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# Effect of the interaction between fungicide application time and rainfall simulation interval on Asian Soybean Rust control effectiveness

# Efeito da interação entre horários de aplicação do fungicida e intervalos de simulação de chuva na eficácia de controle da Ferrugem Asiática da Soja

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## Abstract

Environmental factors affect the performance of fungicides in soybean (*Glycine max* (L.) Merr.). They also influence the residual activity of the products applied to the leaves. The objective of this study was to assess the control effectiveness of the interaction between fungicide application and rainfall simulation on Asian Soybean Rust (ASR). Two experiments were conducted, one in the greenhouse, in a completely randomized design, and the other in the field, in a randomized block design. Both the experiments had the same factorial arrangement of 6x5, with four replications. Factor A: Five fungicide applications time at 0400 h, 0900 h, 1400 h, 1800 h, 2300 h and, a control with no application; Factor B: four intervals of time between the application of fungicide and rainfall simulation at 0, 30, 60 and 120 min for the experiment in the greenhouse and at 2, 30, 60, 120 min for the experiment in the field. A control was included for both the experiments with no rainfall. The number of days to the appearance of the first pustules was determined, along with severity of ASR, relative chlorophyll index and productivity. It was found that the ASR control effectiveness of fungicide applications in soybean plants in sunlight was less efficient with rainfall simulation. The rainfall simulation had greater negative effect on disease control effectiveness in applications conducted at night under dew conditions. The application conducted at 0900 h showed the greatest disease control effectiveness in both greenhouse and in the field conditions. The 1400 h application showed decreased fungicide control residual and ASR control effectiveness, possibly due to a combination of the low relative humidity and high temperature. Rainfall simulation carried out at 120 min after application still had the ability to affect the ASR control effectiveness.
**Key words:** Chemical control. Dew. *Glycine max* L. Night application. *Phakopsora pachyrhizi*.

## Resumo

Os fatores ambientais afetam o comportamento dos fungicidas em soja (*Glycine max* (L.) Merr.). Eles também influenciam a atividade residual de produtos aplicados na folha. O objetivo do estudo

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foi avaliar a eficácia de controle da interação entre a aplicação do fungicida e simulação de chuva na Ferrugem Asiática da Soja (FAS). Foram conduzidos dois experimentos, um em casa de vegetação em delineamento inteiramente casualizado e outro a campo em delineamento de blocos ao acaso. Ambos os experimentos tiveram o mesmo arranjo fatorial 6x5, com quatro repetições. O fator A: cinco horários de aplicação de fungicida às 04h00, 09h00, 14h00, 18h00, 23h00 e uma testemunha sem aplicação; Fator B: quatro intervalos de tempo entre a aplicação do fungicida e a simulação de chuva ao 0, 30, 60 e 120 min para o experimento em casa de vegetação e aos 2, 30, 60, 120 min para o experimento à campo. Um controle sem chuva foi incluído para ambos os experimentos. Os parâmetros avaliados foram o número de dias para o aparecimento das primeiras pústulas, severidade de FAS, índice relativo de clorofila e produtividade. Verifica-se que a eficácia de controle de FAS de aplicações de fungicidas em plantas de soja sob a luz solar foi menos afetada pela simulação de chuva. A simulação de chuva tem maior efeito negativo na eficácia de controle da doença em aplicações realizadas durante a noite sob condições de orvalho. A aplicação realizada às 09h00 apresentou a maior eficácia de controle da doença em ambas às condições de casa de vegetação e a campo. O horário de aplicação das 14h00 reduziu o residual de controle do fungicida e a eficácia de controle de FAS, possivelmente devido à combinação da baixa umidade relativa do ar e alta temperatura. Contudo, a simulação de chuva realizada aos 120 min após a aplicação ainda afetou a eficácia do controle FAS.
**Palavras-chave:** Aplicação noturna. Controle químico. *Glycine max* L. Orvalho. *Phakopsora pachyrhizi*.

## Introduction

Fungicide spray application is a complex process, and its effectiveness can be drastically influenced by temperature, relative humidity, wind speed, and light. The environmental conditions affect both the physical behavior of the spray solutions and the physiological state of the plant affecting leaf absorption and redistribution of substance (RAMSEY et al., 2005; ZABKIEWICZ, 2007).

