a concentration of 3000 mg L^{-1}. After spraying, three samples that were composed of 10 leaflets each were randomly collected from the upper and lower region of the plant canopy in the central lines of each plot, also evaluating the ability of the treatments to penetrate the crop canopy.

The samples were taken to the laboratory and processed according to Palladini et al. [31]. Each sample received 100 mL of distilled water, stirred for 15 s, and the resulting solution was transferred to plastic containers. The quantification of deposits was performed in a spectrophotometer (Shimadzu VIS 1601 PC) with an absorbance reading at a wavelength of 630 nm [31]. After the tracer was washed, the leaf area of each sample was evaluated with the aid of a benchtop leaf area meter (LICOR, model LI-3100). The readings of known concentrations of the dye were correlated to the absorbance values that were obtained in the spectrophotometer and the calibration curve was constructed, obtaining the dye concentration in the target in mg L^{-1}. Finally, the volume that was found in the target was established by correlating the dye concentration in the samples washing solution with the dye concentration in the final spray solution, presenting the data in μL cm^{-2}.

The qualitative analysis of the spraying was carried out through the evaluation of the spray coverage using water-sensitive papers (WSP) (26 × 76 mm) that were distributed in each plot. There were two WSPs that were used attached to a metal rod in the central lines of the plots, one located horizontally at the top and the other at the bottom part of the canopy. Spray coverage and deposit evaluations were performed at the same time.

After spraying, the WSPs were placed in Petri dishes to prevent moisture absorption and taken to the laboratory for analysis. These samples were scanned at a resolution of 600 dpi and the digitalized images were analyzed by the "GOTAS" software (Embrapa®) to obtain the percentage of surface coverage.

The leaf area index (LAI) of the plots was also measured in the period between 50 to 70 days after emergence (DAE) (Field 1) and 50–88 DAE (Field 2), corresponding to the range of all application timings in each field. Therefore, at 50, 60, and 70 DAE of Field 1, and 50, 65, and 88 DAE of Field 2, 10 plants of each cultivar were randomly collected across each experimental field trial. The whole plants were taken to the laboratory, completely defoliated, and the leaves were measured by a benchtop leaf area meter (LICOR, model LI-3100). The total leaf area that was measured of each sample was converted to m^2 and divided by the mean number of plants m^{-1} (Field 1 = 9.93 plants m^{-1} of the susceptible cultivar; 7.75 plants m^{-1} of the partially resistant cultivar; Field 2 = 10.34 plants m^{-1} of the susceptible cultivar; 8.72 plants m^{-1} of the partially resistant cultivar) [32]. The LAI is an important factor to understand spraying quality behavior as well as the capacity of each operational parameter according to the leaf density as a barrier to spraying.

2.4. Assessment of Disease Severity and Control Efficacy

Starting at the V6 growth stage, 10 leaflets were collected weekly per plot from the lower third of the plant and taken to the laboratory for better visualization of fungal structures and symptoms under a stereoscopic microscope. The disease severity (%) was estimated based on the SBR diagrammatic scale that was proposed by Godoy et al. [33], based on visual observation of the symptoms. The Area Under the Disease Progress Curve (AUDPC) was calculated according to Campbell and Madden [34] using the mean values of disease severity that were obtained in the plots and on the respective evaluation dates.

In addition, the disease severity was indirectly assessed by the defoliation level. The evaluations started at 85 DAE, the moment when the highest level of severity began, and no defoliation was still detected. The evaluation was carried out using the ASD FieldSpec Dual

Spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA), with a spectral range from 350 to 1070 nm and 7.5° of field of view. A white panel with approximately 100% reflectance (*Spectralon*) was used as a reference for calibration. Calibration and optimization of the equipment were conducted every 10 min.

The evaluations were conducted weekly under intense sunlight at around 11:00 a.m., performing three readings in the central region of each plot in each experimental trial, maintaining uniform height and equipment position. The reflectance data that were obtained were smoothed by Savitsky–Golay filtering as described above. The reflectance database was then used to calculate the VIs that was the most correlated to the effect of SBR on crop defoliation. For that, the calculation of the LAI through the normalized difference vegetation index (NDVI) and Beer–Lambert law [35] stood out and was used as a reference for evaluating crop defoliation by the disease. Tan et al. [35] proposed the calculation of the LAI through the integration of the Beer–Lambert Law and the NDVI of the samples, also considering its leaf orientation values. Here, the soybean crop was considered as a middle-type plant with leaf orientation values ranging from 30 to 60°, and the whole model is fully described by the authors [35].

2.5. Evaluation of the Effect of Control on Crop Yield

At the end of the crop season, each plot was individually harvested to determine the influence of disease control effectiveness on soybean crop yield. A total of one meter of the three center rows of each plot was manually harvested. The production was weighed on a precision scale to determine the weight of a thousand seeds (TSW) (g) and the crop yield (kg ha^{-1}) of each treatment, considering humidity correction to 13% [36].

2.6. Data Analysis

Statistical analysis for the construction of the disease prediction model was performed according to the procedure that was already described, using the same script for data processing [18]. The prediction model was executed during the experiment to aid in the decision-making of the control and it was cross-validated (n = 10) with the original database that was used for the construction of this prediction model to confirm the disease severity classes identification [18].

The results were analyzed in the factorial scheme that was described and submitted to the analysis of homogeneity and normality. The data were submitted to analysis of variance (ANOVA) using the F test and, when significant, compared by Tukey's test, both at 5% of significance.

Since a significant difference was found between the experimental field replications ($p < 0.01$), both areas were compared separately. In both fields, the spray deposit and coverage mean values were compared separately for each region of the canopy (upper and lower). The percentage of control was calculated based on the AUDPC of the control without application of each cultivar (T1 and T2). All statistical analyses and models were conducted using the R 3.6.3 software [37].

3. Results

3.1. Soybean Rust Detection and Application Timings

The periods of disease detection in the field trials according to each treatment are shown in Figure 2. In Field 1, SBR's first symptoms were detected in both cultivars (Treatments 7 and 8) concomitant to the scheduled application (R1) at 50 DAE, and, therefore, T7 and T8 were sprayed on the same date as T3 and T4. The model was able to detect plants that were classified as "low severity" 6 days after that and in both cultivars when symptoms were about 0.2% severity. In the second experimental replication (Field 2), disease symptoms were detected in susceptible and partially resistant cultivars (T7 and T8) at 70 DAE, as well as the model was also able to detect diseased plants in the susceptible cultivar (T5), which had more characteristic symptoms of the disease at this time. For the partially resistant cultivars, which demonstrated significantly slower disease progression, the model detected diseased plants 7 days after that. The calendarized application (R1) was sprayed at the same time as in Field 1 (50 DAE).

