

Leaf area, spray volumes and applicable quantities of fungicide during the protection period for control of Asian soybean rust

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ABSTRACT

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The purpose of this study was to check the influence of leaf area index, spray volumes and applicable quantities of fungicide during the protection period, and for control of Asian soybean rust. All tests were conducted in the field and in the laboratory between 2014 and 2016. The quantity of fungicide and the spray volume were varied in the application of fungicides on soybean plants with different leaf area index. Rainfall simulation tests were also carried out at

different times after application of fungicide. The spray volume and application rate of fungicide should be adequately proportioned according to the leaf area index of the soybean cultivar. The closer to the time of application that the rain occurs, the lower the control obtained by applying the fungicide. The leaf area index, the fungicide application rates, and the spray volumes influenced the protection period of fungicides and the control of Asian soybean rust.

Keywords: application technology, *Phakopsora pachyrhizi*, rainfall simulation

RESUMO

Chechi, A.; Boller, W.; Forcelini, C.A.; Roehrig, R.; Zuchelli, E. Área foliar, volumes de calda e doses de fungicida no período de proteção e no controle da ferrugem-asiática-da-soja. *Summa Phytopathologica*, v.44, n.4, p.326-331, 2018.

O objetivo deste trabalho foi verificar a influência do índice de área foliar, volumes de calda e de doses de fungicidas no período de proteção e no controle da ferrugem-asiática-da-soja. Os ensaios foram conduzidos a campo e em laboratório, entre 2014 e 2016. Variou-se a dose e o volume de calda na aplicação de fungicidas sobre plantas de soja com diferentes índices de área foliar. Também foi realizado um ensaio com simulação de chuva em

diferentes momentos após a aplicação de fungicidas. O volume de calda e a dose do fungicida devem ser adequados de acordo com o índice de área foliar da cultivar. Quanto mais próximo do momento da aplicação a chuva ocorrer, menor o controle obtido pela aplicação do fungicida. O índice de área foliar, as doses e os volumes de calda influenciam o período de proteção de fungicidas e no controle da ferrugem-asiática-da-soja.

Palavras-chave: *Phakopsora pachyrhizi*, simulação de chuva, tecnologia de aplicação

Asian soybean rust (ASR) caused by the fungus *Phakopsora pachyrhizi* Sydow & Sydow is one of the most severe diseases affecting soybean, with damage varying from 10 to 90% in the different places from where it was reported (20, 22). Santos et al. (19), stated that the control of the disease is mainly based on fungicide applications. The effectiveness of the chemical control of the disease depends on several factors among which are: the choice of effective fungicides; time of application; interval time between applications; spray volume; and the adequacy of the application technology to the target and to the culture (4).

According to Debortoli et al. (8), soybean cultivars with greater branching capacity and leaf quantity need a greater droplet coverage. Soybean cultivars vary in leaf area index, from 3.5 to more than 8.0. It is reasonable to assume that the same spray volume used in spraying does not distribute a uniform quantity of fungicide in plants of different size and leaf area. The same can be inferred from the fungicide rates used

in the soybean crop. In this sense, the spray volume and the quantity of chemicals used should be adjusted in proportion to the leaf area of the plant to be protected. Otherwise, there may be excess coverage in plants of smaller size, and insufficient protection in plants with larger leaf area, which will affect the efficacy of disease control.

The protection period of the fungicides applied on the leaves can be affected by plant characteristics and also by environmental factors. Among the environmental factors, rainfall occurrence may interfere in pesticide performance (18). According to Oliveira (17), rain is one of the main causes of loss, and it is responsible for washing away the fungicide deposits on the plants, thus diluting, redistributing and completely removing the fungicide.

The purpose of this work was to check the influence of leaf area index, spray volumes and applicable quantities of fungicide during the protection period, and for control of ASR.

