ARTIGOS

Curative and eradicant action of fungicides to control *Phakopsora pachyrhizi* in soybean plants

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RESUMO

Reis, E. M.; Zanatta, T.; Zanatta, M. Ação curativa e erradicativa de fungicidas à *Phakopsora pachyrhizi*, em soja. ***Summa Phytopathologica,*** v.42, n.4, p.295-302, 2016.

Em experimentos conduzidos em câmara-de-crescimento e laboratório quantificou-se a ação curativa e erradicante de fungicidas no controle da ferrugem asiática da soja. Os experimentos foram conduzidos com a cultivar CD 214 RR e avaliados os fungicidas isoladamente ou em misturas, clorotalonil, flutriafol, ciproconazol + trifloxistrobina, epoxiconazol + piraclostrobina, ciproconazol + azoxistrobina, ciproconazol + picoxistrobina. Os fungicidas foram aplicados quatro dias após a inoculação (curativo) e nove dias após a inoculação (erradicante). Avaliou-se o efeito dos tratamentos em função da densidade de lesões e de urédias/cm^2 e, no erradicante, a necrose das lesões/urédias e a viabilidade dos uredosporos. Com exceção do fungicida clorotalonil, houve ação curativa sobre as infecções latentes/virtuais pelos demais fungicidas. Os fungicidas penetrantes que são absorvidos tem ação curativa e erradicante na ferrugem da soja.

Palavras-chave: Ação fungicida, controle químico, infecções latentes, ferrugem asiática da soja,

ABSTRACT

Reis, E. M.; Zanatta, T. Curative and eradicant action of fungicides to control *Phakopsora pachyrhizi* in soybean plants. ***Summa Phytopathologica,*** v.42, n.4, p.295-302, 2016.

Experiments were carried out in a growth chamber and laboratory to quantify the curative and eradicant actions of fungicides in Asian soybean rust control. The experiments were conducted with the CD 214 RR cultivar, assessing the following fungicides, separately or in association, chlorothalonil, flutriafol, cyproconazole + trifloxystrobin, epoxiconazole + pyraclostrobin, cyproconazole + azoxystrobin, and cyproconazole + picoxystrobin. The fungicides were applied at four (curative) and nine days after inoculation (eradicant treatment). Treatments were evaluated according to the density of lesions and uredia/cm^2, and the eradicant treatment was assessed based on the necrosis of lesions/uredia and on uredospore viability. Except for the fungicide chlorothalonil, there was curative action of latent/virtual infections by the fungicides. Penetrant fungicides that are absorbed have curative and eradicant action to soybean rust.

Keywords: Fungicide action, chemical control, latent infection, Asian soybean rust.

The Brazilian soybean [*Glycine max* (L.) Merr.] crop has been threatened by several diseases which attack the plant at various development stages (3). Soybean monoculture under no tillage increases the intensity and the damage of diseases caused by necrotrophic parasites.

Emergence of Asian soybean rust (ASR) in Brazil from the 2000/01 season (23) demanded more investment and research into sustainable solutions for its control.

ASR is caused by the basidiomycete fungus *Phakopsora pachyrhizi* Sydow & Sydow. This disease is distinct from the other rusts since it forms necrotic lesions on the host leaves and numerous uredia in each lesion; the spores are hyaline and the fungus penetrates directly through the cuticle (16).

According to Ogle et al. (13), damage caused by ASR is due to a reduction in the number of pods, number of grains, and weight caused by premature grain defoliation. Some published data have estimated the potential damage caused by rust, which can vary from 10 to 90% (19) and, in Brazil, from 30 to 75% (3, 23). Using a scientific methodology, Danelli et al. (5) generated the damage functions to estimate the damage according to the disease intensity in any growth stage: Y = 1,000 – 5.84 (4.53 – 9.02) LI (where y = grain yield normalized to 1,000 kg/ha; LI = central leaflet incidence).

Cultivars with a genetic resistance level sufficient to prevent damage are not available yet. Therefore, control is based on the use of fungicides on aerial organs (17).

Although without scientific basis, several criteria pointing to the beginning of ASR chemical control have been proposed (14). Among them is the preventive onset of diseases based on the application according to the phenological stage (e.g., R1) and pre-closing planting rows. As these criteria are empirical, we searched in the literature for the definition of some of these terms. For example, preventive, curative and eradicant are related to the fungicide action in the infectious process and not to the criteria for decision making. According to Hewitt (7), preventive action occurs when the fungicide is applied on healthy tissues (pre-penetration), curative action when it is applied after the penetration of the fungus, but without the occurrence of symptoms/

signs (pre-symptom/sign), and the eradicant action occurs when the fungicide is applied after symptom manifestation (post- symptoms/signs). In the present study, we have adopted fungicide action concepts according to Hewitt (7).