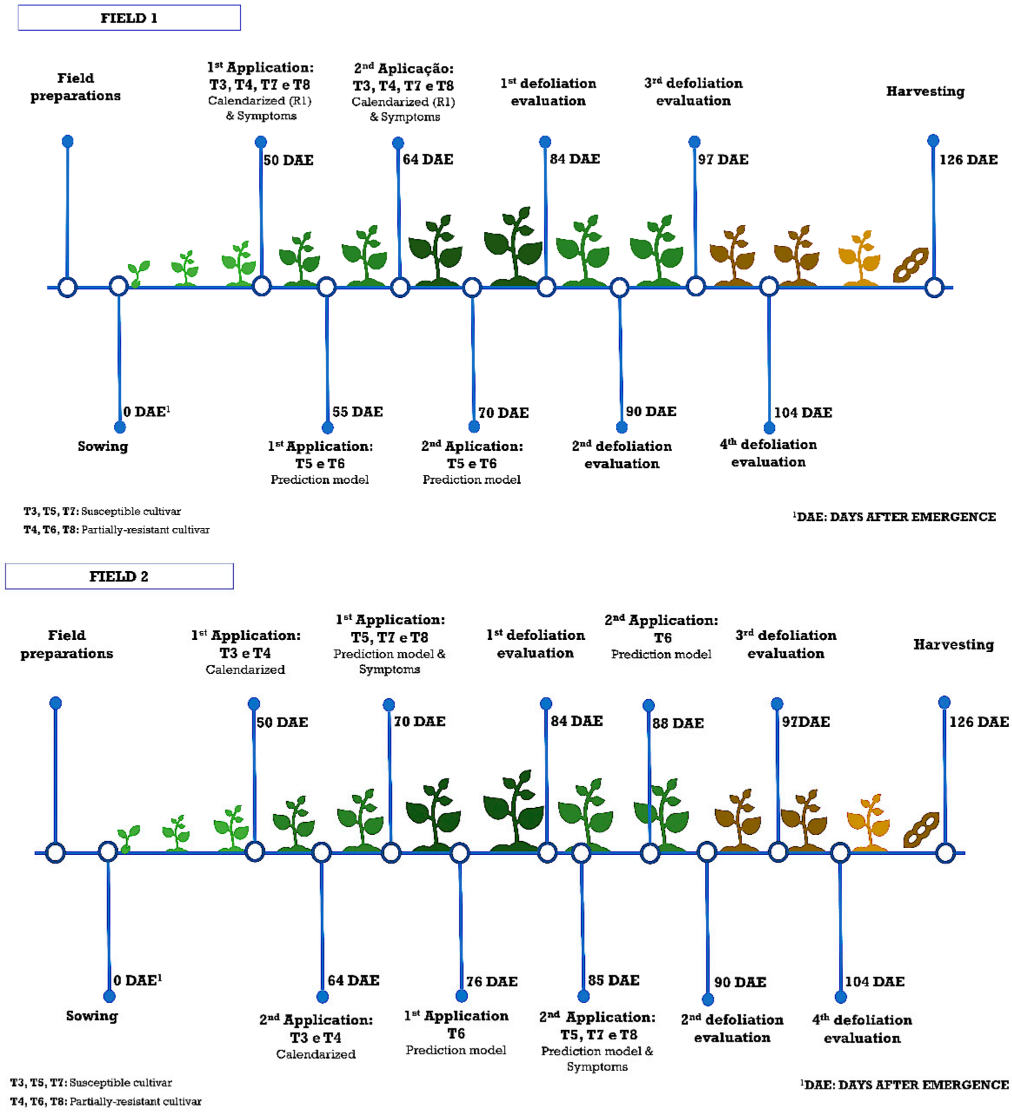

Figure 2. Graphical representation of fungicide application timings along with qualitative and quantitative spraying evaluations as a function of disease detection that was proposed for each treatment.

At both Fields 1 and 2, a peak of LAI was observed around 60–65 DAE, occurring concurrently with the second application of the treatments (Figure 3). This period corresponds to the moment when higher foliar density is found, offering greater deposit and spray penetration challenges.

3.2. Spray Deposit

Consistent results were observed between the field trials and regarding the effect of the application timings on the qualitative and quantitate spraying variables. No significant effect was found in the interaction of the factors for any deposit values in both field trial replications.

In the first application of Field 1, a greater spray deposit was observed in the upper region of the crop canopy when spraying based on the prediction model (0.85 μL cm^{-2}), compared to when based on symptoms (0.65 μL cm^{-2}) and calendarized (0.55 μL cm^{-2}) that were sprayed previously (Figure 4). In the lower region of the canopy, a significant difference was found only between the cultivars, where higher spray deposits ($p < 0.01$)

were found in partially resistant cultivars (0.30 µL cm^{-2}) than in the susceptible cultivars (0.09 µL cm^{-2}) (Figure 5).

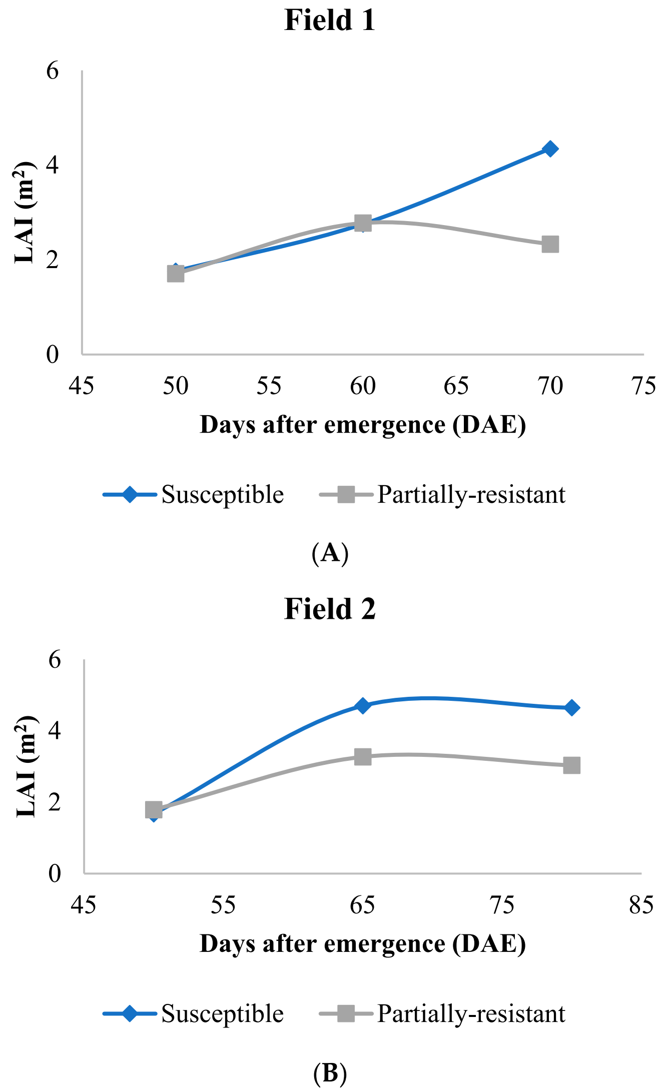

Figure 3. Leaf area index (LAI) of Field Trials 1 (**A**) and 2 (**B**) that were analyzed during the period interval of the fungicide applications.

In the second application, the application timings affected the deposit values at both the upper and lower canopy regions, disregarding the cultivar that was used. Greater spray deposit was found when spraying based on the prediction model at the upper (0.98 µL cm^{-2}) and lower (0.21 µL cm^{-2}) canopy regions (Figure 6). The prediction model application timings (T5 and T6) occurred at a different moment than the other two treatments.