MATERIAL AND METHODS

The experiments were conducted in the experimental field, greenhouse and growth chamber in the University of Passo Fundo, located in the city of Passo Fundo - RS at an altitude of 687 m. The place is located in the latitude of 28°15'S and longitude of 52°24'W.

The field trial was carried out during the 2014/15 season, with three soybean cultivars of indeterminate habit, with IPRO technology (Intacta RR2 PRO), and with different leaf area index (LAI): NS 5445 (LAI<6), BMX Vanguarda (LAI=6 to 7), BMX Ponta (LAI>7). The experimental design was in randomized blocks, with five replications. Each plot measured 2.25 m × 5.0 m with five rows. The distance between blocks was 0.9 m, and was 1.2 m between plots.

The soil is classified as Dystrophic Dark Red Latosol. The accumulated rainfall in the months of December 2014 and January, February and March of 2015 was recorded as 696.8 mm, 73.2 mm above the historical normal for the period in the area. However, almost half of the precipitation occurred in the month of January (333.5 mm). The average temperatures recorded during the period were similar to the historical normal (21°C), being slightly above it in the minimum (17°C), and below the maximum temperatures (28°C) (10, 11).

The sowing was at the end of November, on wheat cultural remains, with spacing of 0.45 m between rows. The sowing density of NS 5445, BMX Vanguarda, and BMX Ponta were 14, 12 and 10.5 seeds per meter, totaling 345,680, 296,296 and 259,260 established plants per hectare. The management of weeds, pests and insects was carried out according to the official technical methods for the crop (16).

Three fungicide applications were administered. In the first application, it was trifloxystrobin + prothioconazole (commercial rate: 60 + 70 g a.i. ha⁻¹) plus vegetable oil composed of methyl ester of soybean oil (360 g a.i. ha⁻¹) at 48 days after sowing (DAS). In the second and third applications, we used azoxystrobin + benzovindiflupyr (60 + 30 g a.i. ha⁻¹) plus mineral oil (256.8 g a.i. ha⁻¹) at 67 and 88 DAS, respectively. The fungicides were applied in three spray volumes (100, 150 and 200 L ha⁻¹) and at three rates (75, 100 and 125% of the rate recommended by the manufacturer), characterizing a 3 × 3 factorial scheme.

A CO₂ pressurized sprayer equipped with a four-nozzle bar (spaced 0.5 m apart) with flat spray tips of Teejet® XR 110015 series at a pressure of 2.0 bar (200 kPa) was used for the application of the spray volumes of 100 and 150 L ha⁻¹. The Teejet® XR 11002 tips at pressure of 3 bar

(300 kPa) were used for the volume of 200 L ha⁻¹. Both tips generated fine-grade drops.

Disease severity assessments were performed biweekly from the date of the first application of fungicide. Three plants were collected per plot, and the severity was evaluated according to the diagrammatic scale proposed by Godoy et al. (12). Among the 55 and 100 DAS, three plants per plot of each soybean cultivar were collected to measure the leaf area index using a LI-3100C® area meter. The leaf area of the plants was multiplied by their populations to estimate the LAI.

The harvest was carried out in 6.75 m² of each plot (three central rows). The humidity of the grains (%), yield (kg ha⁻¹), and mass of one thousand seeds (g), with humidity adjustment to 13% were determined.

For the laboratory test (2015/2016), a completely randomized experimental design was used, employing four replications. Three ND 5445 soybean seeds were sown in pots of 2000 mL in a greenhouse. After germination, only one soybean plant per pot was maintained.

When the plants completed 40 DAS, they were subjected to the same treatments as in the field experiment, using the fungicide azoxystrobin + benzovindiflupyr (60 + 30 g a.i. ha⁻¹) plus mineral oil (256.8 g a.i. ha⁻¹). After the spraying which was carried out in the same manner as in the field experiment, the plants were exposed to an artificial rainfall of 20 mm at different times: 0.5; 1.0; 1.5; 2.0; 2.5 and 3.0 hours after application of the fungicide. The plants remained for 14.5 minutes exposed to rain simulation for the formation of that water slide. The control treatment was kept (one with and the other without fungicide application), both without wetting. Then, the central leaflets of the seventh leaf of each soybean plant were removed. The methodology of detached leaf test used was adapted from Mehl & Scherb (14).

