Pesquisa Agropecuária Tropical
ISSN: 1517-6398
ISSN: 1983-4063
cunhajunior.l.c@ufg.br
Universidade Federal de Goiás
Brasil

Zuntini, Bruno; Félix Alvarez, Rita de Cassia; de Faria Theodoro, Gustavo; Zuffo, Alan Mario

Effect of adding fungicide to mixtures of triazoles and strobilurins in the control of downy mildew and Asian soybean rust

Pesquisa Agropecuária Tropical, vol. 49, 2019
Universidade Federal de Goiás
Goiânia, Brasil

e-ISSN 1983-4063 - www.agro.ufg.br/pat - Pesq. Agropec. Trop., Goiânia, v. 49, e53688, 2019

Research Article

Effect of adding fungicide to mixtures of triazoles and strobilurins in the control of downy mildew and Asian soybean rust[1]

Bruno Zuntini[2], Rita de Cassia Félix Alvarez[2],
Gustavo de Faria Theodoro[3], Alan Mario Zuffo[2]

ABSTRACT

Fungicides used to control the Asian soybean rust are composed almost entirely of mixtures involving the systemic (triazoles) and meso-systemic (strobilurins) modes of action. However, to control the soybean downy mildew, only protective fungicides are available. The present study aimed to evaluate the effectiveness of fungicides with different modes of action in the control of downy mildew and Asian soybean rust. A field experiment was carried out in a randomized block design, with seven treatments [negative control; difenoconazole at V7 + (azoxystrobin + cyproconazole) at R2 and R5.1; (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; carbendazim and mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; (tebuconazole + carbendazim) at V7 + mancozeb in V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1 + mancozeb at R2 and R5.1; (tebuconazole + carbendazim) at V7 + azoxystrobin + tebuconazole at R2 and R5.1 + mancozeb at R2 and R5.1 + carbendazim at R5.1] and four replicates. The following variables were evaluated: disease severity based on diagrammatic scales, phytotoxicity, product effectiveness, defoliation, number of pods per plant, number of seeds per pod, 1,000-seed weight and yield. The treatments with mancozeb addition showed to be more efficient in reducing the severity of mildew and Asian soybean rust and defoliation, providing increased seed yields for the crop. For the soybean downy mildew, the treatments with addition of mancozeb to benzimidazole, strobilurins and triazoles were more efficient in reducing the severity and for the control.

KEYWORDS: *Glycine max*, *Phakopsora pachyrhizi*, *Peronospora manshurica*.

RESUMO

Efeito da adição de fungicida a misturas de triazóis e estrobirulinas no controle de míldio e ferrugem asiática da soja

Os fungicidas utilizados para o controle da ferrugem asiática da soja são constituídos por misturas entre os modos de ação sistêmicos (triazóis) e meso-sistêmicos (estrobilurinas). Entretanto, para o controle do míldio da soja, existem disponíveis apenas fungicidas protetores. Objetivou-se avaliar a eficácia de fungicidas com diferentes modos de ação no controle de míldio e ferrugem asiática da soja. Realizou-se experimento a campo, em delineamento de blocos casualizados, constituído de sete tratamentos [controle negativo; difeconazole em V7 + (azoxystrobina + ciproconazole) em R2 e R5.1; (tebuconazole + carbendazim) em V7 + (azoxystrobina + tebuconazole) em R2 e R5.1; carbendazim e mancozebe em V7 + (azoxystrobina + tebuconazole) em R2 e R5.1; (tebuconazole + carbendazim) em V7 + mancozebe em V7 + (azoxystrobina + tebuconazole) em R2 e R5.1; (tebuconazole + carbendazim) em V7 + (azoxystrobina + tebuconazole) em R2 e R5.1 + mancozebe em R2 e R5.1; (tebuconazole + carbendazim) em V7 + azoxystrobina + tebuconazole em R2 e R5.1 + mancozebe em R2 e R5.1 + carbendazim em R5.1] e quatro repetições. Os parâmetros avaliados foram: severidade das doenças baseada em escalas diagramáticas, fitotoxidez, eficácia de produtos, desfolha, número de vagens por planta, número de grãos por vagem, massa de mil grãos e produtividade. Os tratamentos com adição do mancozebe mostraram-se mais eficientes na redução da severidade de míldio e ferrugem asiática da soja e desfolha, aumentando a produtividade de grãos da cultura. Para o míldio da soja, os tratamentos com adição de mancozebe a benzimidazol, estrobilurinas e triazóis foram mais eficazes na redução da severidade e no controle.