Several authors have assessed the effects of isolated environmental conditions on the behavior of foliar applications. Temperature is related to the permeability of substances through the cuticular waxes associated with the cutin, wherein permeability increases rapidly with increasing temperature (BAUR; SCHÖNHERR, 1995). Relative humidity influences absorption, since it affects cuticle moisture and solute absorption mechanisms (FERNÁNDEZ et al., 2008), such as the evaporation rate of applied solutions (SCHÖNHERR, 2000). In addition to these two factors, light and dew also seem to affect the performance of fungicides in disease control.

The effect of light, in foliar absorption of products, may be indirectly associated with the transpiration rates and metabolic activity of plants, and directly to photo bleaching. For most species, transpiration rate is low at night, close to zero, gradually increasing in the presence of light (TAIZ; ZEIGER, 2013). This difference of plant physiological activity, at different times in the duration of the day, can affect the performance of applications, most likely due to the absorption and translocation of the fungicide.

Nighttime applications are increasingly being used to optimize the time and the window of applications. However, it is uncertain as to how much the absence of light interferes with the effectiveness of fungicides. In addition, lower temperatures, high humidity, and the occurrence of dew in the night, may cause dilution or bleeding of the applied solution (KOGAN; ZÚÑIGA, 2001).

Among all the environmental factors, it is seen that rainfall exerts the greatest influence on residual activity and the effectiveness of fungicides applied on leaves. Based on this, our experiment aimed to assess the effect of the interaction between fungicide application at different times and rainfall simulation intervals on Asian Soybean Rust (ASR) control effectiveness.

## Material and Methods

*Experiment I*

*Growth and management of plants*

The experiment was conducted in the summer of 2012–2013 in the municipality of Itaara, Rio Grande do Sul, Brazil, in partially controlled conditions in the greenhouse. The minimum temperature recorded at night was 16 °C and the maximum temperature during the day was 29 °C, with the maximum regulated through hoods. Relative humidity was controlled by a computerized moisturizing system and maintained at 65 to 90%. The experiment included seeds from cultivar NA 6411 RG, treated with fipronil + pyraclostrobin + thiophanate-methyl (50 + 5 + 45 g a.i. 100 $kg^1$ of seed, respectively) and inoculated before seeding with *Bradyrhizobium japonicum*.

Seeds were sown in pots (5 L) containing soil base substrate + sand (2:1). The chemical composition of the substrate was: pH ($H_2O$) – 4.3, base saturation (V) – 24.1%, cation exchange capacity (CEC) $pH_7$ – 18.0 cmolc $dm^{-3}$, organic matter – 2.7%. The physical fractions of soil; sand, silt and clay were 60.8, 20.3 and 18.9%, respectively. Both pH correction and fertilization were conducted according to the chemical analysis of substrate.

*Treatments and experimental design*

The experiment was conducted in a completely randomized design, in factorial arrangement 6x5, totaling 30 treatments with four replications. Each replicate consisted of a pot with one plant. Factor A comprised five fungicide applications at different times: 0400 h, 0900 h, 1400 h, 1800 h, 2300 h, and a control free of fungicide; Factor B included four time intervals between fungicide application and washing of leaves by rainfall simulation: 0, 30, 60, 120 min and a control with no rainfall. The fungicide used for the treatment was a mixture of trifloxystrobin + prothioconazole (60.0 + 70.0 g a.i. $ha^{-1}$) and soybean oil methyl ester (270 g a.i. $ha^{-1}$).

*Application of treatment and pathogen inoculation*

Fungicide application was carried out when plants reached the R4 stage (FEHR; CAVINESS, 1977), as prevention, with no symptoms of disease. The application was conducted with a knapsack sprayer pressurized with $CO_2$ and equipped with a bar with four spray nozzles (type XR 11002) at a distance of 0.5 m from one another. A fine droplet spectrum was achieved for the application at a speed of 5.4 km $h^{-1}$ and 30 psi pressure in the spray nozzles, providing an application rate of 150 L $ha^{-1}$.

The weather conditions at the time of application are shown in Table 1. After the treatment, rainfall simulation was made at intervals at 0, 30, 60, 120 min, respectively to induce shoot washing and removal of the product not absorbed by the leaflets, using a simulator developed in accordance with Debortoli (2008). The simulator produced a rainfall intensity of 255 mm $h^{-1}$. The equipment worked for four minutes at each interval, totaling a rainfall simulation of 17 mm.