At the laboratory, gerbox boxes were used, in which humidity chambers were created with a unit of polyethylene foam of the size of the gerbox (121 cm²) and two sheets, of the same size, of filter paper. The chambers were moistened with distilled water. Then, the leaflets were placed in the gerbox boxes with the adaxial side facing up, and left in the shade to dry at room temperature. Twenty-four hours after fungicide application, the leaflets were inoculated with a suspension of *P. pachyrhizi* spores (5 × 10⁴ spores mL⁻¹). The inoculum, obtained from soybean leaflets collected in the field, was taken to the laboratory for the preparation of the spore suspension. To a quantity of 500 mL Erlenmeyer, 200 mL of distilled water was added, one drop of Tween® spreader and soybean leaflets with rust. After shaking, the leaves were separated from the spore suspension. With a micropipette, 10 µL aliquot

Table 1 - Spray volumes and fungicide rates used in the applications. Passo Fundo/RS, 2015

Treatment	Spray volume (L ha ⁻¹)	Fungicide commercial rate (%)	Trifloxystrobin + prothioconazole (g a.i. ha ⁻¹)	Azoxystrobin + benzovindiflupyr (g a.i. ha ⁻¹)
1	-	-	-	-
2	100	100	60 + 70	60 + 30
3	100	125	75 + 87.5	75 + 37.5
4	100	75	45 + 52.5	45 + 22.5
5	150	100	60 + 70	60 + 30
6	150	125	75 + 87.5	75 + 37.5
7	150	75	45 + 52.5	45 + 22.5
8	200	100	60 + 70	60 + 30
9	200	125	75 + 87.5	75 + 37.5
10	200	75	45 + 52.5	45 + 22.5

of the suspension was taken and placed on a slide, covered by a cover slip and visualized under an optical microscope for spore counting. This procedure was repeated three times and the mean of the counts was calculated. The suspension was placed in a 500 mL spray bottle and applied to the leaflets.

For germination of the spores, the gerbox boxes were left in the dark for 24 h, with a temperature of 23°C. After this period, they were arranged in benches in a growth chamber, with a photoperiod of 12 h, at the same temperature. Every 2 days, water was added to the gerbox boxes with a pissette, for moisture maintenance. After 20 days of incubation, pustules were counted per cm² of each leaflet. For this, an area of 2.0 cm², being 1.0 cm² on each abaxial half of the leaflets, was evaluated under a stereoscopic microscope.

The disease severity data from the field experiment were submitted to log transformation (log x). For the disease control percentage calculation, the Abbott (1) formula was used. We used the F test for variance analysis, the Skott-Knott test for means classification and polynomial regression.

RESULTS AND DISCUSSION

The fungicide rate and the spray volume influenced the ASR severity, the green leaf area index, and the soybean grain yield in the field experiment, but there were no significant interactions between the factors. The ASR severity in the control reached 52% (cv. NS 5445), 65% (BMX Vanguarda) and 78.5% (BMX Ponta) (Figure 1). These differences on final severity occurred mainly due to differences between cultivars. One of the factors that generated this difference in disease severity was the plant's leaf area.

The most effective treatments in reducing the severity of ASR for cv. NS 5445 were both with 100 and 125% of the commercial rate of the fungicide with the use of the spray volume of 200 L ha⁻¹. For cv. BMX Vanguarda, the best treatments were with the addition of 25% in the commercial rate using the spray volume of the 150 L ha⁻¹ and the rates of 100 and 125% for the spray volume of 200 L ha⁻¹. In cv. BMX Ponta, the treatment with the lowest disease severity was composed of the spray volume of 200 L ha⁻¹ and a 25% increase in the commercial

rate of the fungicide, evidencing that the larger the leaf area, the lower the coverage. These data showed the need to increase the spray volume and, in some cases, the quantity of fungicide.