PALAVRAS-CHAVE: *Glycine max*, *Phakopsora pachyrhizi*, *Peronospora manshurica*.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the most important crops in the world, in terms of cultivated area, with a remarkable participation in human and animal nutrition. One of the main factors limiting high yields in this crop are diseases, which can lead to losses estimated at around 20 %, though some of them may generate losses of almost 100 % (Hartman et al. 2015).

1. Received: Jun. 26, 2018. Accepted: Oct. 31, 2018. Published: Apr. 25, 2019. DOI: 10.1590/1983-40632019v4953688.
2. Universidade Federal de Mato Grosso do Sul, Departamento de Agronomia, Chapadão do Sul, MS, Brasil.
E-mail/ORCID: bruno.zuntini@syngenta.com/0000-0002-9198-9875, ritacfalvarez@gmail.com/0000-0002-1655-9939, alan_zuffo@hotmail.com/0000-0001-9704-5325.
3. Universidade Federal de Mato Grosso do Sul, Departamento de Medicina Veterinária e Zootecnia, Campo Grande, MS, Brasil.
E-mail/ORCID: theodoro.gf@gmail.com/0000-0002-1230-2124.

Asian soybean rust, caused by the *Phakopsora pachyrhizi* (Sydow & Sydow) fungus, is currently the main plant pathological problem for the Brazilian soybean crop, and its proper management is essential to ensure the profitability of producers (Godoy et al. 2016). The disease is favored by well-distributed rains and long periods of continuous foliar wetting, and it can be easily spread through wind. The optimal temperature for its development ranges from 20 ºC to 23 ºC when the minimum dew period is 6 h, and rainfall is an important factor in determining epidemic levels in the field (Melching et al. 1989, Del Ponte et al. 2006).

Soybean downy mildew, caused by *Peronospora manshurica* (Naoumov), is classified as a secondary disease, but its occurrence has increased at a higher frequency in crops grown in the South and Central-West regions of Brazil (Kowata et al. 2008).

Among the methods available for the control of Asian soybean rust, the use of fungicides is the most efficient in situations of high risks of yield drop. Products composed of mixtures of triazoles, strobilurins and carboxamides have been the most widely used, what has led to the emergence of resistant populations of *P. pachyrhizi* (Godoy 2012). However, it is important to know the effects of the application of protective fungicides, such as mancozeb, on the physiological processes of plants and in the absence of Asian soybean rust (Alves & Juliatti 2018).

The successive and non-rational use of these mixtures by producers, coupled with a lack of observation to the resistance management, have resulted in a marked decline in the efficiency of such fungicides at every harvest, especially for the control of Asian soybean rust. This decline in efficiency can be explained by a reduced sensitivity of the fungus to these active ingredients caused by the appearance of new strains of *P. pachyrhizi* (Godoy et al. 2007).

For this reason, alternative modes of action to those traditionally used should be studied to increase the effectiveness of products currently available in the market. Among these, the group of protective fungicides is still not widely used in trials by phytopathologists, although they have already shown a medium efficiency in the control of Asian soybean rust; e.g., in a field trial conducted with the active ingredient mancozeb in Viçosa, Minas Gerais state, Brazil (Duarte et al. 2009). In addition, they have a broad spectrum of action against microorganisms of different kingdoms (Zambolin 2008).

Considering this scenario, several actions have been performed to monitor the resistance of *P. pachyrhizi* to new fungicides over time. Through experiments with Brazilian pathogen populations, Juliatti et al. (2017) showed that multisite fungicides (chlorothalonil, copper oxychloride and mancozeb) can be used in the management of resistance to Asian soybean rust associated with strobilurins, triazoles and carboxamides. However, the risk of emergence of carboxamide resistant populations in Brazil by spores from neighboring countries has already been reported by Furlan et al. (2018).

In view of that, this study aimed to examine if fungicides belonging to the chemical groups of triazoles, strobilurins and benzimidazole, with or without addition of the active ingredient mancozeb, are effective in the control of both downy mildew and Asian soybean rust.

MATERIAL AND METHODS

The experiment was carried out at the Centro de Pesquisa Agropecuária (CPA), in Rio Verde, Goiás state, Brazil (17º46'55"S, 50º59'46"W and 769 m of altitude).

Seeding took place on 10 December 2013, using the NA 7337 RR® cultivar, with rows spaced 0.5 cm apart, at a density of 20 plants m^{-1}. Each experimental plot consisted of six seeding rows, with 5 m in length (18 m^2).

