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CROP PROTECTION

Asian soybean rust control in response to rainfall simulation after fungicide application

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ABSTRACT. Asian soybean rust (*Phakopsora pachyrhizi)* is the main disease that affects soybean in Brazil. Fungicide applications are the main control method, but they can be influenced by the occurrence of rain. We aimed to study the control of Asian soybean rust in response to the occurrence of simulated rainfall at different times after fungicide application. The penetrant fungicides trifloxystrobin + prothioconazole (60 + 70 g a.i. ha^{-1}) and azoxystrobin + benzovindiflupyr (60 + 30 g a.i. ha^{-1}) and the nonpenetrant fungicides mancozeb (1,500 g a.i. ha^{-1}), chlorothalonil (1,440 g a.i. ha^{-1}), and copper oxychloride (672 g a.i. ha^{-1}) were tested using two spray volumes: 70 and 150 L ha^{-1}. Rain was simulated from 30 to 240 minutes after fungicide application. Soybean leaflets were collected and inoculated with a spore suspension of *P. pachyrhizi* (5.0 x 10^4 mL^{-1}) and incubated in plastic boxes for 20 days. The trials were repeated twice. Nonpenetrant fungicides were more susceptible to rain washing, mainly when the 70 L ha^{-1} spray volume was used. For the penetrative fungicides, the best control percentages were obtained when the rainfall occurred between 120 and 180 minutes after application, while the protective fungicides had the best control percentages when the rainfall occurred approximately 240 minutes after application. The Asian rust control is affected by the characteristics of the fungicide applied, by the time interval between fungicide application and rain occurrence and by the spray volume.

Keywords: application technology; chemical control; *Phakopsora pachyrhizi*; spray volume.

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Introduction

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Sydow & Sydow, is one of the most severe diseases affecting soybean crop (Godoy et al., 2016), achieving up to 90% damage in the absence of control measures (Hartmann et al., 2015).

The favorable weather conditions to the development of the pathogen during the growing season make the application of fungicides the best alternative for the management and control of the disease (Levy, 2015). In Brazil, the application of fungicides for the control of Asian rust started in the 2002/03 season (Reis, 2014). Fifty-five fungicides are registered for ASR control (MAPA, 2018) with different modes of action and plant tissue penetrative characteristics. For example, demethylation inhibitors (DMI), quinone outside inhibitors (QoI) and succinate dehydrogenase inhibitors (SDHI) have the ability to be absorbed by the plant tissue. In contrast, multisite fungicides are protective fungicides that are not absorbed by the plants. However, they play an important role in the control of soybean rust, being a fundamental strategy in the management of fungal resistance (Sierotzki & Scalliet, 2013). Fungicides such as mancozeb, chlorothalonil and cooper oxychloride act on multiple metabolic processes of fungi, making it difficult to develop resistance.

The effectiveness of the chemical control of the disease depends on several factors, including the choice of efficient fungicides, timing of application, spray volume and the adaptation of the application technology to the target and to the culture (Cunha, Coelho, & Araújo, 2010). Environmental and weather factors such as rainfall occurrence can affect the control efficacy of fungicides applied in different crops by washing off the fungicide deposits, diluting, redistributing and removing them, and affecting the chemical residual activity (Pigati, Dernoeden, & Grybauskas, 2010; Tofoli, Domingues, Melo, & Ferrari, 2014; Inguagiato & Miele, 2016; Stefanello et al., 2016; Rossouw, Fourie, Van Zyl, Hoffman, & McLéod, 2018).

Summer in Brazil is the time of the year during which fungicide applications are performed in soybean culture, and in the same season, short and nonpredicted periods of rain occurrence are also common. Pluvial precipitation occurring immediately after fungicide spraying, according to the factors described before, can reduce the Asian soybean rust control efficacy, allowing the disease to cause more damage to the culture.

Therefore, it is relevant to study how Asian rust control is affected by rainfall after fungicide application to determine guidelines for its reapplication or the reduction in the time interval between sprays. This work aimed to study the control of ASR in response to the occurrence of simulated rainfall at different times after application of penetrant and multisite protective fungicides using different spray volumes.