The pioneering studies of Godoy & Canteri (6) and Viero & Forcelini (22) brought information about the action of fungicides in ASR control. Nevertheless, many doubts still persist regarding this issue.

Moreover, there is not enough information about the fungicide action on latent infections and its eradicant action on sporulant uredinia. The aim of this study was to quantify the action of a non-penetrant and penetrant fungicides on the infection process of *P. pachyrhizi* in soybeans.

MATERIAL AND METHODS

The experiments were conducted in growth chambers (25ºC and 12 h photoperiod) at the Faculty of Agronomy and Veterinary Medicine, University of Passo Fundo, Passo Fundo, Brazil.

Soybean cultivar. Seeds of soybean cultivar CD 214 RR were weekly sown in polyethylene pots containing 2.0 kg of Tenomax substrate (Ferticel) and maintained in a growth chamber for plant development. Two seeds were placed in each of five holes of 2.0 cm depth which were opened in the substrate. After emergence, seedlings were thinned to five per pot. Plants were inoculated at V3-V4 growth stages (18).

Inoculum. *P. pachyrhizi* uredospore suspension was obtained from soybean leaflets with uredia in abundant sporulation collected from the experimental field of University of Passo Fundo. The leaflets were introduced in a disposable plastic bottle of 500 mL volume containing distilled water and the spreader polyoxyethylene sorbitan monolaurate (Tween 20 – Synth, 240 **µ**L/L water). The bottle was manually shaken to remove uredospores. The uredospore concentration was adjusted after assessing the spore concentration by means of scanning a 10-µL drop under an optical microscope.

For the inoculum maintenance, plants were weekly inoculated with 4 x 10^4 uredospores/mL by using a manual atomizer. Inoculated plants were kept in the dark for 24 hours at 21ºC ± 2 and 24h leaf wetness duration; subsequently, plants were transferred to another growth chamber at 12 h photoperiod and 23 ± 2ºC.

Curative fungicide action. The curative fungicide application (Table 1) on latent infection was performed at four days after inoculation, after penetration and pre-symptom phases. As strobilurins and triazoles are penetrating fungicides, their action was compared to that of a non-penetrating protectant fungicide, chlorothalonil.

Plants were inoculated with 5 x 10^3 uredospores/mL, determined in previous experiments.

Fungicides were sprayed at a rate of 1,000 L/ha, up to run-off, in order to ensure complete leaf coverage, avoiding areas without protection, which could compromise the reliability of results. The application was carried out with a manual atomizer equipped with a cone-type nozzle. Each fungicide was applied onto plants in 10 replicates (10 pots containing five plants each). Central leaflets of the second trifoliate were marked in V3 - V4 stage (18), which safely received the fungicide.

The curative effect of fungicides was quantified based on five leaflets per replicate at 10, 14, 18, 22 and 26 days after inoculation. The disease was quantified as the number of lesions and uredia in two circles of 0.9 cm diameter per leaflet, marked on the central region of each leaflet. Data were expressed as lesion and uredinium number/cm^2. Only previously marked central leaflets that received inoculation and fungicide deposition were evaluated.

Experimental design was in randomized blocks and the units consisted of a pot containing five plants with 10 replicates. Data were subjected to analysis of variance and means compared according to Tukey's test at 5% probability and integrated as the area under the disease progress curve: $\Sigma = (x1 + x2) / 2 * (t2 - t1)$, where x is the intensity of the disease in each interval between assessments; they were subjected to analysis of variance and means compared according to Tukey's test at 5% probability.

Fungicide eradicant action. In this experiment, the same methodology of the previous one was used. For the eradicant effect, application was performed at nine days after inoculation (post-symptom/sign phase) (7) and applied as described for the previous experiment. Only the central leaflets of each leaf in the second trifoliate stage V3 - V4 were assessed (18).

The eradicant activity of the fungicides was measured based on five leaflets per plants at 2, 6, 10, 14, 18, 22 and 26 days after application. The number of lesions and uredinium/cm^2 (health/necrotic) in two leaflet areas, with 0.9 cm diameter marked on the central portion of each leaflet separated from the main rib, was quantified.

Experimental design was arranged in randomized blocks with 10 replicates (pots containing five plants).

The eradicant effect was also quantified based on the effect of fungicides on uredospore germination at 2, 6, 10, 14, 18, 22 and 26 days after fungicide treatments. Five leaflets were collected at random per treatment at each evaluation time. The leaflets were introduced into disposable plastic bottles of 500 mL volume containing 100 ml of distilled water and stirred for uredospore release. A 500-µL suspension was transferred to a Petri plate containing water-agar medium. The plates were incubated in the dark at 24°C for 6 hours. Viability

Table 1. Fungicides, formulation, concentration, and adjuvants.