Furthermore, the cultivar also affected the quantity of the deposits that were found in the lower region of the canopy, in which the partially resistant cultivar (0.17 µL cm^{-2}) significantly overcame the susceptible cultivar (0.05 µL cm^{-2}) (Figure 7). Irrespective of the application timing, an expressive reduction in the spray deposit was found again at the lower region of the canopy, demonstrated by an uneven spray distribution across the canopy regions and reduced the capacity of droplets penetration into the lower regions.

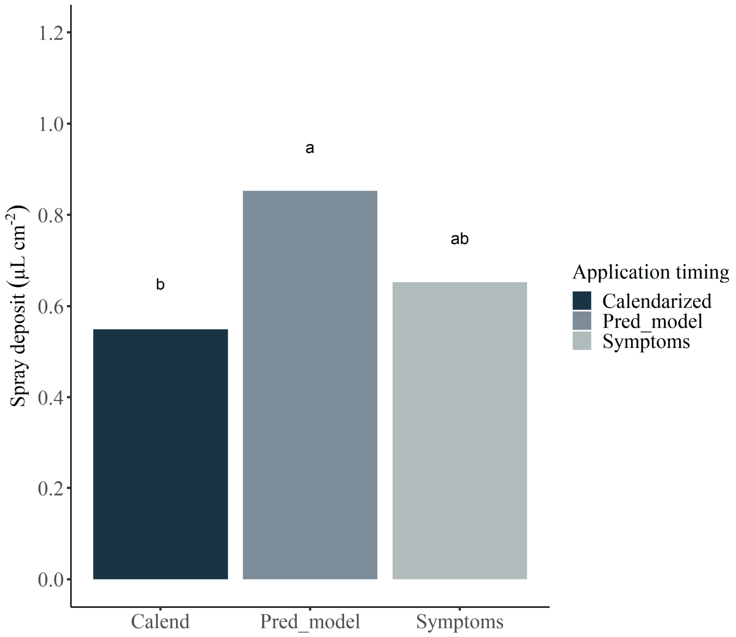

Figure 4. Mean deposit values (μL cm^{-2}) that were collected in the upper region of the soybean canopy at different application timings in the first application of Field 1. The means followed by the same letter did not differ by the Tukey test at 5% probability ($p < 0.05$).

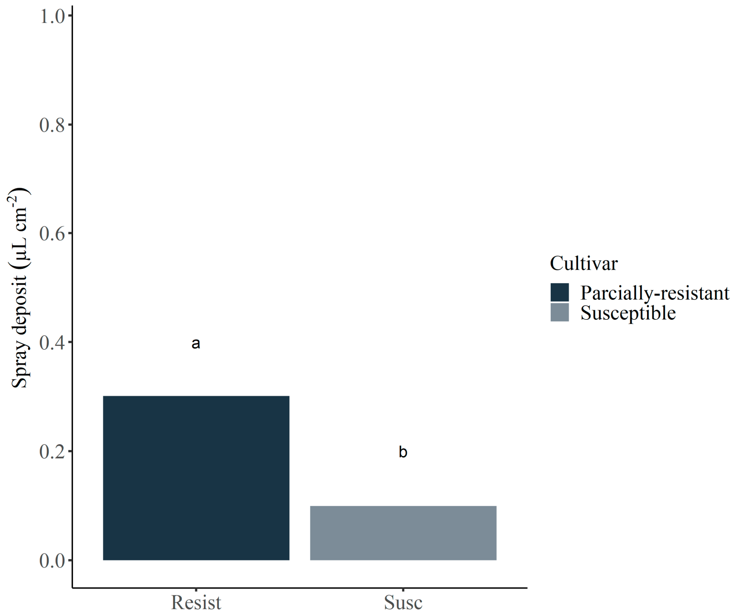

Figure 5. Mean deposit values (μL cm^{-2}) that were collected in the lower region of the soybean canopy of different soybean cultivars in the first application of Field 1. The means followed by the same letter did not differ by the Tukey test at 5% probability ($p < 0.05$).

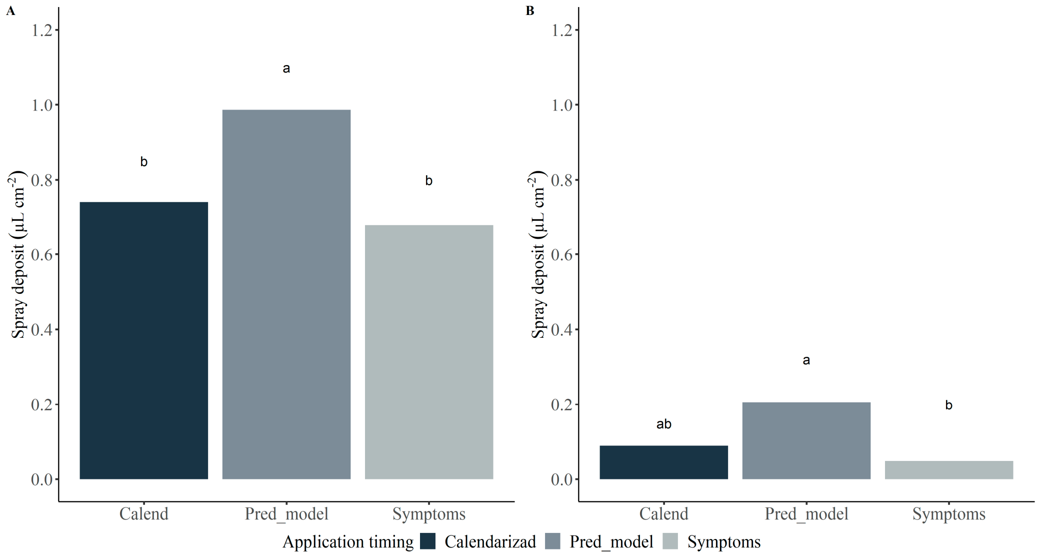

Figure 6. Mean deposit values (μL cm^{-2}) that were collected in the upper (**A**) and lower (**B**) regions of the soybean canopy at different application timings in the second application of Field 1. The means followed by the same letter did not differ by the Tukey's test at 5% probability ($p < 0.05$) in each comparison.