Seeds were treated with imidacloprid (150 g L^{-1}) + thiodicarb (450 g L^{-1}) and carbendazim (150 g L^{-1}) + thiram (350 g L^{-1}). Along with seeding, the planting furrows were fertilized with 300 kg ha^{-1} of the 0-20-20 formulation. Weeds were controlled through glyphosate spray at 20 days after the emergence of the crop, and caterpillars were controlled by two sprays of diflubenzuron (240 g L^{-1}) + methomyl (215 g ha^{-1}) before flowering, and three sprays of chlorantraniliprole (625 g L^{-1}) + chlorpyrifos (480 g L^{-1}) in the reproductive stage. To control *Heteroptera*, an insecticide based on thiamethoxam (250 g kg^{-1}) + lambda-cyhalothrin (250 g L^{-1}) was sprayed once in the phenological stages R4 and R5.3, respectively.

The experiment was set up as a randomized-block design, with seven treatments and four replicates (Table 1). Treatments corresponding to time A were sprayed in the vegetative stage of the crop on 16 January 2014, when the soybean plants had six completely open trifoliates, characterizing the stage V7 (Fehr & Caviness 1977). This spray was aimed at the control of the soybean mildew, since this is the stage when the disease is manifested with greatest intensity. Mckenzie & Wyllie (1971) stressed that *P. manshurica* affects plants under mild temperatures (20-22 ºC) and elevates relative humidity, especially in the vegetative stage.

Sprays were performed using a knapsack sprayer at a constant pressure (CO_2), regulated to 200 Kpa and equipped with a 3-m nozzle-holding bar with six ends spaced 0.5 m apart, flat-jet model for sprayed use (XR 110.05), 200 L ha^{-1} of volume, 0.40 m bar height relative to the crop, and 1 m s^{-1} operator speed. The climatic history of the studied region, based on internal records of the CPA, is shown in Figure 1.

For both diseases, the severity was determined based on diagrammatic scales, where scores ranging from 0 % to 100 % were assigned according to the amount of leaf area showing disease symptoms. For *P. manshurica*, the diagrammatic scale of James (1971) was used, with assessments taking place at 7 and 14 days after the first spray - DAA (time A), (Table 2). For *P. pachyrhizi*, the scale adopted was that proposed by Godoy et al. (2006), and evaluations started at 7 and 14 days after the second spray - DAB (time B) and continued until 7 and 14 days after the last spray - DAC (time C) (Table 2).

The percentage efficiency of the fungicide treatments was measured according to the formula proposed by Abbott (1925): E% = [(T - F)/T] ×100, where *T* is the average severity in the control, *F* the average severity in the treatments and *E%* the percentage effectiveness of each evaluated treatment.

After the severity data were obtained, the Asian soybean rust progress rate was determined based on the area under the disease progress curve (AUDPC), by applying the equation developed by Shaner & Finney (1977).

Phytotoxicity was determined by assigning scores for severity according to the injury symptoms caused by the fungicides on the soybean leaves,

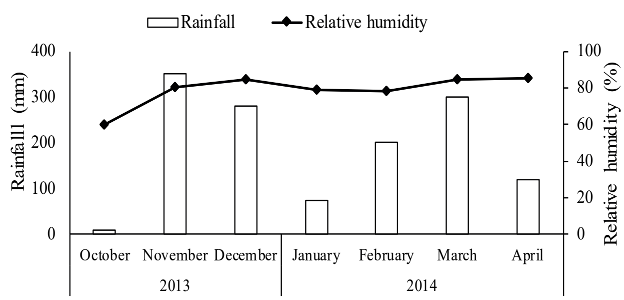

Figure 1. Rainfall and relative humidity data recorded at the Centro de Pesquisa Agropecuária, in Rio Verde, Goiás state, Brazil, from October 2013 to April 2014.

Table 1. Treatments used in the experiment.

Treatment	Spray time[a]	Active ingredient (a.i.)	Concentration	Formulation	A.i. dose (kg ha^{-1})	Mode of action[b]
T1	-	Control	-	-	-	-
T2	A	Difenoconazole	250	EC	0.075	S
	B and C	Azoxystrobin + cyproconazole	200 + 80	SC	0.06 + 0.024	M + S
T3	A	Tebuconazole + carbendazim	125 + 250	SC	0.375	S
	B and C	Azoxystrobin + tebuconazole	120 + 160	SE	0.168	M + S
T4	A	Carbendazim	500	SC	0.250	S
	A	Mancozeb	800	WP	0.800	P
	B and C	Azoxystrobin + tebuconazole	120 + 160	SE	0.168	M + S
T5	A	Tebuconazole + carbendazim	125 + 250	SC	0.375	S
	A	Mancozeb	800	WP	0.800	P
	B and C	Azoxystrobin + tebuconazole	120 + 160	SE	0.168	M + S
T6	A	Tebuconazole + carbendazim	125 + 250	SC	0.375	S
	B and C	Azoxystrobin + tebuconazole	120 + 160	SE	0.168	M + S
	B and C	Mancozeb	800	WP	0.800	P
T7	A	Tebuconazole + carbendazim	125 + 250	SC	0.375	S
	B and C	Azoxystrobin + tebuconazole	120 + 160	SE	0.168	M + S
	B and C	Mancozeb	800	WP	0.800	P
	C	Carbendazim	500	SC	0.500	S