Technical name	Formulation	Concentration a.i. (g)	Commercial formulation (L or kg/ha)	Adjuvant (L)
Chlorothalonil	SC	1.7	2.0	0.0
Flutriafol	CE	62.5	0.5	2.0[w]
Cyproconazole + trifloxystrobin	SE	56.3 + 24.0	0.3	0.5[x]
Epoxiconazole + pyraclostrobin	SC	66.5 + 28.0	0.5	0.05[y]
Cyproconazole + azoxystrobin	SC	60.0 + 24.0	0.3	0.6[z]
Cyproconazole + trifloxystrobin	CE	56.3 + 24.0	0.3	0.5[z]

a.i. – grams of active ingredient; [w]Mineral oil, Oppa; [x]Methylated soybean oil, Áureo; [y]Siliconate adjuvant Break Thru; [z]Mineral oil, Nímbus.

was quantified under an optical microscope, and those uredospores presenting the germ tube larger than the largest spore diameter were considering germinated.

The experiment was repeated twice using the same methodology. Data were subjected to analysis of variance and, when significant, subjected to linear regression analysis.

RESULTS AND DISCUSSION

In recent years, especially with the emergence of ASR, the term curative has been used by farm advisers and some researchers to time fungicide application, unlike the concept proposed by Hewitt (7). Therefore, the three fungicide actions, protectant, curative and eradicant, are distinct and should not be applied to any other sense as the criterion for fungicide application. The fungicide action on the infectious process, in field spraying, is a consequence of the coincidence with the time of application and the infectious phase present at that same moment.

The tested fungicides were those that have been commonly used in soybean farms in recent seasons and shown as most efficient for ASR control. According to Recommendations (17), fungicides should be applied when the leaflet incidence reaches 5%.

The tested concentration of 5 x 10^3 uredospores/mL resulted in a disease intensity similar to that of natural disease occurrence in soybean farms.

Fungicide curative action. The curative action of fungicides was quantified after a single fungicide application at four days after plants have been inoculated.

The initial symptoms in ASR control (tissue yellowing at the penetration site) were observed on the fifth day after the fungus inoculation. Early lesion formation was detected on the 7th day and sporulation started on the 9th day after inoculation. Similar results have been reported by Marchetti et al. (9), Bromfield et al. (2), Melching et al. (12) and Godoy & Canteri (6).

The results for ASR curative control showed that DMI + QoI mixtures reached 100% control of latent infections in all evaluations rated for both uredia (Table 2) and lesions/cm^2 (Table 3).

However, the fungicide flutriafol, also belonging to the demethylation inhibitor (DMI) group, did not provide 100% control of latent/virtual infections. Fungicide-treated plants showed the formation of several lesions but with just a few uredia; thus, the curative action was partial (Tables 2 and 3).

Data showed that the penetrant fungicides, from two different chemical groups and modes of action (DMI and QoI), showed similar performance on latent infections (Table 3). The non-penetrating/protectant/multisite fungicide, chlorothalonil, showed partial curative effect on the 10th day, decreasing statistically from the other times.

ASR curative control was similar in both experiments (I and II). DMI and QoI mixtures showed 100% control of latent infections in all evaluations performed for both uredia and lesions (Tables 3). However, flutriafol which belongs to the triazole family and has DMI action did not provide 100% control of latent infections. The plants treated with this chemical showed numerous lesions (Table 3) but had few uredia inside (Table 2). It is likely that at this moment there was already reduced sensitivity to flutriafol with IC_{50} of 5.61 mg/L for spore germination.

The curative action of chlorothalonil was not as effective as that of other fungicides (Table 3 and 4). Chlorothalonil is a non-penetrating/protectant chemical that did not penetrate the soybean leaf tissue,

Table 2. Curative effect of fungicides on the control of latent infections caused by *Phakopsora pachyrhizi* in soybeans and assessed based on uredinium density (no. /cm^2)

Fungicides	Time (days after application)									
	Experiment 1									
	10		14		18		22		26	
	----------%----------									
Chlorothalonil	90.8	b	4.3	c	9.4	c	2.3	B	8.9	b
Flutriafol	99.8	a	97.9	b	98.7	b	99.4	a	99.5	a
Cyproconazole + trifloxystrobin	99.9	a	100	a	100	a	100	a	100	a
Epoxyconazole + pyraclostrobin	100	a	100	a	100	a	100	a	100	a
Cyproconazole + azoxystrobin	100	a	100	a	100	a	100	a	100	a
Cyproconazole+ picoxystrobin	100	a	100	a	100	a	100	a	100	a
C.V.(%)	5.8		12.3		7.4		17.1		9.6	
	Experiment 2									
Chlorothalonil	46.3	b	14.9	b	7.7	b	2.7	B	2.5	b
Flutriafol	100	a	100	a	100	a	100	a	99.9	a
Cyproconazole + trifloxystrobin	99.9	a	100	a	99.9	a	99.9	a	99.9	a
Epoxyconazole + pyraclostrobin	100	a	100	a	100	a	100	a	100	a
Cyproconazole + azoxystrobin	100	a	100	a	100	a	100	a	100	a
Cyproconazole+ picoxystrobin	100	a	100	a	100	a	100	a	100	a
C.V.(%)	9.0		14.1		21.3		12.0		18.6	