The highest values of leaf area index were 5.9 (cv. NS 5445) 7.2 (BMX Vanguarda) and 8.2 (BMX Ponta) in the best treatments, among stages R1 and R3. For cv. NS 5445, the leaf area index at the end of the grain filling was higher than in the other cultivars, being 2.9 (NS 5445), 2.2 (BMX Vanguarda) and 2.0 (BMX Ponta) (15, 18 and 25% of severity, respectively) confirming the hypothesis that different spray volumes and fungicide rates are required to control the disease in soybean cultivars with a different leaf area index. At the end of the seed filling, the leaf area index showed its lowest value for the control in all experiments. According to Butzen et al. (2) in cultivars with higher quantity of leaves, the increase in the spray volume may provide sufficient coverage and penetration to protect soybean leaves. In a study by Navarini et al. (15), in which different rates of fungicides were evaluated, it was observed that the rate increase was efficient for some fungicides of the triazoles group in the control of soybean rust, which reflected higher grain yield.

The leaf area index and seed yield correlated positively for all cultivars, showing that the higher the leaf area index in R6 stage, the higher the yield. These results were obtained by the fungicide treatment, which better controlled the disease and kept the leaf area healthier.

Plant architecture, which is represented by their stature, number of branches and leaf area, have a significant effect on the coverage and penetration of drops in soybean cultivars. There are lower penetration of canopy drops in those plants with greater branching capacity and greater leaf area (7). The results obtained by Tormen et al. (21) showed that fungicide coverage of leaves of the lower third of the canopy of the soybean plant is dependent on the architectural characteristics of the cultivar at the time of application. Soybean cultivars with upright habit, which do not close completely between rows throughout the soybean cycle are available in the market. In this way, their use could contribute to improving the deposition of the fungicide in the lower parts of the canopy.

The lowest values of seed yield were observed in the control. All other treatments resulted in higher seed yield. Values ranged from 55 to 95% for cv. NS 5445. For cv. BMX Vanguarda, the seed yield was 10 to 66% higher than the control. Already, for the cv. BMX Ponta, the