Material and methods

In a greenhouse, soybean seeds of the BMX Lança cultivar were sown in 2,000 mL pots filled with substrate. After germination, only one soybean plant per pot was retained. The plants received only water during their development. For the tests, a completely randomized experimental design was used with six replications. The trials were conducted in the 2017 season crop and repeated in the same period in 2018.

Forty days after sowing, the plants were submitted to treatments, which were composed of five fungicides, each consisting of an isolated experiment. Among them, two were penetrants: trifloxystrobin + prothioconazole (60 + 70 g a.i. ha^{-1}) plus vegetable oil (180 g a.i. ha^{-1}) and azoxystrobin + benzovindiflupyr (60 + 30 g a.i. ha^{-1}) plus mineral oil (214 g a.i. ha^{-1}), and three were nonpenetrants: mancozeb (1,500 g a.i. ha^{-1}), chlorothalonil (1,440 g a.i. ha^{-1}), and copper oxychloride (672 g a.i. ha^{-1}), being added to all of the protective fungicides the adjuvant composed of synthetic latex, organosilicone fluid and surfactant (128 g a.i. ha^{-1}). The experimental scheme was bifactorial (6 x 2) with six rainfall simulation time intervals after the application of the fungicides (30, 60, 90; 120, 180, and 240 minutes) and two spray volumes: 70 and 150 L ha^{-1}. The controls were composed of one group without fungicide application and another with the application and this last one was not submitted to rainfall simulation.

The applications were performed in the morning, respecting the atmospheric conditions for spraying as temperature lower than 30°C, air humidity above 55% and wind speed between 3 and 10 km h^{-1} (Reunião ..., 2012). A CO_2 pressurized sprayer with a four-nozzle bar (0.5 m distance between nozzles) was used for the applications. Single-jet spray nozzles of the Teejet® XR 110015 series, with a pressure of 3.0 bar (300 kPa) for the 150 L ha^{-1} spray volume and a Magnojet® MCP1 empty conical-jet with a pressure of 3.1 bar (310 kPa) for the 70 L ha^{-1} spray volume, were used, both with a speed of 1.4 m s^{-1}, producing fine spray droplets. For the evaluation of application quality, three hydrosensitive cards were used in each pot at the same plant height.

After spraying, the plants were subjected to an artificial rainfall simulation of 20 mm at different time intervals. For this, we used a tower with a rain simulator on the top, which emits a water slide of 1.38 mm $minute^{-1}$, requiring 14.5 minutes under the rainfall simulator to complete a 20 mm slide of water.

After natural drying, the central leaflets (mean size of 50 cm^2) of the leaves on the upper third of each soybean plant were collected. These were taken to the laboratory. The methodology of the detached leaf test was adapted from Scherb and Mehl (2006). In the Phytopathology Laboratory, plastic boxes were used, in which humid chambers were generated using a unit of polyethylene foam that was the size of the box (121 cm^2) and two sheets of filter paper of the same size. The chambers were moistened with distilled water. Then, the leaflets were placed in the box with the adaxial side facing up. A piece of cotton was added to the petiole, which was saturated with distilled water to maintain the leaflet hydration.

After twenty-four hours, the leaflets were inoculated with a suspension of *P. pachyrhizi* uredospores (5.0 x 10^4 spores mL^{-1}). The inoculum, obtained from soybean leaflets from the field in Passo Fundo, Rio Grande do Sul State, Brazil, and multiplied in soybean healthy plants, was taken to the laboratory for preparation of the spore suspension. In a 500 mL Erlenmeyer flask, 200 mL of distilled water, one drop of Tween[20®] spreader and soybean leaflets with rust were added. After shaking, the spore suspension was filtered, and the uredospore concentration was estimated by a hemocytometer under an optical microscope. The suspension was placed in a 500 mL sprayer and applied to the leaflets. For spore germination, the plastic boxes were left for 24h in the dark at 23°C. After this period, they were placed on benches in a growth chamber with a photoperiod of 12h at the same temperature. Every two days, water was added to the cotton piece, with a wash bottle, to maintain moisture in the chambers.

After 20 days of incubation, the total uredinia number were counted on each leaflet under a stereoscopic microscope. For the control percentage calculation, the Abbott (1925) formula was used. Hydrosensitive cards were scanned, and the leaf cover percentage and density (impacts per cm^2) were evaluated by DropScope software. Data were submitted to the F test for analysis of variance, the Skott-Knott mean comparison test and polynomial regression. The equation obtained from those polynomial regressions was used to calculate the maximum control and maximum time interval.