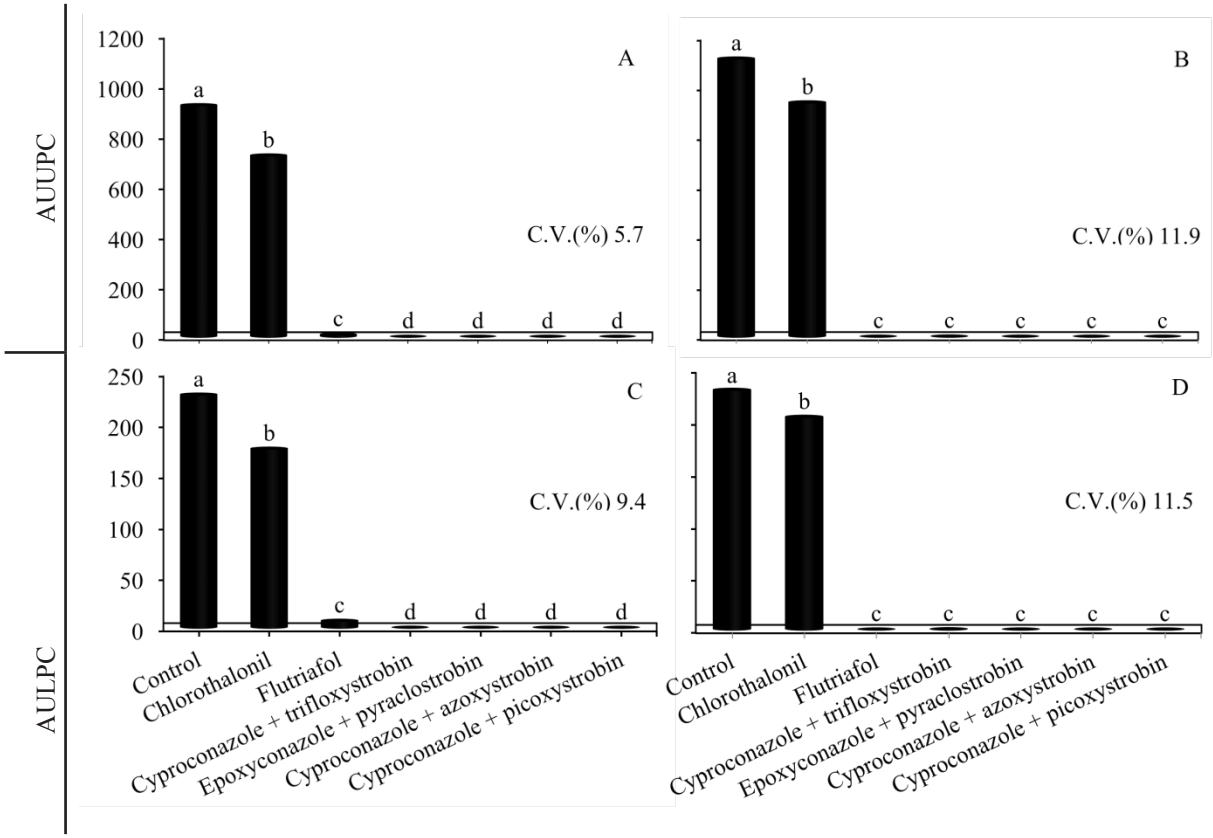
Means followed by the same letter in the columns do not differ according to Tukey's test at 5% probability.

Table 3. Curative effect of fungicides on the control of latent infections caused by *Phakopsora pachyrhyzi* in soybeans and assessed based on lesion density (no./cm^2)

Fungicides	Time (days after application)									
	Experiment 1									
	10		14		18		22		26	
	----------%----------									
Chlorothalonil	32.4	B	12.6	b	5.2	b	7.3	B	5.3	b
Flutriafol	100	a	100	a	100	a	100	a	99.8	a
Cyproconazole + trifloxystrobin	99.8	a	100	a	99.9	a	99.9	a	99.9	a
Epoxyconazole + pyraclostrobin	99.7	a	99.7	a	99.7	a	99.8	a	99.8	a
Cyproconazole + azoxystrobin	99.5	a	100	a	100	a	100	a	100	a
Cyproconazole+ picoxystrobin	100	a	100	a	100	a	100	a	100	a
C.V.(%)	24.2		8.6		13.5		7.3		51	
	Experiment 2									
Chlorothalonil	87.7	B	8.5	b	10.5	b	2.7	B	4.3	b
Flutriafol	98.2	a	98.7	a	98.1	a	92.6	a	98.1	a
Cyproconazole + trifloxystrobin	100	a	100	a	100	a	100	a	100	a
Epoxyconazole + pyraclostrobin	100	a	100	a	100	a	100	a	100	a
Cyproconazole + azoxystrobin	100	a	100	a	100	a	100	a	100	a
Cyproconazole+ picoxystrobin	100	a	100	a	100	a	100	a	100	a
C.V.(%)	7.9		3.8		5.3		19.1		14,2	