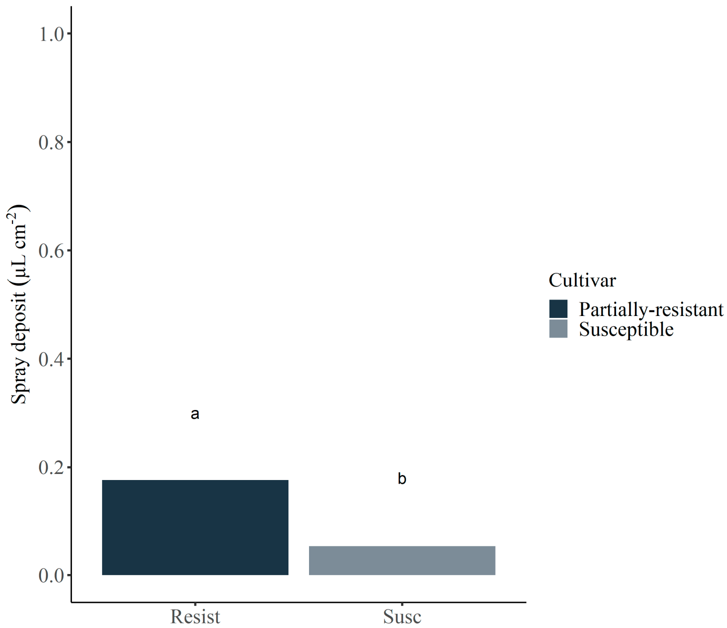

Figure 7. Mean deposit values (μL cm^{-2}) that were collected in the lower region of the soybean canopy of different soybean cultivars in the second application of Field 1. The means followed by the same letter did not differ by the Tukey's test at 5% probability ($p < 0.05$).

The second experimental field trial presented a similar trend in deposit values. In the first application, a significantly greater deposit was found in the upper region in treatments that were sprayed at the first symptoms appearance (0.82 μL cm^{-2}) and prediction model decision (0.84 μL cm^{-2}) (Figure 8). These applications happened 20 days after the calendarized application (0.66 μL cm^{-2}), once more demonstrating better spray deposit in the region with the later application. Moreover, a higher mean deposit value was found in the lower region of partially resistant cultivars (0.13 μL cm^{-2}) than those found in the susceptible cultivars (0.05 μL cm^{-2}) (Figure 9).

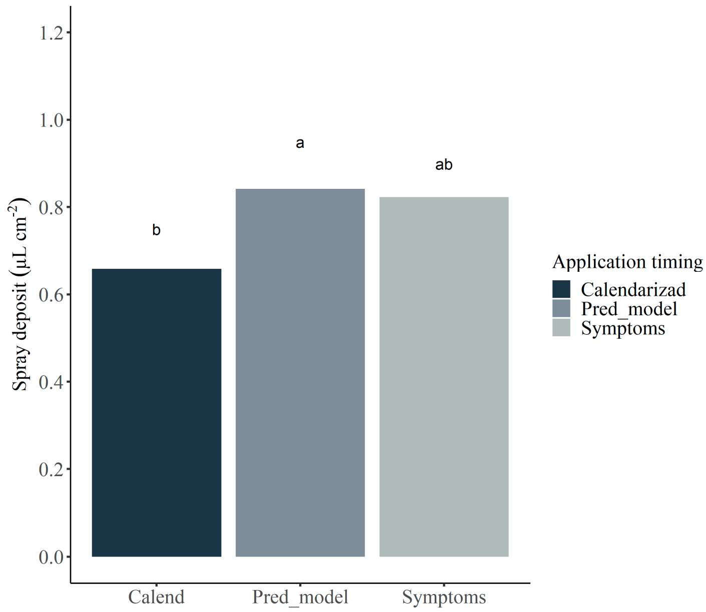

Figure 8. Mean deposit values (µL cm^{-2}) that were collected in the upper region of the soybean canopy at different application timings in the first application of Field 2. The means followed by the same letter did not differ by the Tukey's test at 5% probability ($p < 0.05$).

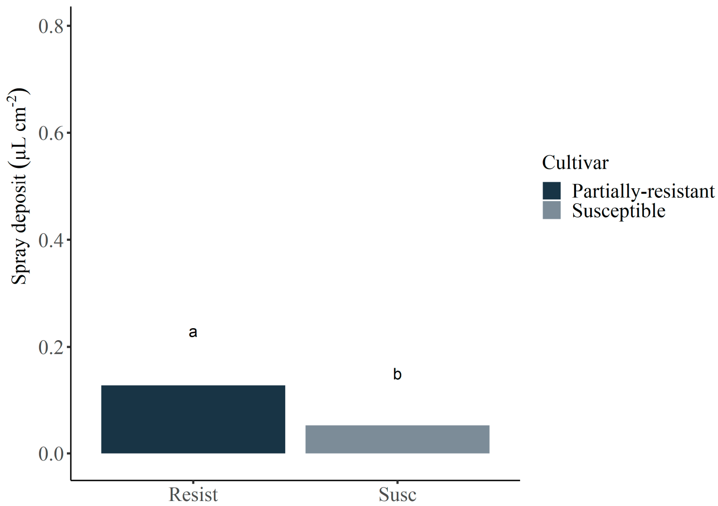

Figure 9. Mean deposit values (µL cm^{-2}) that were collected in the lower region of the soybean canopy of different soybean cultivars in the first application of Field 2. The means followed by the same letter did not differ by the Tukey's test at 5% probability ($p < 0.05$).

In the second application of Field 2, significant differences were found only in the lower region of the canopy. The application timings that were based on the prediction model and symptoms that were applied on the same date, obtained significantly higher deposition than the scheduled application (Figure 10). The higher mean deposit values were also found in the lower region of the canopy of partially resistant cultivars (Figure 11).

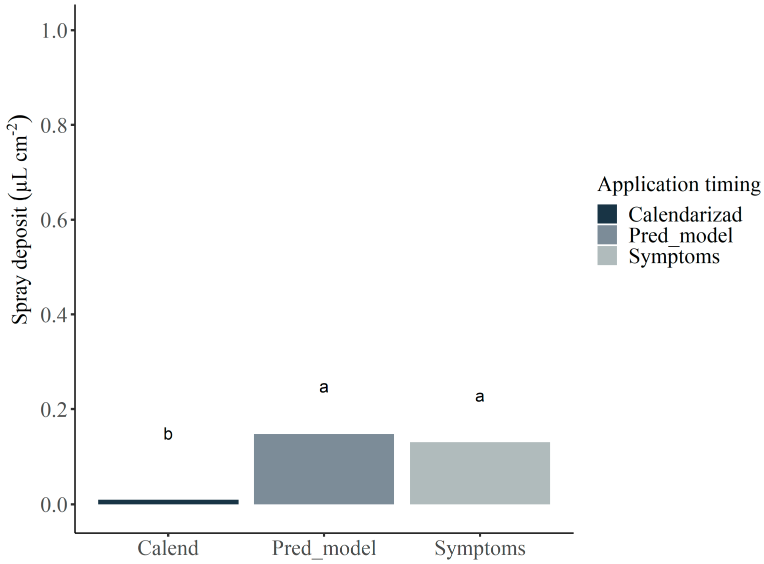

Figure 10. Mean deposit values (µL cm^{-2}) that were collected in the upper region of the soybean canopy at different application timings in the second application of Field 2. The means followed by the same letter did not differ by the Tukey's test at 5% probability ($p < 0.05$).