Results and discussion

There was a relation of cause and effect, showing that the sooner rainfall occurred after an application, the lower the Asian soybean rust control, which, in this case, was represented by an increase in the number of uredinia per leaflet in all trials. The influence of rain on the protection period of the plants was highly dependent on the time interval between the application of the products and rain occurrence (Lenz et al., 2012; Tofoli et al., 2014). The factors of spray volume and time interval did not have a statistical significant interaction for all fungicides tested, and then these variables were analyzed separately (Table 1). The control without fungicide application showed an average of 98 and 117 uredinia per leaflet for the 2017 and 2018 trials, respectively. In addition, all regression analyses for the Asian soybean rust control were significant ($p < 0.01$) (Figure 1, Table 2).

Table 1. Number of uredinia of *Phakopsora pachyrhizi* per soybean leaflet observed after fungicide application with artificial rain occurring at different time intervals after spraying (20 mm). Passo Fundo/UPF, 2019.

Time interval (minutes)	Trifloxystrobin + prothioconazole			
	----- number of uredinia per leaflet-----			
	2017		2018	
	70 L ha^{-1}	150 L ha^{-1}	70 L ha^{-1}	150 L ha^{-1}
30	74.0 a A*	55.3 a B	77.3 a A	63.7 a B
60	57.3 b A	40.0 b B	65.0 b A	56.0 b B
90	35.3 c A	25.7 c B	48.7 c A	35.3 c B
120	28.3 c A	15.0 d B	37.7 d A	28.3 d B
180	15.3 d A	8.0 e B	34.3 d A	24.3 d B
240	14.7 d A	7.3 e B	30.7 e A	23.0 d B
No rain	12.7 d A	7.4 e B	30.0 e A	23.3 d B
CV (%)	18.1	15.4	10.4	13.4
Time interval (minutes)	Azoxystrobin + benzovindiflupyr			
	2017		2018	
	70 L ha^{-1}	150 L ha^{-1}	70 L ha^{-1}	150 L ha^{-1}
30	85.7 a A	71.0 a B	93.7 a A	78.7 a B
60	67.7 b A	60.0 b B	83.7 b A	65.7 b B
90	56.7 c A	46.0 c B	60.3 c A	48.7 c B
120	48.0 d A	38.7 d B	50.7 d A	42.0 d B
180	38.0 e A	29.7 e B	41.3 e A	31.0 e B
240	33.3 e A	26.7 e B	39.0 e A	29.0 e B
No rain	31.3 e A	25.9 e B	37.0 e A	27.7 e B
CV (%)	11.9	9.8	11.1	11.9
Time interval (minutes)	Mancozeb			
	2017		2018	
	70 L ha^{-1}	150 L ha^{-1}	70 L ha^{-1}	150 L ha^{-1}
30	97.7 a A	93.7 a A	113.3 a A	110.0 a A
60	87.3 b A	80.0 b A	104.7 b A	97.3 b A
90	75.8 c A	60.7 c B	84.7 c A	74.3 c B
120	56.0 d A	47.3 d B	68.3 d A	50.7 d B
180	47.0 e A	38.7 e B	52.0 e A	42.0 e B
240	37.3 f A	28.0 f B	42.7 f A	30.0 f B
No rain	35.3 f A	28.6 f B	42.3 f A	26.0 f B
CV (%)	10.2	11.0	7.9	8.9
Time interval (minutes)	Chlorothalonil			
	2017		2018	
	70 L ha^{-1}	150 L ha^{-1}	70 L ha^{-1}	150 L ha^{-1}
30	96.8 a A	94.3 a A	111.8 a A	108.6 a A
60	85.3 b A	78.0 b A	100.3 b A	96.3 b A
90	71.5 c A	64.3 c B	81.1 c A	71.0 c A
120	52.6 d A	45.0 d B	61.6 d A	53.3 d B
180	44.6 d A	35.6 e B	48.3 e A	36.6 e B
240	34.0 e A	25.0 f B	37.3 f A	26.7 f B
No rain	35.2 e A	23.8 f B	36.3 f A	24.6 f B
CV (%)	14.1	11.0	9.4	9.4
Time interval (minutes)	Cooper oxychloride			
	2017		2018	
	70 L ha^{-1}	150 L ha^{-1}	70 L ha^{-1}	150 L ha^{-1}
30	97.1 a A	94.6 a A	116.3 a A	111.3 a A
60	89.0 b A	80.7 b B	107.6 b A	99.3 b B
90	79.3 c A	69.0 c B	100.3 b A	91.3 c B
120	60.0 d A	51.6 d B	79.3 c A	69.0 d B
180	48.0 e A	38.7 e B	66.7 d A	56.7 e B
240	38.0 f A	32.0 f B	56.3 e A	48.6 f B
No rain	36.6 f A	30.5 f B	56.8 e A	46.9 f B
CV (%)	7.0	7,1	7.5	8.2