*Phakopsora pachyrhizi* was artificially inoculated onto both sides of the leaves, 12 hours after the last fungicide application time. Inoculation was based on the methodology used by Lenz et al. (2011). Urediniospores of *P. pachyrhizi* were at the concentration of 2 $x10^5$ spores $ml^{-1}$ of distilled water plus Tween adhesive spreader (80 ppm).

**Table 1.** Application time, date, soybean development stage, temperature (Temp.), relative humidity (RH), wind speed and dew at each application time in greenhouse and field conditions. Itaara, 2013.

| Application Time | Date | Development stage | Temp. (°C) | RH (%) | Wind speed (km $h^{-1}$) | Dew |
|---|---|---|---|---|---|---|
| ---------- Greenhouse ---------- | | | | | | |
| **1400 h** | 22 mar. 2013 | R4[1] | 26.5 | 42.4 | 1.8 | absent |
| **1800 h** | 22 mar. 2013 | R4 | 25.0 | 60.3 | 1.3 | absent |
| **2300 h** | 22 mar. 2013 | R4 | 19.8 | 76.1 | 1.2 | absent |
| **0400 h** | 23 mar. 2013 | R4 | 16.8 | 86.2 | 0.7 | absent |
| **0900 h** | 23 mar. 2013 | R4 | 24.5 | 66.4 | 1.0 | absent |
| ---------- Field ---------- | | | | | | |
| **1400 h** | 07 mar. 2013 | R2 | 34.1 | 39.1 | 3.1 | absent |
| **1800 h** | 06 mar. 2013 | R2 | 25.2 | 55.6 | 2.5 | absent |
| **2300 h** | 06 mar. 2013 | R2 | 19.4 | 76.2 | 2.3 | absent |
| **0400 h** | 07 mar. 2013 | R2 | 16.9 | 88.2 | 2.7 | present |
| **0900 h** | 07 mar. 2013 | R2 | 20.9 | 81.5 | 2.9 | absent |

[1]Soybean development scale proposed by Fehr and Caviness (1977).

*Assessments*

The last two fully expanded leaflets of each plant were marked with colored tape and repeatedly evaluated. The number of days to the appearance of the first pustules (NDAFP) was recorded. In order to do so, daily observations were made from the second day of inoculation using a 20X magnifier to examine the initial symptoms. This methodology also allowed for estimation of the fungicide residual.

At 30 days after the application of the fungicide, the severity of ASR was assessed, along with relative chlorophyll index (RCI). Severity was determined by assigning visual scores of percentage of diseased area versus the healthy areas, using the scale proposed by Godoy et al. (2006). The control effectiveness was determined by comparing the fungicide treatments severity scores compared to the control without fungicide. RCI was calculated based on indexes generated by a SPAD-502® portable chlorophyll measurer (Konica Minolta Sensing Americas, Inc., New Jersey, United States of America), with the mean of 10 readings per trefoil. The productivity of the plants was determined in g plant$^{-1}$ from the harvesting and manual threshing of pods of each plant and grain moisture adjusted to 13%.

*Experiment II*

*Growth and management of plants*

The second experiment was conducted under field conditions during the 2012-2013 harvest in the municipality of Itaara, Rio Grande do Sul, Brazil, latitude 29° 35'10.2" S, longitude 53° 48'36.6" W, altitude of 451 m. The soil was a typical eutrophic litholic neosol and the climate, according to the Köppen classification, was humid subtropical. Seeds used in the field were from cultivar FCEP 64 RR treated in accordance with experiment I. The seeding rate was 16 seeds m$^{-1}$ at a spacing of 0.5 m, resulting in an average population of 280,000 plants ha$^{-1}$. A 300 kg ha$^{-1}$ NPK base fertilization was used, along with formula 04-24-18.

*Treatment and experimental design*

The experimental design, as well as levels for factors A and B, were the same as described for experiment I, totaling 30 treatments and four replications, in a randomized block design with split plots. The plot consisted of factor A, application time, and the subplot consisted of factor B, time intervals for rainfall simulation. The experimental units consisted of six sowing lines spaced at 0.5 m. The length of the lines was five meters, with a total of 15 $m^2$. Assessments disregarded the two outer lines and 0.5 m from each end, covering an area of 8 $m^2$.

*Application of treatments and pathogen inoculation*

The application of fungicide at different times in a day, levels of factor A, was done preventively, when the plants were in the phenological stage of full flowering (R2) (FEHR; CAVINES, 1977). At 21 and 42 days after treatments, fungicide applications without rain simulation were done in the entire area. Thus, the results obtained in the experiment were affected only by the first application. The method employed and fungicide compositions were the same as described for Experiment I. The environmental conditions at the time of application are shown in Table 1.