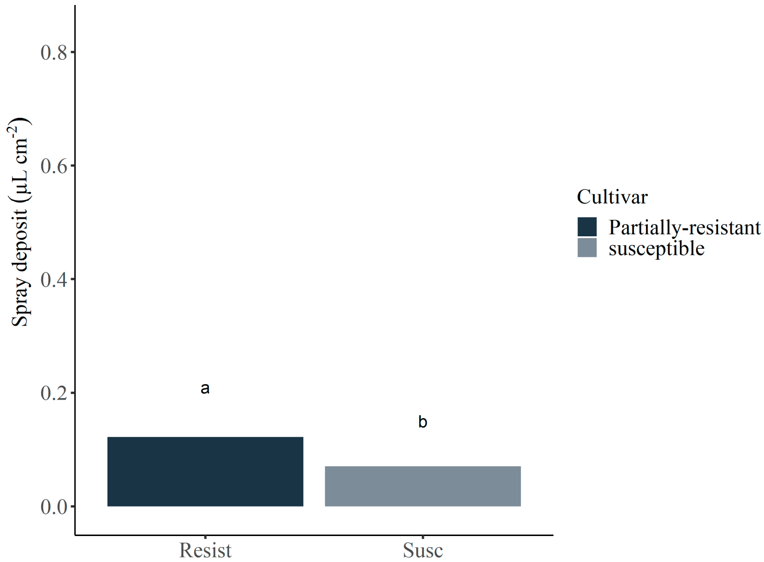

Figure 11. Mean deposit values (µL cm^{-2}) that were collected in the lower region of the soybean canopy of different soybean cultivars in the second application of Field 2. The means followed by the same letter did not differ by the Tukey's test at 5% probability ($p < 0.05$).

3.3. Spray Coverage

Finally, the qualitative evaluations that were based on the percentage of spray coverage were also consistent between the experimental trials. For Field 1, no significant difference was found in the first application, irrespective of application timing, cultivar, or canopy region. In the second application, a higher percentage of coverage was observed in the lower region of the canopy according to the cultivar, in which there was greater coverage in the partially resistant cultivars (42.3%) compared to the susceptible ones (31.8%) (Figure 12).

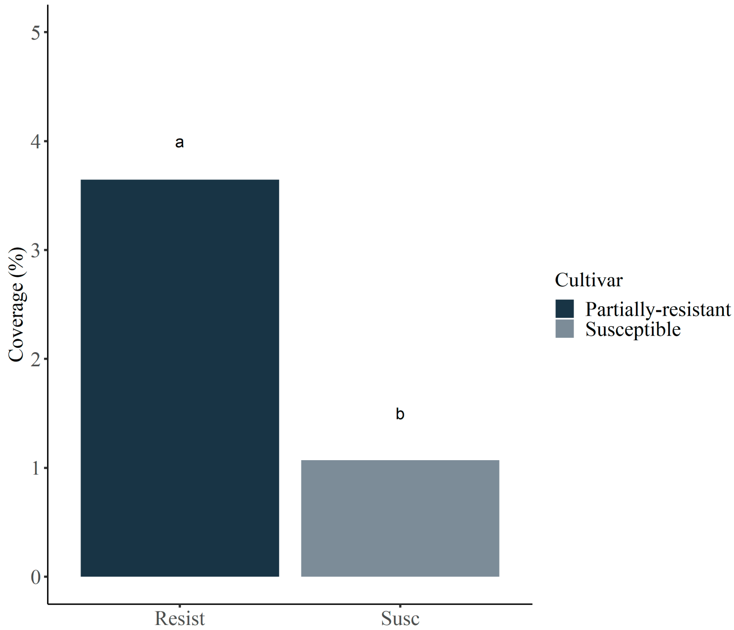

Figure 12. Mean percentage of coverage (%) in water-sensitive papers that were located at the lower region of the soybean canopy, according to different soybean cultivars in the second application of Field 1. The means followed by the same letter did not differ by the Tukey's test at 5% probability ($p < 0.05$).

On the other hand, a significant difference was found in the interaction between the factors in the first application of Field 2 only in the lower region of the canopy (Table 3). A higher percentage was found at the lower-positioned targets of partially resistant cultivar treatments (21.9%) compared to the susceptible cultivars (5.14%) when spraying at a calendarized timing. Moreover, the coverage of the partially resistant cultivars were also significantly higher compared to the other application timings. In the second application, another difference was found in the lower region of the canopy as a function of application timings, in which the scheduled spraying timing presented significantly lower values compared to the others (Figure 13).

Table 3. Mean percentage coverage (%) on water-sensitive papers in the first application of Field 2, according to different application timings and soybean cultivars in each canopy region.

Application Timing	**Cultivar**	
	Susceptible	**Partially Resistant**
Calendarized	5.14 ^{aB}	21.97 ^{aA}
Prediction model	6.57 ^{aA}	2.06 ^{bA}
Symptoms	6.74 ^{aA}	4.27 ^{bA}
Cause of Variation	**F**	**P**
Application timing (AT)	2.885	0.087 ^{NS}
Cultivar ©	0.922	0.352 ^{NS}
AT x C	3.958	0.042 *

^{NS}: Not significant; * significant a $p \leq 0.05$ by F test. Means followed by the same letter did not differ according to Tukey's test at 5% probability ($p < 0.05$). Lowercase letters compare between the means of application timings at each cultivar level (lines). Uppercase letters compare between the means of cultivars at each application timing (columns). Each statistical comparison was conducted separately for each canopy region (upper and lower).

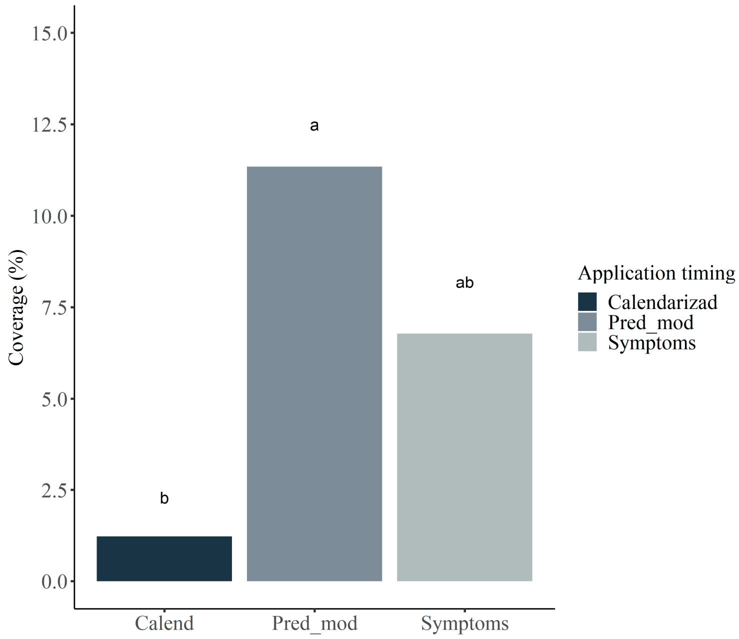

Figure 13. Mean percentage of coverage (%) in water-sensitive papers that were located at the lower region of the soybean canopy, according to different application timings in the second application of Field 2. The means followed by the same letter did not differ by the Tukey's test at 5% probability ($p < 0.05$).