*Means followed by the same lower letter in the column and the capital letter in the line did not differ statistically by the Scott-Knott test (p < 0,05).

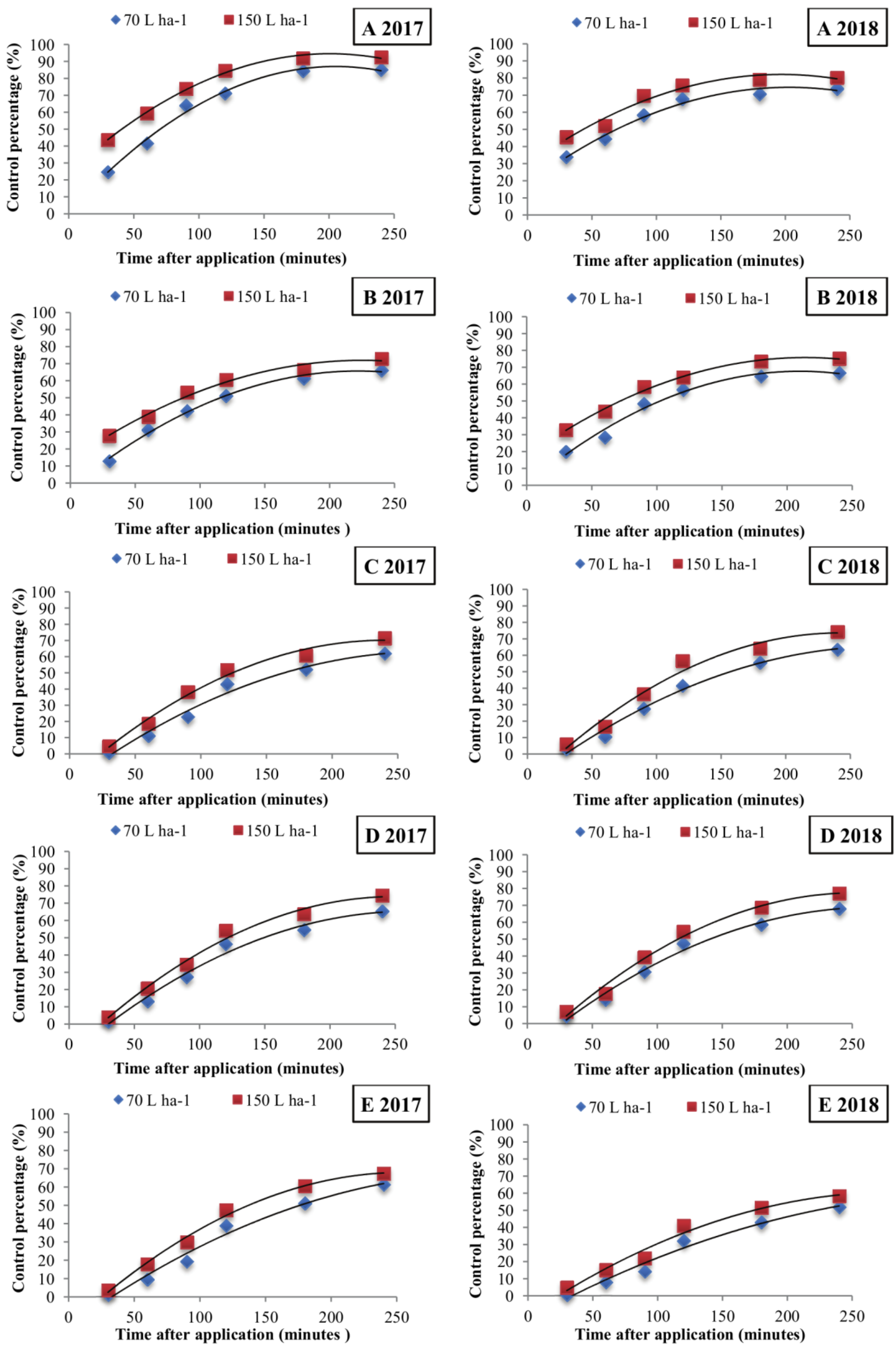

Figure 1. Polynomial regression for the Asian soybean rust mean control percentage according to the occurrence of rainfall at different times after fungicide application using two spray volumes in the 2017 and 2018 seasons.

Table 2. Equations and coefficients of regression of each polynomial regression for the Asian soybean rust mean control percentage according to the occurrence of rainfall at different times after fungicide application using two spray volumes in the 2017 and 2018 seasons. Passo Fundo/UPF, 2019.

A 2017 - Trifloxystrobin + prothioconazole	A 2018 - Trifloxystrobin + prothioconazole
70 L ha^{-1}	70 L ha^{-1}
$y = -0.002x^2 + 0.8363x + 1.285$	$y = -0.0014x^2 + 0.556x + 18.153$
$R^2 = 0.991$	$R^2 = 0.980$
150 L ha^{-1}	150 L ha^{-1}
$y = -0.0017x^2 + 0.7009x + 24.325$	$y = -0.0014x^2 + 0.5361x + 29.39$
$R^2 = 0.996$	$R^2 = 0.961$
B 2017 – Azoxystrobin + benzovindiflupyr	B 2018 – Azoxystrobin + benzovindiflupyr