After the application of the fungicide, considering the different times of application, rainfall was simulated at 2, 30, 60, 120 min, respectively and a control was not subjected to simulated rainfall. Due to the methodology used in the field, it was not possible to simulate rain immediately (0 min) after application, thus, rainfall was simulated at 2 min after application. For field rainfall simulation, we used micro sprinklers installed in PVC pipes (32 mm) suspended from a wooden structure. The micro sprinklers were spaced at 1.5 m and positioned at 1.6 m from the soil surface. Pressure was adjusted manually by a switch in the pump outlet, and the intensity set at 51 mm $h^{-1}$. The equipment worked for 20 minutes at each rainfall simulation period, totaling 17 mm. Disease in the field occurred naturally.

*Assessments*

The severity assessments to determine the control effectiveness and RCI were performed at 21 days after application (DAA). The control effectiveness was determined through fungicide treatment severity scores and compared to a control, without fungicide. In both experimental conditions, in the greenhouse and in the field, the disease severity was determined by visual scores for the percentage of diseased leaf area using the scale proposed by Godoy et al. (2006). The RCI was determined through the mean of 20 readings per plot at random, considering the median third of the plants. Productivity was determined by the harvesting plants from the useful area of each experimental unit. The volume of grain obtained was weighed and moisture adjusted to 13% for final yield (kg $ha^{-1}$).

*Statistical analysis*

The variables; the number of days to the appearance of the first pustules, ASR control effectiveness, RCI, and productivity were submitted to an analysis of variance (ANOVA), and the interaction between the factors and their means were compared by Tukey's test ($P \leq 0.01$), using the statistical package Assistat® version 7.7 Beta (SILVA; AZEVEDO, 2002).

## Results and Discussion

The analysis of variance of variables number of days to appearance of the first pustule (NDAFP) in greenhouse, ASR control effectiveness, relative chlorophyll index (RCI), and productivity in both the greenhouse and the field experiments showed significance in interaction between the factors.

The number of days to the appearance of the first pustule (NDAFP) is an important variable to estimate the fungicide control residue (Table 2). In general, the greater the time interval from the application of the fungicide and rain simulation, the greater is the number of days for the appearance of symptoms in the plant. Measures to ensure a delay in the onset of the first symptoms represent a definite step in controlling the disease.

**Table 2.** Number of days to the appearance of the first pustule of *Phakopsora pachyrhizi* on soybean leaves at each application time and rainfall simulation interval after application in greenhouse conditions. Itaara, 2013.

| Application time | Rainfall simulation intervals[1] | | | | |
|---|---|---|---|---|---|
| | 0' | 30' | 60' | 120' | No rain[2] |
| **0400 h** | 9.00 aE[4] | 10.63 cD | 13.00 cC | 18.00 bB | 20.00 cA |
| **0900 h** | 9.38 aE | 13.63 aD | 14.75 aC | 20.25 aB | 22.00 aA |
| **1400 h** | 9.38 aE | 12.63 bD | 13.88 bC | 17.00 cB | 19.00 dA |
| **1800 h** | 9.50 aE | 12.63 bD | 14.25 aC | 20.00 aB | 21.00 bA |
| **2300 h** | 9.00 aE | 10.88 cD | 13.00 cC | 18.00 bB | 21.00 bA |
| **Control[3]** | 7.13 bA | 7.25 dA | 7.13 dA | 7.00 dA | 7.00 eA |
| C.V. (%) = 1.80 | | | | | |

[1]0', 30', 60', 120' time in minutes elapsed between fungicide application and rainfall simulation; [2]No rain: Control without rainfall; [3]Control: without fungicide; [4]Lowercase letters in column (compare application times in each combination of rainfall simulation intervals); uppercase letters in lines (compare rainfall simulation intervals in each combination of application time); means followed by the same letters do not differ by Tukey's test ($P \leq 0.01$).

Taking into consideration the rainfall simulation intervals at 30 and 60 min, a delay was observed in the appearance of the first pustules in treatments with application of fungicide in the presence of light (0900 h, 1400 h and 1800 h) as compared to the applications at night (0400 h and 2300 h) (Table 2). This suggests that leaf fungicidal absorption occurs faster in the presence of light, ensuring greater product residue. Thus, rainfall simulation allows for inferences on speed of product absorption and its persistence on the leaves and optimizes estimation of residual activity. Control of residue of fungicides is highly correlated with the rate of leaf absorption (WANG; LIU, 2007).