Means followed by the same letter in the columns do not differ according to Tukey's test at 5% probability.

Figure 1. Effect of curative fungicide application on latent infections caused by *Phakopsora pachyrhizi*, assessed based on the area under uredinium progress curve (AUUPC) and the area under the lesion progress curve (AULPC). First experiment (A-C) and second experiment (B-D). Columns followed by the same letter do not differ according to Tukey's test ($p<0.05\%$).



remaining on the surface, not acting directly on internal fungal structures. The curative action of chlorothalonil was slighter than that of other fungicides (Table 3).

Curative action was also evidenced when ASR was rated by AUUPC and AULPC (Figure 1). Fungicide co-formulations controlled 100% latent infections in both the first and the second experiments, significantly differing from the control and from chlorothalonil. Moreover, flutriafol generated an AULPC of 8.46 units and an AUUPC of 6.84 units, differing from the control. In this case, it is likely that the leaf area was not completely covered by the fungicide, which allowed the development of some uredia. In the second experiment, the fungicide flutriafol showed 100% curative action.

In this study, fungicide was applied with a 1,000 L/ha spray volume to avoid the presence of uncovered areas that could compromise the reliability of results. However, the reports by Godoy & Canteri (6), Ugalde (20) and Viero & Forcelini (22) showed that some fungicides belonging to the DMI and QoI groups, as well as the mixture of epoxiconazole + pyraclostrobin, did not show 100% control of latent infections when sprayed up to the second and fourth day after the fungus inoculation, respectively. The discrepancy between our results and those of the above-mentioned authors may be due to the different water volume of 200 L/ha used by them, which did not promote complete coverage of the soybean leaves, resulting in unprotected areas and allowing the disease onset. Therefore, the low performance could be due to incomplete coverage and not to fungicide efficiency.

Even if fungicides have the ability to move (translocate) through the xylem vessels, and although there are differences between molecules on this particularity, they hardly have the ability to correct any gaps in the plant canopy coverage resulting from spraying.

The results of the present study and those reported by Godoy & Canteri (6), Ugalde (20), and Viero & Forcelini (22) partially explain the success and the failure found in commercial fields in controlling ASR. Therefore, a soybean crop that has been treated with fungicides with proven curative action will not have full curative control of latent infections if the application results in incomplete leaflet surface coverage, allowing the disease to develop and increase its intensity even a few days after fungicide application.

Even if the fungicides penetrate the leaves, they have the ability to show deep movement in the leaf tissue and apical movement through the xylem vessels, and although there are differences between the molecules with respect to this characteristic, they hardly have the ability to correct possible failures in coverage. It is still difficult to completely cover the soybean canopy with the available spray technology. Even those penetrant/mobile fungicides require full leaf coverage. Leaf parts or those leaves that are not covered will not be protected because there is no fungicide movement through the phloem or complete systemicity in broad-leaved crops. Penetrant mobile fungicides do not move from one leave to the other, but only to their tips.

Our results demonstrated that maximum efficiency of ASR curative control was obtained by applying DMI + QoI mixtures. Thus, under field conditions, curative and eradicant control requires that the application results in complete leaf coverage, which directly involves high-quality spray technology.

The curative action of fungicides, under field conditions, has little practical importance since the exact time of fungus deposition and penetration in the host tissues cannot be defined.

In the evaluations, lesions/uredia of brown/dark color at the same time were considered eradicated. Except for chlorothalonil, DMI + QoI co-formulations and isolated triazole showed similar performance on eradicant action (Figure 2).

The eradicant effect of fungicides was quantified after a single application on sporulating uredia at nine days after the fungus inoculation.

The eradicant action on lesions and uredia was more pronounced from 14 days after the application. It is likely that the lesion aging over time helped increase the fungicide eradicant action (Figure 4).

Finally, at 26 days after the fungicide application, there was no eradicant control (100%) of the lesions and sporulating uredia. Similar results were reported by Godoy & Canteri (6), who found that the eradicant effect of some fungicides belonging to the DMI and QoI group was little or not evident until 14 days after application.