[a] Time A (36 days after planting - DAP): spray of the treatments at V7 - six completely open trifoliates (first spray); time B (58 DAP): spray of the treatments at R2 - full flowering (second spray); and time C (78 DAP): spray of the treatments at R5.1 - start of grain filling (third spray). [b] M + S = systemic; P = protective. Spray volume: 150 L ha^{-1}.

Table 2. Dates, phenological stages and heights of the soybean crop (cm), during the evaluations.

Evaluations	7 DAA	14 DAA	7 DAB	14 DAB	7 DAC	14 DAC	Harvest
Date	23 Jan.	30 Jan.	14 Jan.	21 Mar.	06 Mar.	13 Mar.	03 Apr.
Phenological stage	V7	R1	R4	R5.1	R5.3	R5.5	R8
Height(1)	49	50	67	80	81.5	81.5	-

(1) Obtained from ten plants per treatment. DAA: days after the first spray - time A; DAB: days after the second spray - time B; DAC: days after the third spray - time C.

using an adapted version of the diagrammatic scale developed by Frans et al. (1986).

Defoliation was evaluated at 14 DAC, when leaf fall in the control plots reached 80 %. The methodology consisted of assigning a visual score to the damage caused by Asian soybean rust from 0 % to 100 % for each plot, using an adapted scale of Hirano et al. (2010).

The total number of pods formed per plant and total number of seeds were obtained in the stage of physiological maturation by directly counting all pods and seeds present in 10 plants harvested at random from the usable area. Simultaneously, to determine the 1,000-seed weight, three random samples of 1,000 seeds were separated from each experimental unit.

Harvest was performed manually on 04 April 2014, and all plants in the 4.5-m^2 usable area were harvested and threshed using a stationary thresher. The seed yield of each plot was measured and the obtained value (in kg plot^{-1}) was transformed into kg ha^{-1} after the moisture was corrected to 13 % (Saraiva et al. 2009).

The data were subjected to analysis of variance (Anova) and means were compared by the Tukey test at 5 % of probability. Severity means were transformed by the formula $(x + 1)^{0.5}$. Statistical analyses were undertaken using the SAS software, version 9 (SAS Institute Inc., Cary, CN, USA).

RESULTS AND DISCUSSION

All treatments in which the plants received fungicide spray during the vegetative stage (time A) led to significantly lower disease severity than in the control treatment (Table 3). In the evaluation performed at 7 DAA and 14 DAA, the plots treated with mancozeb + tebuconazole and carbendazim (T5) showed a 5 % lower severity for *P. manshurica*, on average, than those treated with the same active ingredients without mancozeb addition (T3). Silva et al. (2013) also described a reduction in the severity of soybean downy mildew with the use of fungicide associations.

At 14 DAA, the severity in T5 was also 7 % lower than that obtained with the sole spray

Table 3. Severity scores for soybean downy mildew (*Peronospora manshurica*) [$(x+1)^{0.5}$ transformed] and effectiveness of control by fungicides sprayed at the vegetative stage of soybean in two periods of evolution.

Treatment	Severity (%)		Effectiveness control (%)	
	7 DAA(1)	14 DAA	7 DAA	14 DAA
T1(2)	49.7 c*	50.8 d	-	-
T2	31.3 ab	33.4 c	37.1 a	34.0 b
T3	31.5 b	32.1 bc	36.6 a	37.0 b
T4	27.1 ab	28.1 ab	45.4 a	46.5 a
T5	26.1 a	26.1 a	47.4 a	48.4 a
T6	30.0 ab	30.0 abc	39.6 a	40.7 ab
T7	28.9 ab	28.9 abc	41.9 a	43.0 ab
P-value	< 0.001	< 0.001	0.0308	0.0019
LSD	0.48	0.47	11.02	9.96
CV (%)	3.66	3.54	11.60	10.43

* Means followed by the same letter in the columns do not differ by the Tukey test at 5 %. (1) DAA: days after spray. (2) T1 = negative control; T2 = difenoconazole at V7 + (azoxystrobin + cyproconazole) at R2 and R5.1; T3 = (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T4 = carbendazim and mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T5 = (tebuconazole + carbendazim) at V7 + mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T6 = (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1 + mancozeb at R2 and R5.1; T7 = (tebuconazole + carbendazim) at V7 + azoxystrobin + tebuconazole at R2 and R5.1 + mancozeb at R2 and R5.1 + carbendazim at R5.1.

of difenoconazole (T2). The low efficiency of the triazoles sprayed alone may be explained by the fact that downy mildew is considered a false fungus, belonging to the Chromista kingdom, with some morphological and physiological differences in relation to the fungi, what hampers its control by fungicides specific for the Fungi kingdom (Rossman & Palm 2006).