Currently, there is a great deal of concern on the effectiveness of products in nighttime applications. This study reveals that fungicide absorption in the applications conducted at night, is slower than in daytime applications. Such nocturnal applications are more susceptible to the damaging effects of rain due to slower absorption. However, when there is no rain after night application, the ASR control residual is seen to be greater than the application at 1400 h, similar to the residual at 1800 h and lower than at 0900 h.

Because of plant water loss by transpiration, the dynamics of leaf fungicide absorption are also subject to oscillations throughout the day. Solar radiation is one of the key environmental factors affecting transpiration rate (MEDRANO et al., 2005). Water content in intercellular spaces is reduced with transpiration, establishing a water concentration gradient between the inner leaf and the sprayed droplets on its surface. This concentration difference is assumed to increase product absorption in the leaf. This justifies the increased rate of absorption in applications throughout the day. At night, the leaves recover turgidity when the temperature is lower, humidity is slightly higher, and wind velocity is low (TAIZ; ZEIGER, 2013) reducing fungicide absorption because of a lack of a concentration gradient.

The daytime (0900 h, 1400 h, and 1800 h) temperatures in the greenhouse, were 24.5, 26.5 and 25.0 °C, respectively, much higher than the temperatures recorded at night (2300 h and 0400 h) (Table 1). Leaf absorption of fungicide is affected by temperatures during applications. Moderately warm temperatures stimulate foliar absorption by increasing the rate of physiological processes, such as photosynthesis and translocation in the plant (CURRIER; DYBING, 1959). In the absorption process, the temperature is usually expressed as the diffusion activation energy. The diffusion activation energies are interpreted as the energy needed to produce free volume in a sufficiently large polymer to accommodate the diffusion molecule (BAUR et al., 1997). Therefore, temperature is an important factor because it affects the chemical reactions and physical properties of cells, organs and the whole plant (GRUDA, 2005).

In the application conducted at 1400 h (26.5 ºC and relative humidity of 42.4%) with no simulation of rainfall, there was a reduction of the fungicide control residual in two and three days when compared to applications made at 1800 h and 0900 h, respectively (Table 2). Both in the greenhouse and field conditions, the application made at 1400 h showed less ASR control effectiveness compared to other application times (Table 3). This might be because the beneficial effects of temperature are recorded up to a threshold value and are dependent on other parameters, such as moisture. The effect of high temperature associated with low moisture, as seen in the 1400 h applications, can limit the absorption rate due to the rapid drying of the solution and minimum levels of cuticle hydration (CURRIER; DYBING, 1959).

In study has been observed that relative humidity is an important factor for effective control of disease. An increase of relative humidity from 50% to 90% is seen to double the $CaCl_2$ penetration rate constants (SCHÖNHERR, 2000), increase the IPA-glyphosate penetration rate by a factor of seven when increased from 70% to 100% (SCHÖNHERR, 2002). High humidity also enhances the transport capacity of the penetration of hydrophilic solutes by increasing size or number of polar pores (SCHÖNHERR, 2001).

The data of application at 0900 h reveal a greater delay in the onset of pustules of ASR in the greenhouse (Table 2) and more ASR control effectiveness in the field (Table 3). With the exception of simulation at 0 min (greenhouse) and at 2 min (field), the most ASR control effectiveness occurred in this fungicide application in all rainfall simulation levels. It is possible therefore, that the plants in the 0900 h are physiologically more active or more pliant to spraying. Nonetheless, the environmental conditions of temperature and humidity prevailing at this time prove favorable to fungicide applications (Table 1).

The ASR control effectiveness in the interaction between application time and rainfall simulation intervals after application at 30 DAA in greenhouse and at 21 DAA in field conditions is shown in Table 3. The most ASR control effectiveness is shown for applications made in daylight with subsequent rainfall simulations compared to nighttime applications.

In the field, the disease control effectiveness was negatively influenced by rain simulation after the application at 0400 h in the presence of dew compared to other times of application. The presence of dew eases flow of product and hence removal from the leaves. Dew also contributes to slow diffusion of the fungicide into the plant by decreasing the concentration gradient of water between the inner leaf and the sprayed droplets on the leaf surface. The duration of the wetting period and the amount of dew on the leaves affect the behavior and the leaf absorption of phytosanitary treatments (BAKER; HUNT, 1985).