70 L ha^{-1}	70 L ha^{-1}
$y = -0.0014x^2 + 0.6166x - 2.7135$	$y = -0.0015x^2 + 0.6417x + 0.2896$
$R^2 = 0.994$	$R^2 = 0.979$
150 L ha^{-1}	150 L ha^{-1}
$y = -0.0012x^2 + 0.5187x + 13.545$	$y = -0.0013x^2 + 0.5473x + 17.26$
$R^2 = 0.985$	$R^2 = 0.993$
C 2017 – Mancozeb	C 2018 – Mancozeb
70 L ha^{-1}	70 L ha^{-1}
$y = -0.0011x^2 + 0.5955x - 18.172$	$y = -0.0011x^2 + 0.5897x - 16.392$
$R^2 = 0.981$	$R^2 = 0.988$
150 L ha^{-1}	150 L ha^{-1}
$y = -0.0015x^2 + 0.7172x - 16.007$	$y = -0.0016x^2 + 0.7549x - 17.77$
$R^2 = 0.987$	$R^2 = 0.977$
D 2017 – Chlorothalonil	D 2018 – Chlorothalonil
70 L ha^{-1}	70 L ha^{-1}
$y = -0.0012x^2 + 0.6395x - 18.109$	$y = -0.0012x^2 + 0.6369x - 15.83$
$R^2 = 0.983$	$R^2 = 0.988$
150 L ha^{-1}	150 L ha^{-1}
$y = -0.0015x^2 + 0.7258x - 16.777$	$y = -0.0015x^2 + 0.7443x - 16.477$
$R^2 = 0.989$	$R^2 = 0.989$
E 2017 – Cooper oxychloride	E 2018 – Cooper oxychloride
70 L ha^{-1}	70 L ha^{-1}
$y = -0.0008x^2 + 0.5129x - 16.011$	$y = -0.0006x^2 + 0.415x - 13.445$
$R^2 = 0.981$	$R^2 = 0.976$
150 L ha^{-1}	150 L ha^{-1}
$y = -0.0013x^2 + 0.6604x - 16.115$	$y = -0.0009x^2 + 0.5083x - 11.378$
$R^2 = 0.993$	$R^2 = 0.978$