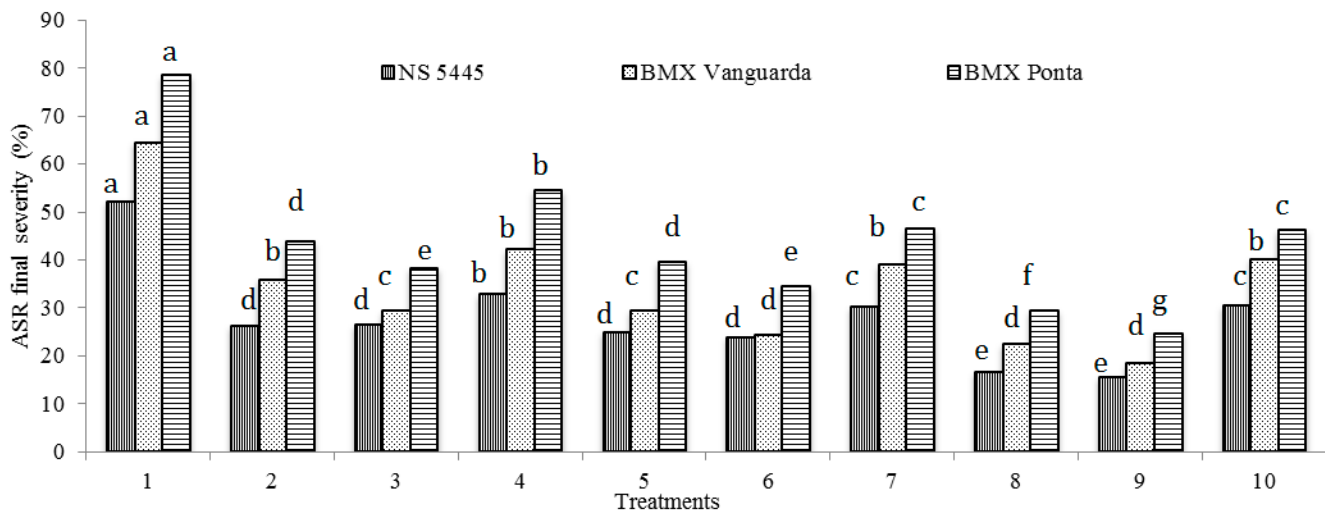


Figure 1 – Final severity of the Asian soybean rust according to each cultivar and treatment.

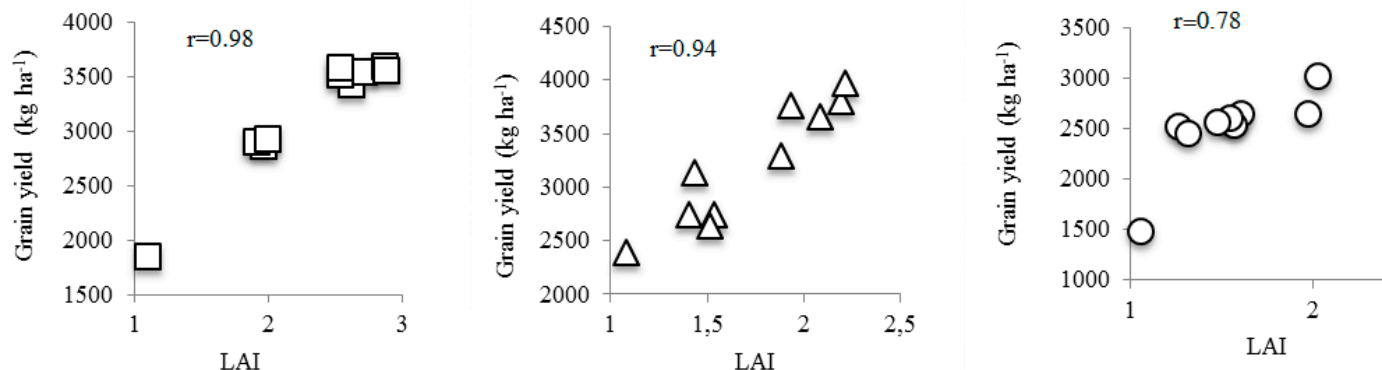


Figure 2 - Correlation between the leaf area index at the grain final filling (R6 stage) with the grain yield of each soybean cultivar (square = NS 5445; triangle = BMX Vanguarda; circle = BMX Ponta)

yield ranged between 65 and 103% when compared to the control treatment. Regarding yield, for cv. NS 5445, the treatment with the 100 L ha⁻¹ spray volume and the commercial rate of the fungicide proved to be the most appropriate since there was no difference between the spray volumes nor between the commercial rate and the one 25% higher. For cv. BMX Vanguarda, a spray volume of at least 150 L ha⁻¹ was required along with the commercial rate of the fungicide for a satisfactory control of the disease. The volume of 200 L ha⁻¹ and the rate 25% higher were statistically similar to them. In this case, the spray volume of 100 L ha⁻¹ was shown to be lower in the ASR control. Already, for cv. BMX Ponta, the best control was obtained with 200 L ha⁻¹ and a 25% increase in the commercial rate. The rates of 75% of the chemical did not present efficient control for the disease, regardless of the spray volume applied, with damages of more than 500 kg ha⁻¹ when compared to the most effective treatments.