The inefficient control when mancozeb (800 g ha^{-1} of a.i.) was added to the triazole + strobilurins mixtures at 14 DAA can be observed in Table 3. Results for the triple mixture, with mancozeb addition, corroborate the field trials conducted by Dunleavy (1987) in Iowa, USA, which showed that mildew can be effectively controlled with the use of maneb (manganese ethylene-bis-dithiocarbamate), whose mode of action is the same as that of mancozeb. Plants that were sprayed with this fungicide yielded 7 % more than the control treatment. In Brazil, Balardin (2004) also mentioned the good effectiveness of protective fungicides in the control of this disease. In addition to mancozeb, chlorothalonil can also be used to control *P. manshurica*.

Kumagai et al. (1991) stressed that this active principle has a broad range of action and effectiveness in the control of microorganisms of the Chromista kingdom, as is the case of *P. manshurica*. Zambolin (2008) also indicated mancozeb as the recommended fungicide in the control of mildews and foliar diseases in general, because its mechanism of action is multisite, what inhibits enzymes and interferes with the metabolic processes of the fungal cell.

In all evaluations, there was a significant difference between the treatments (Table 4). In these assessments, the control treatment (T1) showed statistically the highest severity indices at all evaluated times. Severity for the control treatment was, on average, 45 % higher than that of the fungicide treatments. These results are similar to those observed by Alves & Juliatti (2017), who described that the addition of mancozeb to mixtures of fungicides (fluxapyroxad + pyraclostrobin, azoxystrobin + benzovindiflupyr, trifloxystrobin + prothioconazole and tebuconazole + picoxystrobin) was effective in reducing the soybean rust severity, when compared with the control treatment.

In the evaluation at 7 DAB, there was no significant difference between the treatments that received fungicide spray. At 14 DAB, however, the treatments composed of triple mixtures (with a systemic, meso-systemic and protective mode of action), such as tebuconazole + azoxystrobin + mancozeb (T6) and tebuconazole + azoxystrobin + mancozeb + carbendazim (T7), displayed significantly lower severity values (around 20 %), when compared with the treatments with double mixtures (systemic and meso-systemic modes of action): azoxystrobin + cyproconazole (T2) and azoxystrobin + tebuconazole (T3) (Table 4). A more effective reduction of Asian soybean rust severity was obtained when triple mixtures (with systemic, mesosystemic and protective modes of action) were sprayed.

The interference of the triple mixture in reducing the disease progress can be better

Table 4. Severity of Asian soybean rust at four evaluation times and control efficiency of the products after sprays of fungicides during the reproductive stage of the crop.

Treatment	Severity of *P. pachyrhizi* (%)				Control efficiency of *P. pachyrhizi* (%)			
	7 DAB	14 DAB	7 DAC	14 DAC	7 DAB	14 DAB	7 DAC	14 DAC
T1[(1)]	62 b*	80 e	81 c	85 c	-	-	-	-
T2	27 a	35 cd	36 b	38 b	56.2 b	55.9 c	55.5 b	50.1 b
T3	23 a	37 d	40 b	41 b	61.9 b	52.9 bc	50.9 b	46.1 b
T4	25 a	32 bcd	35 b	36 b	57.4 b	59.2 abc	57.0 ab	51.3 ab
T5	22 a	25 ab	32 ab	37 ab	65.5 ab	65.4 ab	60.1 ab	50.3 ab
T6	21 a	27 abc	30 ab	31 ab	69.3 ab	68.7 a	63.1 ab	57.6 a
T7	17 a	23 a	25 a	30 a	81.1 a	70.2 a	69.3 a	59.1 a
P-value	< 0.001	< 0.001	< 0.001	< 0.001	0.005	< 0.001	0.007	0.005
DMS	1.06	0.79	0.90	0.73	18.61	10.69	13.29	9.89
CV (%)	8.57	5.65	6.18	4.83	12.41	7.49	9.75	8.20