The disease control was similar to the treatment with fungicide that was not submitted to rainfall for the trials in which the rain occurred around 180 minutes after trifloxystrobin + prothioconazole (60 + 70 g a.i. ha^{-1}) application in 2017. In 2018, the treatments that had similar number of uredinia when compared to the control without rainfall were those in which the rain occurred 120 minutes after fungicide application for the 150 L ha^{-1} spray volume and 240 minutes for the 70 L ha^{-1} spray volume. In general, these results agreed with those obtained by Stefanello et al. (2016), in which it was observed that it was necessary to have at least 120 minutes without rain occurrence after the same fungicide application. The control treatments, which were not submitted to rain simulation, showed 85.0% and 92.2% control using spray volumes of 70 L ha^{-1} and 150 L ha^{-1} for the 2017 trials. In 2018, the values were 74.4% and 80.1%, respectively. The maximum control percentages obtained from the spray volume of 70 L ha^{-1} were 88.7% at 209 minutes (2017) and 73.4% at 198 minutes (2018) without rainfall occurrence after spraying. The values reached 96.6% (2017) and 80.7% (2018) at 206 and 191 minutes for the 150 L ha^{-1} spray volume. Moreover, for the same fungicide, the spray volume of 150 L ha^{-1} when compared to the spray volume of 70 L ha^{-1} provided an increase varying from 8.0% to 43.5% in the disease control in 2017. For 2018, the values varied from 7.2% to 25.7%.

For the azoxystrobin + benzovindiflupyr (60 + 30 g a.i. ha^{-1}) fungicide, when the rain was simulated 180 minutes after application, lower values of uredinia per leaflet were observed, and these treatments (180 and 240 minutes) were statistically similar to those that were not subjected to rainfall in both years. This result showed that it was necessary to have a time interval of at least 180 minutes between azoxystrobin + benzovindiflupyr application and rainfall occurrence (Figure 1B), which was in agreement with the results obtained by Chechi, Boller, Forcelini, Roehrig, and Zuchelli (2018). The treatments that were not submitted to rain simulation presented 67.1% and 73.5% control for the spray volumes of 70 L ha^{-1} and 150 L ha^{-1} in

2017 and 68.4% and 76.4% in 2018. For the 70 L ha^{-1} application rate, the maximum control was 65.2% after 220 minutes (2017) and 68.9% after 214 minutes (2018). For 150 L ha^{-1}, 69.6% control was observed after 216 minutes, and 74.9% control was observed 210 minutes after application for the same years. Additionally, in 2017, the application rate of 150 L ha^{-1} compared to 70 L ha^{-1} provided a 7.5% to 53.6% increase in disease control. For the 2018 trials, the increase in disease control ranged from 10.4% to 39.1%.

For mancozeb, a multisite fungicide (1,500 g a.i. ha^{-1}), the treatments that were not submitted to rainfall had 64.1% and 73.5% control for the volume of 70 L ha^{-1} and 150 L ha^{-1} in 2017 and 63.8% and 77.8% in 2018, respectively, and these treatments were statistically similar to those that received simulated rainfall at 240 minutes after the fungicide application. When comparing the application rates, the higher application rate (150 L ha^{-1}) presented an increase of 19.2% to 85.1% in disease control in 2017. In 2018, the maximum disease control increase was 47.6% using the 150 L ha^{-1} spray volume compared to the 70 L ha^{-1} spray volume. In a recent study conducted by Rani, Sharma, Kumar, and Mohan (2015), the highest values of disease severity were found in tomatoes when rainfall occurred immediately after the application of the fungicide mancozeb. Hunsche, Damerow, Shmitz-Eiberger, and Noga (2007) found that the reduction in mancozeb deposits on leaves of apple seedlings ranged from 55.0% to 80.0% with a rain simulation of 1 to 5 mm at a speed of 5.0 mm h^{-1}. Rossouw et al. (2018) verified that 1, 5, and 10 mm of rain reduced the mancozeb fungicide residue by 32.9, 37.8, and 41.1%, respectively.

For chlorothalonil (1,440 g a.i. ha-1), the control percentages of the treatments that were not submitted to rainfall were 65.0% and 68.9% for the application rate of 70 L ha-1 in 2017 and 2018. The control values were 75.7% and 78.9% when using 150 L ha-1 for the same years, respectively, and these treatments were statistically similar to the those in which rain occurred at 240 minutes after fungicide application. The higher spray volume (150 L ha-1) showed a better disease control, with the control 12.3% to 63.4% greater than the one achieved with 70 L ha-1 in 2017 and 11.8% to 38.0% in 2018. Ingugiato and Miele (2016) found that the control efficacy of the chlorothalonil chemical for leaf spot in plants of the Poaceae family was significantly reduced when rain occurred less than sixty minutes after the fungicide application. Pigatti et al. (2010) reported that the reduction in disease control in the same crop was 67.0% after rainfall occurrence.