These results may have been influenced by the environment where the experiments were conducted. In the growth chamber, the weather conditions (temperature, leaf wetness and relative humidity) were favorable and stable, which possibly explains the slow eradicant action of fungicides in both experiments. On the other hand, eradicant applications on sporulating uredia and lesions of soybean plants grown in the field led to an eradicant effect of these fungicides on the seventh day after application. However, under field conditions, environmental factors may have helped reduce the epidemic progress (1, 10, 11, 12, 19). In the same context, some reports have indicated the deleterious effect of increased solar irradiation in reducing uredospore viability, a factor that can contribute to greater and faster eradicant action of fungicides under natural conditions (8).

Fungicide mixtures showed similar results on uredospore viability. On the 2nd and 26th days after application, viability was 39.5 and 14.8% for epoxiconazole + pyraclostrobin, 42.3 and 11.3% for cyproconazole + azoxystrobin, 54.1 and 7.5 % for cyproconazole and picoxystrobin, and 61.0 and 15.2% for cyproconazole + trifloxystrobin, respectively. On the other hand, the action of flutriafol was less efficient, compared to that of DMI and QoI mixtures, which resulted in uredospore viability of 73.2 and 18.8% on the 2nd and 26th days, respectively.

Either the fungicide eradicant action on ASR uredia/lesions or spore viability in water-agar substrate was evaluated. As the time passed, fungicides progressively reduced uredospore viability by following a linear model (Figure 3).

The eradicant effect of the fungicide chlorothalonil averaged, on all ratings, a reduction of 88.4% and 77.2% in the first and second experiments, respectively. As a non-penetrant or protectant fungicide, it is not taken up by the leaf tissue, remaining on the leaf surface; thus, when the leaflets were immersed in distilled water for uredospore release, the toxic substance was probably diluted in the spore suspension, preventing germination (15).

Moreover, the fungicide co-formulations showed similar results for uredospore viability. The viability of uredospores on the 2nd and 26th days after application was 39.5 and 14.8% for pyraclostrobin + epoxyconazole, 42.3 and 11.3% for cyproconazole + azoxystrobin, 54.1 and 75.0% for cyproconazole + picoxystrobin, and 61.0 and 15.2% for cyproconazole and trifloxystrobin. Furthermore, the fungicide flutriafol was less efficient in relation to the other DMI and QoI mixtures, which resulted in uredospore viability of 73.2 and 18.8% on the 2nd and 26th days after application, respectively. In a pioneer study, Godoy & Canteri (6) also showed that the use of DMIs or QoIs reduced to a greater extent the uredospore viability.

The eradicant action of fungicides showed effects on lesions, uredia and uredospore germination. The treatment with chlorothalonil resulted in the smallest AUUPC, differing from the other treatments. The mixture of picoxystrobin + cyproconazole differed from the other DMIs in Experiments 1 and 2, except for the mixture of cyproconazole + azoxystrobin in Experiment 2. Mixtures of the two chemical groups