**Table 3.** Asian Soybean Rust control effectiveness (%) at each application time and rainfall simulation interval after application at 30 DAA in greenhouse and at 21 DAA in field conditions. Itaara, 2013.

| Application time | Greenhouse (%) — Rainfall simulation intervals[1] 0' | 30' | 60' | 120' | No rain[2] |
|---|---|---|---|---|---|
| **0400 h** | 32.9 cE[3] | 56.6 cD | 75.0 cC | 80.6 cB | 88.8 bcA |
| **0900 h** | 40.8 bE | 67.1 aD | 85.5 aC | 90.8 aB | 95.0 aA |
| **1400 h** | 44.1 aD | 61.8 bC | 80.9 bB | 83.6 bB | 87.5 cA |
| **1800 h** | 39.5 bE | 61.8 bD | 80.9 bC | 86.2 bB | 91.2 bA |
| **2300 h** | 35.5 cE | 59.2 bcD | 78.3 bC | 83.6 bB | 91.1 bA |
| | C.V.(%) = 1.99 | | | | |
| **Field (%)** | **2'** | **30'** | **60'** | **120'** | **No rain[2]** |
| **0400 h** | 3.3 cE[3] | 7.2 eD | 12.9 eC | 18.2 eB | 74.5 cA |
| **0900 h** | 14.7 aD | 51.9 aC | 70.6 aB | 80.4 aA | 81.2 aA |
| **1400 h** | 14.2 aD | 37.0 cC | 57.8 cB | 69.9 cA | 70.5 dA |
| **1800 h** | 14.3 aE | 42.6 bD | 62.4 bC | 74.4 bB | 78.2 bA |
| **2300 h** | 6.8 bE | 16.2 dD | 27.7 dC | 43.3 dB | 76.3 bcA |
| | C.V.(%) A = 2.25 C.V.(%) R = 2.78 | | | | |

[1]0' or 2', 30', 60', 120' time in minutes elapsed between fungicide application and rainfall simulation; [2]No rain: Control without rainfall; [3]Lowercase letters in column (compare application times in each combination of rainfall simulation intervals); uppercase letters in lines (compare rainfall simulation intervals in each combination of application time); means followed by the same letters do not differ by Tukey's test ($P \leq 0.01$).

In addition, a comparison in disease control effectiveness between the applications at 2300 h with the application at 0400 h in the field experiment showed the influence of dew in the behavior of nocturnal applications. The data suggest that at night, with the presence of dew, leaf fungicidal absorption can be even slower than application performed overnight without the presence of dew. When a large volume of spray is applied in leaves wet by rain or dew, there is a greater reflection potential, which decreases the retention of the product (ZABKIEWICZ, 2000).

As expected, the simple simulation of rainfall on the control groups that received no application of fungicide at each level of rainfall simulation had no effect on the development of the disease. Considering the ASR control effectiveness, data show that the negative effect of rainfall is directly related to the interval between the application and its occurrence. Even the rainfall simulation at 120 min after the applications at different times had the ability to affect the ASR control effectiveness. Here, the 120 min rainfall after application affected the retention of the product on the leaf and was expressed by the lower control effectiveness values compared to treatment with no rainfall, in both experiments (greenhouse and field). Rainfall affects the removal of fungicide from crop foliage (FIFE; NOKES, 2002) and consequently affects the ASR control effectiveness.

The occurrence of rainfall immediately after the application of the fungicide (0 or 2 min) also showed disease control effectiveness. This indicates that some of the applied active ingredient is readily absorbed by plant tissue. These results are in agreement with Lenz et al. (2011), who compared the droplet spectrum and leaflets age on the rate of absorption and fungicide residual effect in soybean. However, in the field, at 2, 30, 60, and 120 min rain simulation after application at 0400 h, we observed a decrease in control of effectiveness compared to the other application times. This confirms the negative effects of dew, on absorption rate of fungicide.

Rainfall and dew are climatic factors that require attention at the time of application. The minimum time interval between the application and the occurrence of rainfall must be maintained in order to allow for absorption of active ingredients by foliage. The occurrence of rainfall can affect the structure and activity of the product by means of dilution, redistribution, physical removal or removal from plant tissue (THACKER; YOUNG, 1999).