For the laboratory experiment, a cause and effect relationship was found, showing that the sooner rainfall occurs after an application, the lower the disease control, in this case, represented by the increase in the number of pustules per cm² (Figure 3). The influence of rain during the plant protection period is highly dependent on the time interval between the application of the products and its occurrence, besides the product being applied (13).

The control, without application of fungicide, presented an average of 97 pustules per cm². For the spray volume of 100 L ha⁻¹, the number of pustules per cm² was lower for treatment with 125% of the commercial rate, as compared to the 100% rate of treatment. With the use of 150 L ha⁻¹, the 25% higher fungicide rate presented a lower number of pustules per cm² when rainfall occurred in the first few hours after application. When the rain occurred 2 hours after spraying, the treatment with 100% of the rate presented the lowest number of pustules per cm². For the volume of the 200 L ha⁻¹, in the first few hours of rainfall after application, the 25% increase in commercial rate showed the lowest number of pustules per cm². However, 2 hours after spraying, the pustules values per cm² were similar for 100 and 125% of the fungicide rate.

The treatment with 75% of the commercial rate, regardless of the volume applied, generated the highest values of pustules per cm², being the treatments less effective in the control of soybean rust, well agreeing with the experiment in the field. These results reinforce the use of adequate rates and spray volumes to the conditions, both environmental and the characteristics of the cultivar to be used.

The Asian rust control ranged from 90.7 to 97.4% for treatments that were not submitted to artificial rainfall. As for the treatments that were submitted to the rainfall simulator, the percentage of control varied between 24.7 and 55.7% (0.5 h after application); 41.8 and 61.9% (1.0 h); 44.8 and 71.1% (1.5 h); 67.0 and 78.4% (2.0 h); 74.2 and 84.5% (2.5 h); 80.7 and 94.6% (3.0 h). Debortoli (6) found that the occurrence of rain had a negative effect on the control of Asian rust, and it is directly related to the interval between the application and the occurrence of rainfall. The author observed that the simulation of rain at 0 and 30 minutes after the application of fungicide showed the greatest influence on the efficacy of the disease control.

When estimating the percentage of reduction in the effectiveness of the fungicide caused by rainfall, losses were observed between 45.1 and 66% (0.5 h after application of the fungicide); 35.6 and 50.0% (1.0 h); 26.3 and 45.9% (1.5 h); 18.3 and 26.8% (2.0 h); 12.6 and 18.6% (2.5 h); 2.8 and 10.1% (3.0 h). With the occurrence of rainfall only 1 hour after application, the majority of the treatments presented about 50% reduction in the control effectiveness of Asian rust, while the treatments composed of spray volume of 200 L ha⁻¹ and 100 and 125% of the fungicide rate showed losses around 35%. An explanation for such situations is that the use of a larger volume of spray provides a greater coverage and penetration of the active ingredient in the leaflet, resulting in a greater percentage of disease control. In works carried out by Cunha et al. (3, 5), it has been observed that there is a tendency towards increase in the deposition of the product with the increase of the spray volume. Derksen & Sanderson (9) reported higher coverage and lower fungicide deposition variations throughout the canopy of soybean plants, with increasing volume of application.

Even 3 hours after application of the fungicide, there was still a reduction in the amount of active ingredient in the leaflets. In an experiment conducted by Oliveira (17), the values observed 1, 2 and 48 hours after application did not reflect the common sense of the fungicide market, which recommends a minimum interval of 2 hours between application and rainfall.

Rational use of fungicides requires adjusting the fungicide rate and the spray volume to the leaf area index of the soybean cultivar for a better and more efficient control of Asian rust. The occurrence of rain until 3 hours after the application of fungicide reduced the effectiveness of disease control.