3.4. Effect of Application Timings on SBR Control and Crop Defoliation

The disease severity (AUDPC) and the control efficacy in both experimental fields are shown in Table 4. In general, a significant reduction in the disease severity was observed in all treatments with the fungicide application compared to the control. In addition, there was also a significant difference between the cultivars, in which the partially resistant cultivar presented lower AUDPC, regardless of the application timing. For the percentage of control, a difference was found only in Field 2, where a higher disease severity was observed in all the treatments and, therefore, with higher disease pressure. In this field trial, the percentage of control with the scheduled application (R1) in the partially resistant cultivar was significantly lower than in the others. Although the percentage of control seems much lower in the partially resistant cultivars, it is possibly due to the lower severity that was found even for the control treatment without applications.

The disease progress curves were considerably similar between both experimental fields (Figure 14). It is possible to observe greater development starting at 42 days after the first application (DAA) and rapid growth after this moment. On the other hand, greater severity progress was found at Field 2, where other treatments were also affected by the disease and promoted greater disease development. Overall, susceptible cultivars showed greater development, especially the control without fungicide application.

For the level of defoliation as an indirect severity evaluation, the spectroradiometer proved to be effective in representing the leaf stand level of the treatments (Figure 15). As the crop moved towards the end of the season, a clear reduction in the LAI was observed in both field trials regardless of the treatment.

3.5. Effect of SBR on Crop Yield

Regarding the effect of the disease on the crop yield (kg ha^{-1}), no significant differences were found in the interaction of factors. However, the application timings affected the crop yield in Field 2, in which the control treatment without application presented a lower crop yield ($p < 0.05$) compared to the other application timings (Table 5). The numerical difference was kept similar in Field 1.

Table 4. Mean values of disease severity (AUDPC) and control (%) according to the application timings and susceptible and partially resistant soybean cultivars, for each field trial replication.

Field	Application Timing	AUDPC		Control (%)	
		Cultivar		Cultivar	
		Susceptible	P. Resistant	Susceptible	P. Resistant
Field 1	Control	200.80 aA	40.50 aB	-	-
	Calendarized	68.90 bA	18.10 aB	64.40	51.50
	Prediction model	54.60 bA	15.90 aB	71.60	59.20
	Symptoms	63.00 bA	15.00 aB	67.90	58.90
	Causes of Variation	**F**	**P**	**F**	**P**
	App. Timing (AT)	53.450	<0.001 ***	0.576	0.574 NS
	Cultivar ©	177.450	<0.001 ***	3.772	0.071 NS
	AT x C	26.450	<0.001 ***	0.042	0.959 NS
Field 2	Control	519.20 aA	65.70 aB	-	-
	Calendarized	138.90 bA	32.50 aB	72.50 aA	47.80 bB
	Prediction model	142.50 bA	22.40 aB	71.70 aA	65.70 aA
	Symptoms	191.00 bA	27.10 aB	62.10 aA	56.74 abA
	Causes of Variation	**F**	**P**	**F**	**P**
	App. timing (AT)	43.076	<0.001 ***	143.823	<0.001 ***
	Cultiv©(C)	188.949	<0.001 ***	8.577	0.008 *
	AT x C	28.402	<0.001 ***	5.019	0.009 *

NS: Not significant; * significant a $p \leq 0.05$; *** significant a $p \leq 0.01$ by F test. Means followed by the same letter did not differ according to Tukey's test at 5% probability ($p < 0.05$). Lowercase letters compare between the means of application timings at each cultivar level (lines). Uppercase letters compare between the means of cultivars at each application timing (columns). Each statistical comparison was conducted separately for each canopy region (upper and lower).

Table 5. Mean values of soybean crop yield (kg ha^{-1}) and thousand seeds weight (TSW) (g), according to soybean rust effect of different application timings and soybeans cultivars, for each field tria repetition.

Application Timing	Crop Yield		TSW	
	kg ha^{-1}		g	
	Field 1	Field 2	Field 1	Field2
Control	2393.714	2799.833 b	2393.71	2799.83 b
Calendarized	3143.054	3462.89 a	3143.05	3462.89 a
Prediction model	2738.061	3055.73 ab	2738.06	3055.73 ab
Symptoms	2817.034	3169.037 ab	2817.03	3169.04 ab
F value	2.713 NS	4.092 *	5.510 ***	1.804 NS
CV (%)	19.05	12.31	6.47	8.54

NS: Not significant; *** significant at $p \leq 0.01$; * significant at $p \leq 0.05$ by F test. Means followed by the same letter did not differ according to Tukey's test at 5% probability ($p < 0.05$).

Likewise, a lower TWS was observed for the control treatment without control, significantly ($p < 0.05$) for Field 1 (Table 5). Furthermore, the effect of the cultivars was also observed for TWS in Field 2, in which the susceptible cultivar presented lower TWS (160.9 g) than the partially resistant cultivar (178.8 g) (Figure 16).

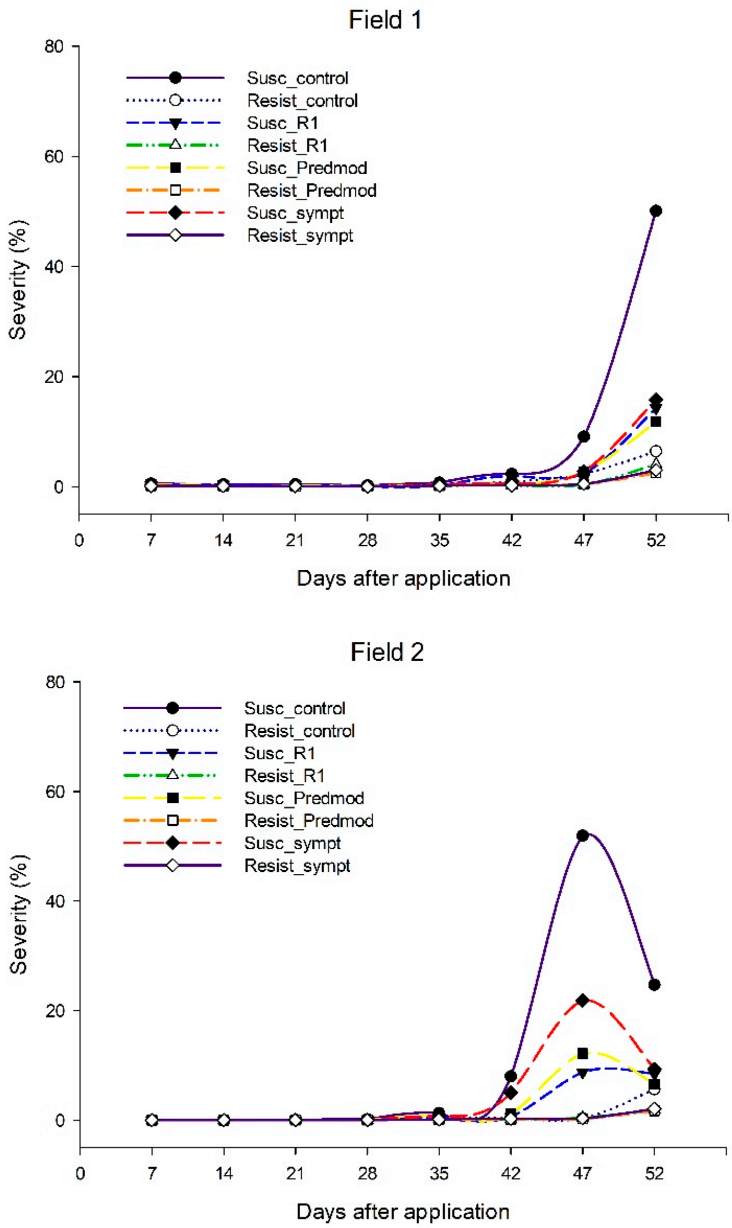

Figure 14. Soybean rust disease progress curve at Field 1 and Field 2 trial repetitions, according to the different soybean cultivars and application timings (treatments). Note: Susc_: susceptible soybean cultivar (DS6217); Resist_: partially resistant soybean cultivar (TMG7063). Control: without fungicide application; Calend: calendarized application (reproductive growth stage R1); Model: application timing based on the prediction model; Sympt: application timing based on the appearance of the first symptoms.