By observing the data for relative chlorophyll index (RCI), through rainfall simulations after applications, it is possible to elicit higher values for applications made during the day as compared to those carried out at night (Table 4). The simulation of rainfall shortly after the applications in the dark has a great negative impact on control effectiveness, providing greater severity values of the disease in leaves which leads to smaller RCI values.

**Table 4.** Relative chlorophyll index on soybean leaves at each application time and rainfall simulation interval after application at 30 DAA in greenhouse and at 21 DAA in field conditions. Itaara, 2013.

| Application time | Rainfall simulation intervals[1] | | | | |
|---|---|---|---|---|---|
| **Greenhouse** | | | | | |
| | **0'** | **30'** | **60'** | **120'** | **No rain[2]** |
| **0400 h** | 17.1 bcD[4] | 20.0 bD | 23.5 bC | 27.4 cB | 32.0 abA |
| **0900 h** | 20.1 abD | 25.1 aC | 29.7 aB | 33.1 aA | 34.6 aA |
| **1400 h** | 19.3 abC | 24.4 aB | 28.5 aA | 31.8 abA | 30.1 bA |
| **1800 h** | 21.2 aC | 24.2 aC | 29.1 aB | 32.0 aAB | 33.5 abA |
| **2300 h** | 17.1 bcD | 19.3 bD | 23.3 bC | 28.5 bcB | 32.0 abA |
| **Control[3]** | 14.4 cA | 14.4 cA | 14.5 cA | 14.5 dA | 14.0 cA |
| C.V.(%) = 6.76 | | | | | |
| **Field** | | | | | |
| | **2'** | **30'** | **60'** | **120'** | **No rain[2]** |
| **0400 h** | 13.13 bD[4] | 13.38 cD | 15.48 cC | 17.13 dB | 25.65 bcA |
| **0900 h** | 15.70 aE | 20.65 aD | 23.65 aC | 26.50 aB | 29.75 aA |
| **1400 h** | 15.25 aD | 20.05 aC | 22.93 aB | 24.25 bAB | 24.60 cA |
| **1800 h** | 15.38 aE | 20.73 aD | 23.48 aC | 25.08 abB | 27.08 bA |
| **2300 h** | 13.25 bE | 16.15 bD | 18.58 bC | 21.80 cB | 26.33 bA |
| **Control[3]** | 13.05 bA | 12.88 cA | 12.48 dA | 13.15 eA | 12.93 dA |
| C.V.(%) A = 4.67 C.V.(%) R = 4.17 | | | | | |

[1]0' or 2', 30', 60', 120' time in minutes elapsed between fungicide application and rainfall simulation; [2]No rain: Control without rainfall; [3]Control: without fungicide; [4]Lowercase letters in column (compare application times in each combination of rainfall simulation intervals); uppercase letters in lines (compare rainfall simulation intervals in each combination of application time); means followed by the same letters do not differ by Tukey's test ($P \leq 0.01$).

The application conducted at 1400 h without rain showed the lowest RCI values in both greenhouse and the field conditions. This is the outcome of application at highest temperature and lowest humidity, resulting in lower disease control compared to the other application times. At rainfall simulations at 30 and 60 min after application of fungicide in the greenhouse and the field conditions, larger RCI values were observed in soybean leaves for applications made during the day. This greater RCI is due to the higher disease control effectiveness in leaves. As a result, it provides better control of the disease by delaying the appearance of the first pustule of *P. pachyrhizi* and thus, lowers disease severity, prolonging the green leaf tissue. A study that assesses the action of defense activator and foliar fungicide on the control of ASR and on yield and quality of soybean seeds has also found that the highest chlorophyll content was related to the lowest number of injuries caused by the pathogen (CARVALHO et al., 2013).

Productivity in soybean plants under fungicide applications at different times can be seen in Table 5. In the greenhouse, reduced productivity was found in plants with fungicide application at 0400 h and 2300 h and rainfall simulations at 30 and 60 min after application compared to the applications at 0900 h, 1400 h and 1800 h. This same phenomenon can be observed in the field experiments at 2, 30, 60, and 120 min of rainfall simulation after application.