* Means followed by the same letter do not differ by the Tukey test at 5 %. (1) T1 = negative control; T2 = difenoconazole at V7 + (azoxystrobin + cyproconazole) at R2 and R5.1; T3 = (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T4 = carbendazim and mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T5 = (tebuconazole + carbendazim) at V7 + mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T6 = (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1 + mancozeb at R2 and R5.1; T7 = (tebuconazole + carbendazim) at V7 + azoxystrobin + tebuconazole at R2 and R5.1 + mancozeb at R2 and R5.1 + carbendazim at R5.1.

understood by analyzing the area under the disease progress curve, which is the best representation of an epidemic (Table 5). The same afore-described treatments, T6 and T7, in which mancozeb was added to the mixtures, significantly slowed the disease progress, as compared with the double mixtures of triazoles and strobilurins (T3 and T4), reaching an up to 75 % lower area under the curve than the control. According to Zambolim et al. (2008), the multisite action of this fungicide inhibits enzymes and interferes with metabolic processes of the fungal cell, rather than following the specificity of action presented in mixed fungicide formulations sprayed separately.

To further elucidate the efficiency gain, when the three modes of action are used together (Table 5), treatments T6 and T2 can be used as an example. These treatments consist of the same active ingredients and differ only in the addition of the protective fungicide in both sprays. Treatment T6, to which the active ingredient mancozeb was added, showed a 15 % greater reduction in the AUDPC than T2, differing significantly by the Tukey test at 5 % of probability. Results for the triple mixture suggest a broad possibility of new studies on the effectiveness of the active ingredient mancozeb at the level of 800 g ha^{-1} of a.i., used in a mixture or even alone, aiming at reducing the severity of *P. manshurica* and mainly of *P. pachyrhizi.*

The control efficiency values of each treatment are described in Table 4. The data corroborate the previously discussed severity analysis, in which the association of mancozeb with traditional mixtures of triazoles and strobilurins (T6 and T7) also increased the efficiency of these products by more than 15 %, regardless of the use of triazole in the double mixture (cyproconazole or tebuconazole + azoxystrobin). This fact may be related to the synergistic effect and increase in the control, when associated with the traditional fungicide mixtures.

Although the outcomes of adding the protective fungicide to the mixtures can be promising, one fundamental factor should be taken into account in this study: the rainfall occurring in the experimental area in the months when sprays were performed was low. The periods in which the spraying/assessments took place (January and February) had the lowest volumes of rain throughout the experiment (Figure 1).

According to the internal records of the CPA, in the last five years, the average monthly rainfall in the region of the experimental station, especially in the month of February (spray times B and C, specific for Asian soybean rust), has been higher than 300 mm. During the experiment, rainfall was only 179 mm, characterizing a decline of almost 40 %.

These conditions are favorable for a better efficiency of the protective fungicides (Kumar & Agarwal 1992), since the proper deposition of mancozeb on the leaf surface is essential for the effective control of this molecule (Zambolin 2008). If rainfall had been constant, the ideal deposition would be compromised and the product might have been removed from the leaves. In the experimental conditions, mancozeb provided the double mixtures with an increased residual power, what would hardly occur in rainy years. In the study conditions, the triple mixture

Table 5. Area under the disease progress curve (AUDPC) of Asian soybean rust, defoliation percentage, production components and seed yield of soybean plants, after foliar spray of fungicides.

Treatment	AUDPC	Defoliation	Number of pods per plant	Number of seeds per pod	Yield (kg ha^{-1})
T1[(1)]	1,649.3 e	90.0 d	20.6 b	33.2 b	1,129.4 d
T2	743.7 cd	55.5 bc	24.6 ab	42.7 a	1,557.3 bc
T3	787.5 d	59.5 c	23.4 ab	42.9 a	1,291.3 cd
T4	706.1 cd	50.0 bc	25.7 ab	40.6 ab	1,305.7 cd
T5	647.5 bc	55.0 bc	24.9 ab	42.9 a	1,326.3 cd
T6	577.5 ab	50.0 ab	28.2 a	44.0 a	1,731.2 ab
T7	525.0 a	45.0 a	29.1 a	47.0 a	1,883.7 a
P-value	< 0.001	< 0.001	0.0112	0.0046	< 0.001
LSD	103.88	7.51	6.79	9.21	60.53
CV (%)	5.52	5.49	11.52	9.39	8.29

* Means followed by the same letter in the columns do not differ by the Tukey test at 5 %. [(1)] T1 = control; T2 = difenoconazole at V7 + (azoxystrobin + cyproconazole) at R2 and R5.1; T3 = (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T4 = carbendazim and mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T5 = (tebuconazole + carbendazim) at V7 + mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T6 = (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1 + mancozeb at R2 and R5.1; T7 = (tebuconazole + carbendazim) at V7 + azoxystrobin + tebuconazole at R2 and R5.1 + mancozeb at R2 and R5.1 + carbendazim at R5.1.

provided a greater protection to the crop precisely during the peak period of the disease, after the crop flowering and pod-filling stages.