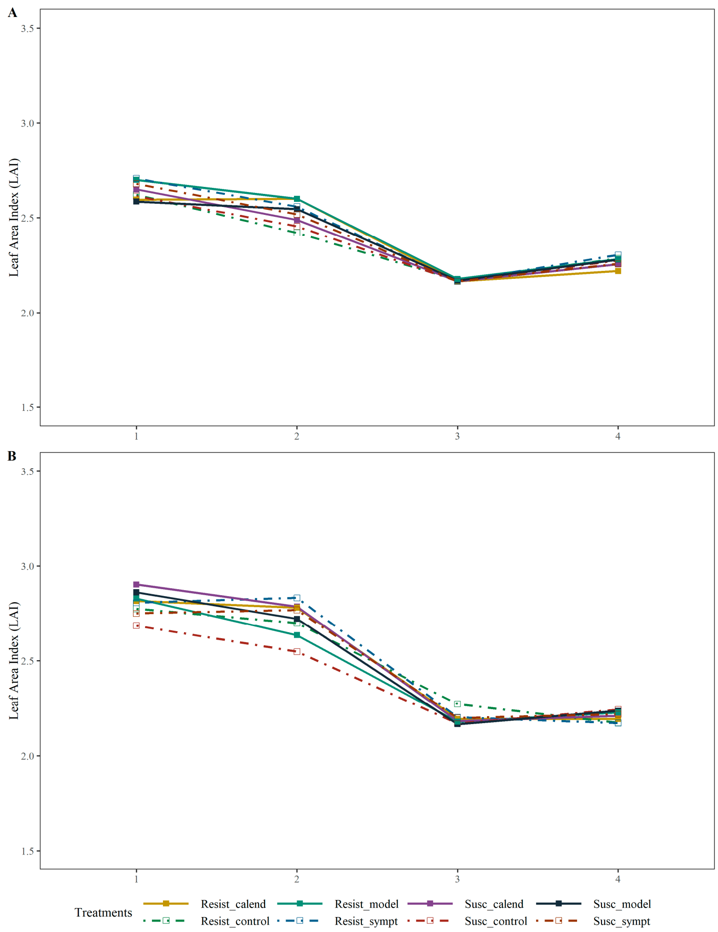

Figure 15. Defoliation assessment based on the leaf area index (LAI) through the integration of NDVI and Beer–Lambert law, according to the spectral curves of the susceptible and partially resistant soybean cultivars under soybean rust effect, across experimental evaluation periods of Field 1 (**A**) and Field 2 (**B**). Notes: Susc_: susceptible soybean cultivar (DS6217); Resist_: partially resistant soybean cultivar (TMG7063). Control: without fungicide application; Calend: calendarized application (reproductive growth stage R1); Model: application timing based on the prediction model; Sympt: application timing based on the appearance of the first symptoms. LAI calculated according to Tan et al. [35].

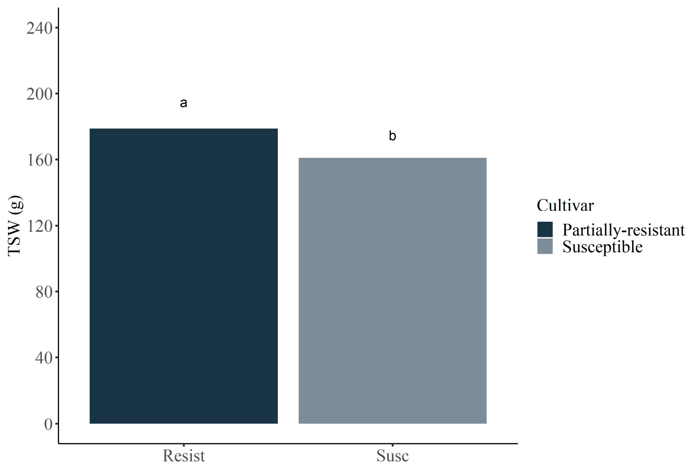

Figure 16. Mean values of thousand seeds weight (TSW) (g) as a function of soybean rust effect over different soybean cultivars in the experimental Field 2. The means followed by the same letter did not differ by Tukey's test at 5% probability ($p < 0.05$).

4. Discussion

This study evaluated the applicability of an SBR prediction model and the effect of different fungicide application timings on the spraying quality as well as on disease control and crop yield. Based on the disease epidemiology, it is important to provide proper SBR control as soon as possible, aiming to avoid the quick dispersal of spores and the emergence of epidemics in the field [2,38]. In this scenario, adequate disease monitoring is key to improving the application timing accuracy, spraying quality, and control efficacy. Besides, remote sensing and the usage of prediction models as support decision systems showed to be a valuable tool for IDM of SBR.

The prediction model that was applied here was able to identify plants with severity levels as low as 0.2% severity. The disease detection matched with the first appearance of symptoms, which can be considered an advantage since remote sensing may be applied in a simpler and faster way than extensive scouting in the field [15], especially with innovations in technology such as hyperspectral cameras and drone imaging of wide fields in lesser times [13]. The possibility of identifying the disease as quickly as possible can provide greater chances of a successful disease management program.

The application timings impacted the spraying quality, resulting in significant differences in the spray deposit and coverage at different parts of the crop canopy. A difference in the quantity of product that was deposited in the leaves, as well as the area that was covered by the spraying, may play a significant effect on the control of the biological agent, interfering with the control effectiveness and epidemic management. For instance, Berger-Neto et al. [39] reported higher white mold (*Sclerotinia sclerotiorum*) incidence in soybean with treatments that produced lower spray deposits, especially in lower canopy regions.

In our study, a marked difference was found mainly with later application timings. It promoted greater retention of spray deposit on the upper region of the canopy, therefore demonstrating a greater barrier for spray penetration. This outcome was also observed as the percentage of coverage. In Field 1, spraying that was based on the prediction model was conducted 6 days later than the others, which already promoted slightly greater retention in the upper section. None of the treatments were able to promote proper deposition and coverage in the lower region of the canopy. Meanwhile, the second application was conducted when a reduction of LAI was already started due to crop defoliation [23], influencing the penetration of the spray into the canopy and, therefore, promoting better deposition.