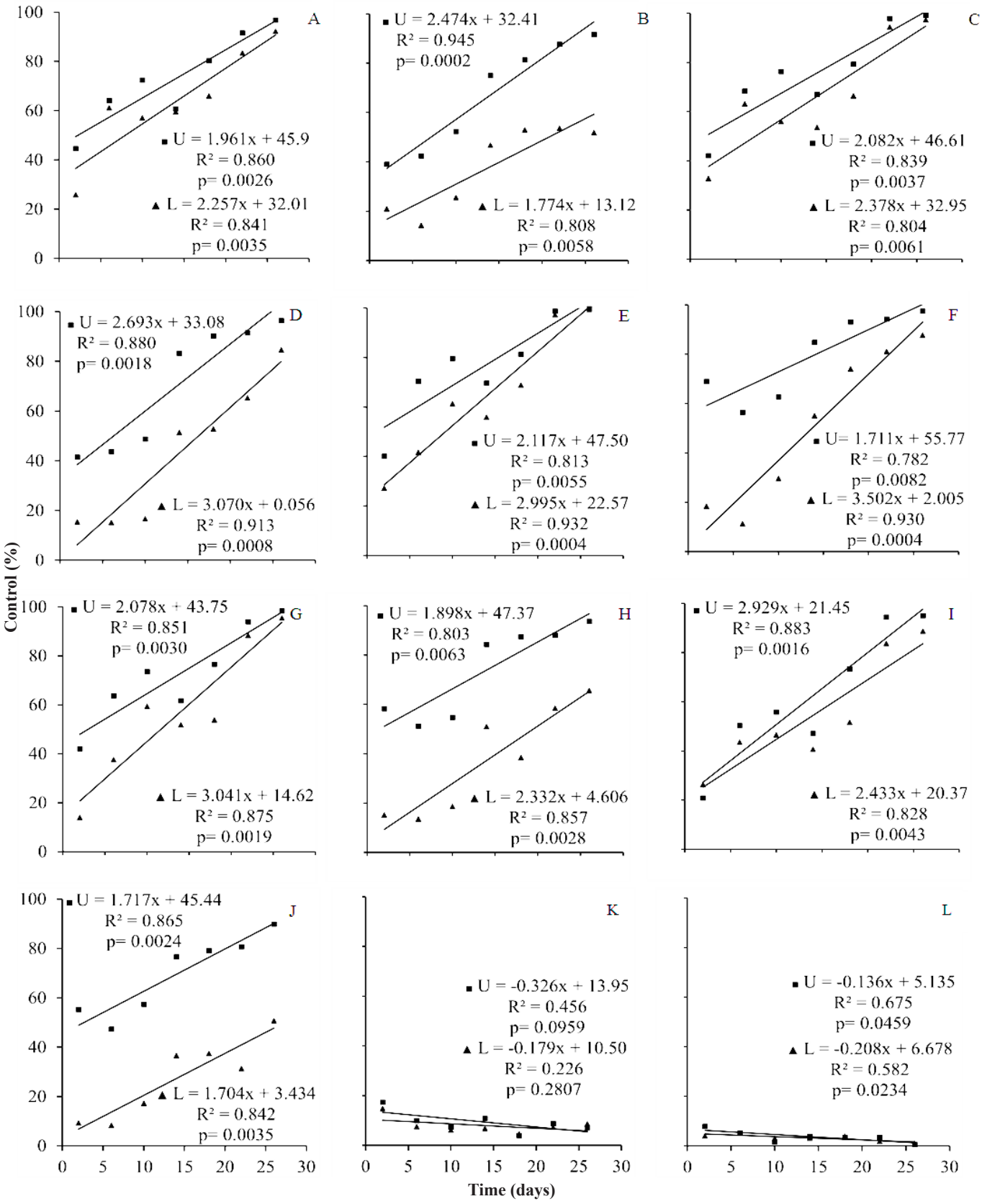


Figure 2. Effect of eradicant fungicide application on *Phakopsora pachyrhizi* control. (Rated by U= uredia/cm^2; L= lesions/cm^2). First experiment (A-C-E-G-I-K) and second experiment (B-D-F-H-J-L). Epoxiconazole + pyraclostrobin, (A-B) cyproconazole + azoxystrobin, (C-D) cyproconazole + picoxystrobin, (E-F) cyproconazole + trifloxystrobin, (G-H) flutriafol, and (I-J) chlorothalonil (K-L).