The same reason can be used to explain the lower improvement in the Asian soybean rust control when mancozeb was applied in the vegetative period of the crop (T4 and T5), with six completely open trifoliates (V7), at 36 days after planting. Because that is the month of highest rainfall in the period, the product was washed away faster, leaving the crop unprotected upon the arrival of *P. pachyrhizi* in the experimental area. Therefore, new studies in years with higher rainfall rates are required to confirm the good results of the triple mixtures presented here.

Phytotoxicity from the use of fungicides in the soybean plants was significant in all the evaluations (Table 6). Azoxystrobin + cyproconazole (T2) did not lead to plant phytotoxicity symptoms, whereas all other treatments composed of azoxystrobin + tebuconazole (T3 to T7) led to phytotoxicity. Therefore, it can be inferred that the active ingredient tebuconazole was responsible for the observed injuries. All treatments in which the active ingredient was present led to mild symptoms of phytotoxicity on the soybean leaves, whose peak occurred after the second spray of the tebuconazole + azoxystrobin mixtures (phenological stage R5.2), with an average near 10 % in all plots.

The data agree with Barros (2009), who conducted a trial in Maracaju, Mato Groso do Sul state, Brazil, and diagnosed symptoms of phytotoxicity caused by tebuconazole of the order of 10 % in the CD 219RR cultivar, although the factor did not affect the crop yield.

Table 6. Scores for the phytotoxicity caused after foliar sprays of fungicides.

Treatment	Phytotoxicity			
	7 DAB	14 DAB	7 DAC	14 DAC
T1[1]	no	no	no	no
T2	0.00 a*	0.00 a	0.00 a	0.00 a
T3	2.33 b	2.77 b	3.31 b	3.38 b
T4	2.39 b	3.00 b	3.21 b	3.28 b
T5	2.44 b	2.88 b	3.09 b	3.17 b
T6	2.43 b	2.98 b	3.31 b	3.38 b
T7	2.53 b	2.90 b	3.11 b	3.19 b
P-value	< 0.001	< 0.001	< 0.001	< 0.001
LSD	0.40	0.88	0.77	0.80
CV (%)	8.58	16.06	12.78	13.08

* Means followed by the same letter do not differ by the Tukey test at 5 %. (1) T1 = control; T2 = difenoconazole at V7 + (azoxystrobin + cyproconazole) at R2 and R5.1; T3 = (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T4 = carbendazim and mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T5 = (tebuconazole + carbendazim) at V7 + mancozeb at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1; T6 = (tebuconazole + carbendazim) at V7 + (azoxystrobin + tebuconazole) at R2 and R5.1 + mancozeb at R2 and R5.1; T7 = (tebuconazole + carbendazim) at V7 + azoxystrobin + tebuconazole at R2 and R5.1 + mancozeb at R2 and R5.1 + carbendazim at R5.1; no = not observed.

The toxicity of triazoles may appear in soybean under certain conditions; e.g.: cultivars more sensitive to this group of fungicides, spray at elevated temperatures and/or in plants under water stress. Problems are further aggravated when sprays are consecutive, especially after the stage R5 of the crop (Barros 2009). Such conditions, time and number of sprays are similar to those occurring during the development of the present experiment.

Of all members of the triazoles group, tebuconazole causes most the problems, if determinations are not followed (Zambolin 2008). Because the product has a faster absorption and a lower translocation speed through the vascular bundles, it can accumulate on the leaves, resulting in a toxic action to plants, mainly if sprayed at high temperatures. Another factor for the appearance of phytotoxicity symptoms is a possible elevated dose of the product, suggesting that triazoles should be used cautiously in disease management (Zambolin 2008).

Although occasional phytotoxicity symptoms may appear, it should be noted that systemic products like tebuconazole are traditionally used in disease control and are, in most cases, associated with strobilurins (Schmitz et al. 2014).

The treatments in which mancozeb was added at times B and C (T6 and T7) also showed a significant difference, in terms of defoliation, as compared with the use of the mixtures alone, regardless of the triazole used (cyproconazole or tebuconazole + azoxystrobin), as described in Table 5.

The control treatment resulted in a 90 % defoliation at 14 DAC, which is 45 % higher than that seen for the treatments composed of the triple mixture. Treatments T6 and T7 also displayed defoliation levels statistically lower than those of the treatments with double mixtures (T2 and T3), which did not receive the addition of the protective fungicide and in which defoliation levels reached 56 % and 60 %, respectively.