Nonetheless, the recommended timing of application at the end of the vegetative growth stage and reproductive growth stage is due to the possibility of still reaching the entire vertical profile of the plants and, therefore, achieving a better spray distribution, besides targeting the period of greater disease development [38]. However, it was seen that even spraying on R1 did not provide a uniform distribution. Furthermore, most fungicides that are recommended for SBR control act preventively and curatively, while most applications aim to act preventively to avoid any disease incidence and proliferation throughout the field [1]. Oppositely, when sprayed too far away from the first disease incidence in the field it is possible that the residual of the fungicide may not still be as active as needed, leading to lower efficacy [40].

Müller et al. [8] reported similar results of soybean rust severity at different application timings. Higher SBR severity (AUDPC) was observed when the application was the furthest from the first disease incidence. So, an application after R3 produced the best control when the disease incidence happened only after this period, whereas an application at R1 resulted in the best control efficacy when the disease was first seen before this growth stage and, therefore, closer to the disease incidence. Although an earlier application during the vegetative growth stage may provide better fungicide distribution in the canopy [24], the residual effect of the fungicide may not last until the period of higher disease incidence [40].

Moreover, Müller et al. [8] found that applications that were conducted closer to the first disease observations resulted in higher crop yields compared to other treatments that were sprayed after a longer time. This information also reassures the importance of proper disease monitoring to reduce both unnecessary fungicide applications in the field and to improve application timing accuracy and disease epidemic control. This is especially true when considering a country with proper environmental conditions for SBR development, along with fungicide application as the main source of control that is majorly characterized by several applications of only a few chemical choices (triazole, strobilurin, and carboxamides) that contributes to the selection of resistant populations [1,5,6].

In this study, especially for Field 2 where the disease started only 20 days after the calendarized application, at least one application would have been saved considering the whole crop season and the 15-day interval between applications. A smaller number of applications could reduce the chemical usage and, therefore, decrease fungal exposure to these fungicides, while a more accurate application having the potential to cease fungal development at determinant points that can increase control efficacy and decrease the chance of fungal survival in the field [8]. Finally, a better distribution throughout the crop canopy could also ensure a lower risk of over- or under-application rate, which could also lead to fungicide resistance [41]. Therefore, the benefits of sensor-based prediction models might come from the reduction of the number of applications or the fungicide efficacy that it might achieve.

The structural differences of the cultivars also played a major role in the spraying technology. The partially resistant cultivar (TMG7063) visually had less inter-row canopy closure compared to the susceptible cultivar (DS6217), in addition to a lower LAI. Therefore, the architecture format of the cultivar allowed a better penetration into the canopy, represented by a better spray distribution. Thereby, in most spraying that was conducted, better spray deposition and coverage were observed in the lower section of the plants.

A study unveiled the dynamic spray deposition according to different soybean cultivars' architecture, reporting a significant increase in the spray coverage and overall penetration capacity when spraying in cultivars with lower height, LAI, and numbers of branches [22]. An increase in the coverage of almost 96% at the lower canopy regions was found for these cultivars.

The coverage and spray deposit results were very similar and consistent. The significant reduction in spraying reaching capacity into the innermost regions of the canopy stood out in all cases. Therefore, it highlights the need to adjust the technology according to the application timing, especially based on the LAI and canopy closure [22]. Alternatives such as using specialized spray nozzles or higher application volumes can be very responsive to provide significant improvements [9,39,42,43].

The results that were obtained from the application technology corroborate the disease severity that was found. The treatments with higher spray deposit and coverage also promoted slightly lesser disease severity, such as those that were observed when spraying based on the prediction model and first symptoms. Therefore, in general, the spraying decision-making that was based on remote sensing data improved or at least maintained the control effectiveness level of conventional methods. Furthermore, greater disease development was observed 42 days after the first application (DAA). Therefore, the earlier applications may have promoted a lower control rate due to greater distance from the peak of disease severity and lower fungicide residual.

With regards to the disease severity, the partially resistant cultivar was also considered to be highly effective in terms of suppressing the level of disease growth, with a marked progress curve reduction and lower severity indexes, even when without application. Along with the aforementioned cultivar structure that allowed a better spray distribution, all these features contribute to disease management and are, therefore, considered an excellent tool for the IDM.

To date, seven soybean resistance loci have been identified [44,45]. Vittal et al. [11] identified varied infection capacities of *P. pachyrhizi* among different soybean genotypes that were tested, reporting reduced hyphae development in resistant soybean cultivars. Resistant (immune reaction) and partially resistant cultivars are known to produce fewer pustules, lesions, and longer latent periods [10].

Another important factor is that, in this study, the disease incidence happened later than the usual first appearance, which can be visually identified in the curve progress. In this study, the later application timings at the disease onset provided better control. It is possible that in regions where there is higher disease pressure, monitoring by remote sensing can even anticipate the application compared to the calendarized applications. Improvements in disease monitoring to supply information to the decision support system have the potential to improve control efficacy as well as to even reduce the number of applications in regions of lower disease pressure [13,46–48].

Moreover, the use of remote sensing to assess the disease severity and defoliation was also considered successful, as reported by other authors [35,49]. Although no significant difference was found between the treatments, the LAI that was calculated through the integration of NDVI and the Beer–Lambert law [35] properly represented the defoliation levels, allowing better visualization of the effect of the disease on the crop leaf mass throughout the crop season.

The fungicide application promoted a reduced disease effect on the soybean crop yield, allowing greater yields irrespective of the cultivar or application timings. However, the differences that were found for each treatment control and spraying quality were not fully correlated to the crop yield, despite the proximity of the values that were found. For example, in both field repetitions, the calendarized application which obtained poorer spraying quality and higher disease severity, also achieved slightly higher crop yield than the others, even though it was only numerical. The crop yield is mostly affected when disease pressure is high, such as in the values that were observed in the control treatment without application. Besides, the effect of SBR on crop yield is most pronounced when its progression occurs at pod formation and filling [44,50]. Other injuries than visual symptoms may also play an important role in affecting the crop yield, such as on the carbon exchange rates [51].

Overall, the model was considered an effective tool and showed promising results to be used as a tool in the integrated management of SBR, since the detection periods were similar to those that were based on visual diagnosis and with potential for maintenance or improvement of disease control. In addition, improvements in assessment techniques are reported as a result of time savings compared to visual assessments. For cultivars with lower disease severity, or situations where there is a lower risk of epidemic or lower disease severity and incidence, it could be considered even more effective. It is important to state that the applicability of remote sensing techniques and data obtainment to supply these types of prediction models is difficult since many variables can interfere with the optical analyses, such as climate conditions and the presence of other injuries [13,19]. More studies are encouraged to be conducted to improve the prediction model database as well as to implement it in IDM programs.

Our results denote the practicality of using remote sensing and prediction models in the integrated management of the disease. Positive results were found, indicating the possibility of integrating it among the currently present management techniques. In addition, the timing of application impacted the application technology, resulting in significant differences in spray deposition and coverage, which have a high potential to also interfere with the effective control and management of epidemics. The application at lower LAI promoted better spray distribution and better SBR control efficacy. Finally, partially resistant cultivars also played a major role in the SBR control and as a powerful tool of the IDM.

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