**Table 5.** Soybean yield in greenhouse and field conditions at each application time and rainfall simulation intervals after application. Itaara, 2013.

| Application time | Greenhouse (g plant$^{-1}$) — Rainfall simulation intervals[1] | | | | |
|---|---|---|---|---|---|
| | 0’ | 30’ | 60’ | 120’ | No rain[2] |
| **0400 h** | 7.79 bC[4] | 8.31 bC | 9.24 bB | 10.16 aA | 10.20 aA |
| **0900 h** | 8.46 aC | 9.29 aB | 9.95 aA | 10.32 aA | 10.36 aA |
| **1400 h** | 8.42 aC | 9.27 aB | 9.87 aA | 10.19 aA | 10.18 aA |
| **1800 h** | 8.41 aC | 9.26 aB | 9.84 aA | 10.21 aA | 10.26 aA |
| **2300 h** | 7.99 abC | 8.46 bC | 9.24 bB | 10.16 aA | 10.31 aA |
| **Control[3]** | 7.60 bA | 7.58 cA | 7.52 cA | 7.51 bA | 7.62 bA |
| C.V.(%) = 3.10 | | | | | |
| | **Field (kg ha$^{-1}$)** | | | | |
| | 2’ | 30’ | 60’ | 120’ | No rain[2] |
| **0400 h** | 1806.96 bC[4] | 1821.50 bcC | 1869.50 dC | 1993.35 dB | 2407.76 abA |
| **0900 h** | 2020.16 aD | 2135.94 aC | 2311.56 aB | 2471.59 aA | 2506.42 aA |
| **1400 h** | 2025.65 aC | 2087.63 aC | 2186.12 bB | 2333.70 bA | 2345.55 bA |
| **1800 h** | 2021.36 aC | 2128.56 aB | 2222.49 abB | 2393.28 abA | 2464.13 aA |
| **2300 h** | 1834.91 bC | 1907.57 bC | 2076.84 cB | 2155.47 cB | 2441.33 abA |
| **Control[3]** | 1783.82 bA | 1804.06 cA | 1806.42 dA | 1799.03 eA | 1804.06 cA |
| C.V.(%) A = 2.62 C.V.(%) R = 2.26 | | | | | |

[1]0’ or 2’, 30’, 60’, 120’ time in minutes elapsed between fungicide application and rainfall simulation; [2]No rain: Control without rainfall; [3]Control: without fungicide; [4]Lowercase letters in column (compare application times in each combination of rainfall simulation intervals); uppercase letters in lines (compare rainfall simulation intervals in each combination of application time); means followed by the same letters do not differ by Tukey’s test ($P \leq 0.01$).

The rainfall simulation in different time intervals after the application of the fungicide reduced the control effectiveness in leaves in the dark, enabling entry of pathogen by reducing residual disease control which in turn, increased the severity of disease in the plants. The damage observed by foliar diseases results from the injury and necrosis of plant tissue that limits the interception of solar radiation resulting in reduced grain yield (BORDIN et al., 2014).

In applications conducted at 0400 h and 2300 h, with subsequent simulation of rainfall at zero min in the experiment in greenhouse and at 2 min in the experiment in the field, there was no increase in plant productivity over the control. However, when comparing the value of the control with the values of applications carried out in sunlight, on the same simulations, there was increased productivity on applications made during the day.

When analyzing productivity data in the field experiment, it is possible to note differences among nighttime applications and subsequent rainfall simulation. The data suggest that the rainfall simulations after the application at 0400 h, with the occurrence of dew, further reduces the fungicide, providing lower ASR control effectiveness than application at 2300 h, without dew. This lower control effectiveness in treatment with application at 0400 h reduced the productivity as compared with the application at 2300 h. Mueller et al. (2009) reported that soybean rust is a devastating foliar disease of the soybean plant that may cause significant yield losses if not managed by well-timed fungicide applications.

Knowledge of the disease control effectiveness of fungicide applications and the effect of rainfall is essential to optimize their use. This envisages the precise estimation of their residual activity and helps establish parameters to define the reapplication after rainfall.

## Conclusions

In sunlight, the Asian Soybean Rust control effectiveness of fungicide applications in soybean plants was less affected by rainfall simulation. The rainfall simulation had greater negative impact on disease control effectiveness in applications conducted at night under dew conditions. The application conducted at 0900 h showed the greatest disease control effectiveness in both the greenhouse as well as the field conditions. The 1400 h application time showed decreased fungicide control residual and ASR control effectiveness, possibly due to the combination of the low relative humidity and high temperature. Rainfall simulation carried out at 120 min after application still had the ability to affect the ASR control effectiveness.

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