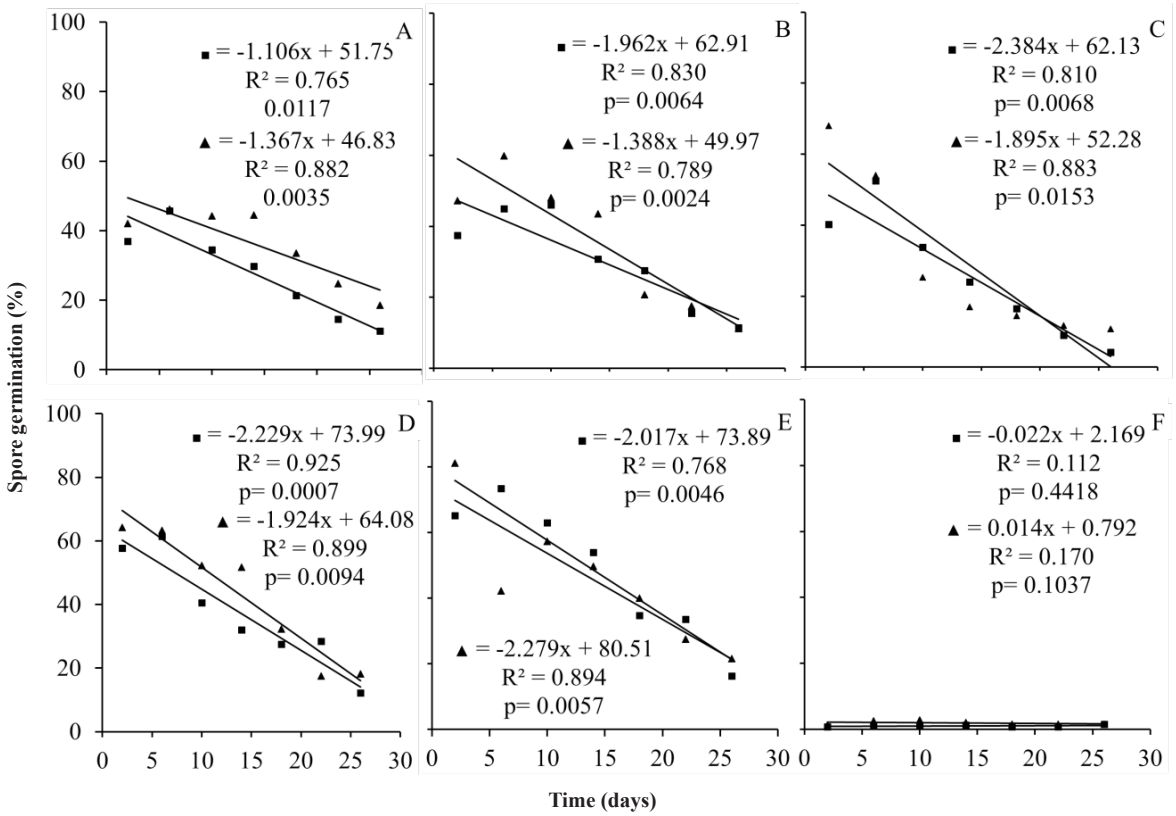


Figure 3. Eradicant effect of fungicide application on uredospore germination of *Phakopsora pachyrhizi*. First experiment (■) and second experiment (▲). Epoxiconazole + pyraclostrobin, (A) cyproconazole + azoxystrobin, (B) cyproconazole + picoxystrobin, (D) cyproconazole+ trifloxystrobin, (E) flutriafol and (F) chlorothalonil (G).

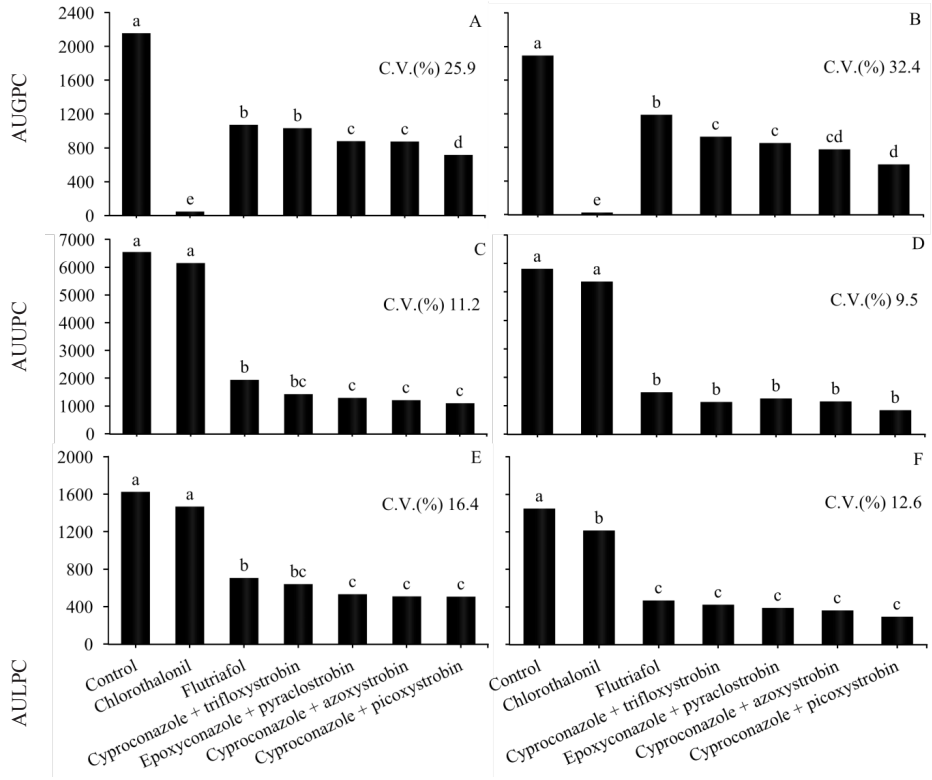


Figure 4. Eradicant effect of fungicide on the area under uredospore germination progress curve (AUGPC), area under uredinium progress curve (AUUPC) and area under lesion progress curve (AULPC) of Asian soybean rust caused by *Phakopsora pachyrhizi*. First experiment (A-C-E) and second experiment (B-D-F).

showed trends in reducing both the AUUPC and AULPC after eradicant fungicide application.

QoIs act on spore germination, interfering in the energy production in the fungal cell by blocking the electron transfer, which prevents ATP formation (21). However, triazoles act in the membrane synthesis which occurs only 24 hours after the initiation of spore germination (7).

DMI and QoI co-formulations showed curative action on *P. pachyrhizi;* however, with flutriafol, the action was less evident. The curative action of chlorothalonil, as expected, was less evident. All fungicides reduced spore viability.

Mixtures of triazoles plus strobilurins have complete curative action on *P. pachyrhizi*. The eradicant action on lesions is difficult to evaluate due to their brown color; however, on uredinia, based on the uredospore color shift, it was clearly evident. The eradicant action is dependent on the fungus exposure time to the fungicide.

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