For the last trial with copper oxychloride fungicide (672 g a.i. ha^{-1}), the treatments that did not receive simulated rain showed disease control percentages of 63.2% and 69.7% for the spray volumes of 70 L ha^{-1} and 150 L ha^{-1} in 2017. In the next year, a reduction in disease control ranging from 13.4% to 18.7% was observed, with 51.4% control for 70 L ha^{-1} and 60.4% control for 150 L ha^{-1}. The treatments mentioned above were statistically similar to those in which the rainfall simulation occurred at 240 minutes after fungicide application. In this study, cooper oxychloride had the worst performance in the control of ASR. The higher spraying rate (150 L ha^{-1}) still showed better results in disease control than the lower spraying rate (70 L ha^{-1}). The increase in disease control ranged from 9.0% to 68.2% in 2017 and from 11.2% to 88.2% in 2018 when comparing the 150 L ha^{-1} spray volume to the 70 L ha^{-1}. Perez-Rodriguez, Soto-Gomez, Lopez-Periago, and Paradelo (2015) reported the removal of copper-based fungicides in vineyards after rainfall occurrence. However, the authors concluded that this removal depended on the formulation of the product, being higher for Bordeaux mixture than for copper oxychloride (Perez-Rodriguez et al., 2016). According to Vincent, Armengol, and García-Jiménez (2007), the effect of rain on the reduction in the tenacity of copper deposits in plant tissues is not completely understood yet; however, most copper formulations are removed even with the use of larger spray volumes in the applications.

We can affirm that it is necessary to have at least 120 and 180 minutes between fungicide application and rainfall occurrence for both penetrative fungicides tested (trifloxystrobin + prothioconazole and azoxystrobin + benzovindiflupyr). In the case of trifloxystrobin + prothioconazole, we observed different results in the fungicide performance in the 2018 season when compared to the 2017 season, reducing the disease control by 12.5% to 17.3%. This may have occurred because sometimes prothioconazole was used more than twice on each crop season and was applied isolated in the field. These attitudes can lead to a reduction in fungus susceptibility to the fungicide active ingredient, as previously reported by Godoy et al. (2018), in which the fungicide mixtures containing prothioconazole (DMI) showed a decrease in disease control efficacy in some Brazilian areas of soybean production in the 2017/2018 season. For the mixture of azoxystrobin + benzovindiflupyr, it was known that mutations that cause resistance in *P. pachyrhizi* had previously been detected for both active ingredients, such as the F129L mutation for azoxystrobin

(Klosowski et al., 2016) and the I86F mutation for benzovindiflupyr (Klappach, 2017). However, for this fungicide mixture, the disease control rate was similar for both years, indicating that it did not get worse in the context of resistance during this time.

Similar behaviors were observed among the three multisite protective fungicides. All of them presented the highest percentages of disease control when rain occurred only at 240 minutes after their application. Mancozeb, chlorothalonil and copper oxychloride are naturally protective, being neither absorbed nor translocated and remaining on the surface of the plant, where they were deposited.

When studying fungicide washing caused by rain on potatoes, Tofoli et al. (2014) observed that systemic or translaminar fungicides were less affected by the occurrence of rainfall when compared to protective or nonsystemic fungicides. Moreover, the same authors found that the longer time interval between application and rainfall occurrence favored the retention and absorption of the products in the plants, presenting a positive and direct relation with the disease control. When comparing the application rates, the use of 150 L ha^{-1} presented higher reduction of uredinia number when compared to 70 L ha^{-1} for both the protective and penetrant fungicides. This behavior indicates that an increase in spray volume may promote greater leaf coverage, reducing the impact of rain on fungicide washing. Thus, the greater spray volume was even more relevant when using protective fungicides, which are not absorbed and are more easily removed by rain than the penetrative fungicides.