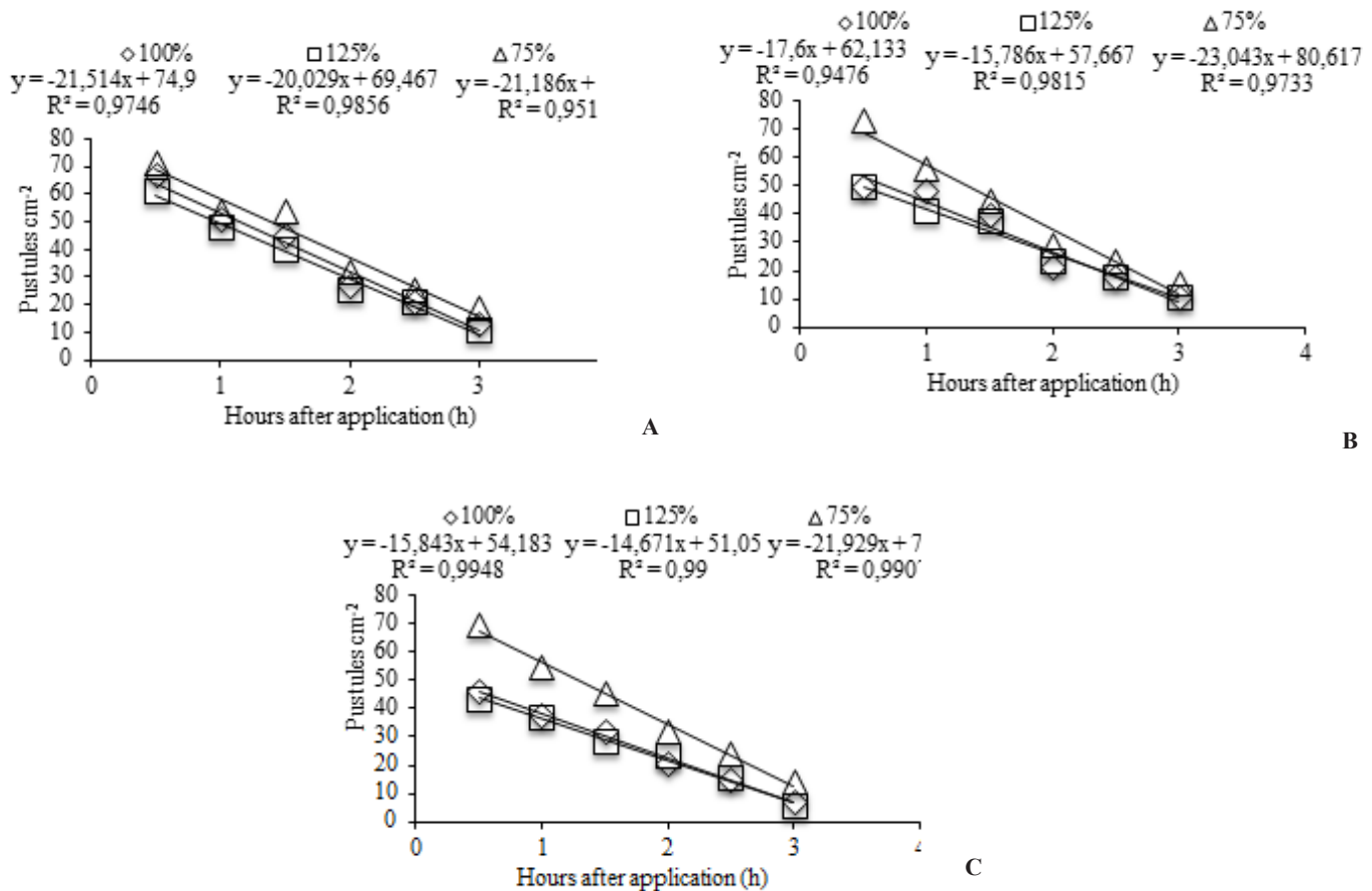


Figure 3 - Simple linear regression for *Phakopsora pachyrhizi* pustules number in soybean, in function of the rainfall interval time after application for different quantities of fungicide, with the spray volume of 100 (A), 150 (B) e 200 (C) L ha⁻¹.

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