Based on the results from the hydrosensitive cards, the statistical analysis showed that there were interactions between the factors (fungicide and volumes) for drop density and leaf coverage. For drop density, all of the fungicides showed superior results using the application rate of 150 L ha^{-1} when compared to 70 L ha^{-1} (Table 3). For leaf coverage, 150 L ha^{-1} trifloxystrobin + prothioconazole, 150 L ha^{-1} cooper oxychloride and 70 L ha^{-1} and 150 L ha^{-1} chlorothalonil showed the best performance, followed by 150 L ha^{-1} azoxystrobin + benzovindiflupyr and 150 L ha^{-1} and 70 L ha^{-1} mancozeb. The worse treatments for leaf coverage were 70 L ha^{-1} trifloxystrobin + prothioconazole and 70 L ha^{-1} azoxystrobin + benzovindiflupyr, reinforcing the idea of not using low spray volumes for these mixtures application. For mancozeb and chlorothalonil fungicides, no significant differences were found for the coverage with the use of different spray volumes. This was probably due to the addition of the surfactant adjuvant to the fungicide mixture. The treatment with 70 L ha^{-1} cooper oxychloride was inferior to all treatments mentioned above. The worst treatments for this variable were those with penetrant fungicides trifloxystrobin + prothioconazole and azoxystrobin + benzovindiflupyr with an application rate of 70 L ha^{-1}.

Table 3. Leaf coverage and impacts density according to each treatment. Passo Fundo/UPF, 2019.

Treatment	Coverage (%)				Impacts density (impacts cm^{-2})			
	70		150		70		150	
	------------ L ha^{-1} ------------				------------ L ha^{-1} ------------			
Trifloxystrobin + prothioconazole	15.2	cB	39.4	aA	260.4	bA	396.1	aA
Azoxystrobin + benzovindiflupyr	8.0	cB	31.3	bA	188.6	bB	371,7	aA
Mancozeb	22.0	bA	29.2	bA	261.9	bA	468,5	aA
Chlorothalonil	29.3	aA	31.6	aA	312.5	aB	552.9	aA
Cooper oxychloride	21.9	bB	43.7	aA	296.4	bA	322.1	aA
	CV (%)		17.45		CV (%)		24.52	

Means followed by the same lower-case letter in the column (between fungicides) and upper-case letter (between spray volumes) in the rows are not different by the Scott-Knott test ($p < 0.05$).

The leaf coverage values and impacts density values were 2.6 and 2.5 times (trifloxystrobin + prothioconazole), 3.9 and 1.9 times (azoxystrobin + benzovindiflupyr), 1.08 and 1.77 times (chlorothalonil), 1.3 and 1.8 times (mancozeb), and 2.0 and 1.1 times (copper oxychloride) higher for the application rate of 150 L ha^{-1} than to 70 L ha^{-1}. An explanation for this fact is that the use of larger spray volumes can promote greater leaf coverage and penetration of the product in the plant profile, resulting in greater disease control effectiveness. In a trial testing spray volume between 40 and 160 L ha^{-1}, Roehrig, Boller, Forcelini, and Chechi (2018) verified that an increase in the spray volume promoted an increase in the drop density in the profile of the soybean plant, with an increase of 2.1 times in the number of drops per cm^{2}. The authors also demonstrated that a reduction in application rate presented negative results on leaf coverage, resulting in a reduction in Asian soybean rust control. For soybean yield, the best results were found with the 130 L ha^{-1} application volume, which was statistically similar to the 160 L ha^{-1} application volume. In tests conducted by Cunha, Moura, Silva, Zago, and Juliatti (2008), Cunha, Juliatti, and Reis (2014), it was observed that there was a trend of increasing drop deposition of the product with greater spray volumes. Prado et al. (2015), when comparing application rates of 60 to 160 L ha^{-1}, observed that the 160 L ha^{-1} spray volume delivered the highest values of disease control and soybean yield.

Finally, we also emphasize the importance of monitoring the rainfall occurrence before and after the application of fungicides in the field, and they should not be applied when the rainfall is imminent, since the residues are taken directly into the soil, causing soil contamination and unnecessary expenses to the farmers.

Conclusion

The control of Asian soybean rust is affected by the characteristics of the fungicide that is applied, by the time interval of rain occurrence after application and by the spray volume. The sooner rainfall occurs after the application of fungicides, the greater their washing, reducing the disease control percentage. In addition, nonpenetrant fungicides were more susceptible to rain washing among the tested fungicides, mainly when using a spray volume of 70 L ha^{-1} compared to 150 L ha^{-1}.

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