The number of pods per plant, number of seeds per pod and seed yield also differed significantly between the factors, according to the Tukey test, at 5 % of probability (Table 5). Once again, treatments T6 and T7 stood out with an approximately 30 % higher number of pods and yield per pod than the control treatment. The other fungicide treatments did not differ statistically from each other for either number of pods or seed yield per pod.

The beneficial effect of the triple mixture also reflected in the crop yield, with treatments T6 and T7 being significantly more productive (1,731 kg ha^{-1} and 1,883 kg ha^{-1}, respectively). Overall, the mancozeb addition to a triple mixture, aiming at the control of Asian soybean rust, resulted in 300 kg ha^{-1} or six more bags than the traditional double mixtures.

This study opens new possibilities for research investigating the addition of protective fungicides, mainly the active ingredient mancozeb, in association with traditional mixtures of triazoles and strobilurins under different environmental conditions for this crop. Further results may reinforce the present findings on the control of not only downy mildew and Asian soybean rust, but also other important pathogens affecting soybean. Silva et al. (2013) observed that separate sprays of mancozeb (2.0 kg ha^{-1}) reduced the severity of Asian soybean rust and increased crop yield.

Because mancozeb is a protective fungicide with a broad spectrum of action, and because no cases of emergence of resistant fungi are known, it is an important tool for the management of resistance to *P. pachyrhizi*, as it can extend the shelf life of fungicides that act on specific sites in pathogens (Gullino et al. 2010).

CONCLUSIONS

1. Treatments with mancozeb addition to triazoles and strobilurins are more efficient in reducing the Asian soybean rust disease severity and defoliation, thereby increasing the grain yield of the soybean crop;
2. For soybean downy mildew, treatments with the addition of mancozeb to benzimidazole, strobilurins and triazoles are more efficient in reducing severity.

REFERENCES

ABBOTT, W. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, v. 18, n. 3, p. 265-267, 1925.

ALVES, V. M.; JULIATTI, F. C. Fungicidas no manejo da ferrugem da soja: processos fisiológicos e produtividade da cultura. *Summa Phytopathologica*, v. 44, n. 3, p. 245-251, 2018.

BALARDIN, R. S. Doenças de final de ciclo e ferrugem. In: REIS, E. M. *Doenças na cultura da soja*. Passo Fundo: Aldeia Norte, 2004. p. 97-108.

BARROS, R. Doenças da cultura da soja. In: BARROS, R. *Tecnologia e produção*: soja e milho 2008/2009. Maracaju: Fundação MS, 2009. p. 109-122.

DEL PONTE, E. M. et al. Predicting severity of Asian soybean rust epidemics with empirical rainfall models. *Phytopathology*, v. 96, n. 7, p. 797-803, 2006.

DUARTE, H. S. S. et al. Silicato de potássio, acibenzolar-S-metil e fungicidas no controle da ferrugem da soja. *Ciência Rural*, v. 39, n. 8, p. 2271-2277, 2009.

DUNLEAVY, J. M. Yield reduction in soybeans caused by downy mildew. *Plant Disease*, v. 71, n. 12, p. 1112-1114, 1987.

FEHR, W. R.; CAVINESS, C. E. *Stages of soybean development*. Ames: Yowa State University of Science and Technology, 1977.

FRANS, R. et al. Experimental design and techniques for measuring and analyzing plant responses to weed control practices. In: CAMPER, N. D. (Ed.). *Research methods in weed science*. 3. ed. Champaign: Southern Weed Science Society, 1986.

FURLAN, S. H.; CARVALHO, F. K.; ANTUNIASSI, U. R. Strategies for the control of Asian soybean rust (*Phakopsora pachyrhizi*) in Brazil: fungicide resistance and spray efficacy. *Outlooks on Pest Management*, v. 29, n. 3, p. 120-123, 2018.

GODOY, C. V. et al. Asian soybean rust in Brazil: past, present, and future. *Pesquisa Agropecuária Brasileira*, v. 51, n. 5, p. 407-421, 2016.

GODOY, C. V. et al. *Eficiência de fungicidas para controle da ferrugem asiática da soja, Phakopsora pachyrhizi, na safra 2006/07*. Londrina: Embrapa Soja, 2007.

GODOY, C. V. Risk and management of fungicide resistance in the Asian soybean rust fungus *Phakopsora pachyrhizi*. In: THIND, T. S. (Ed.). *Fungicide resistance in plant protection*: risk and management. London: CAB, 2012. p. 87-95.

GODOY, C. V.; KOGA, L. J.; CANTERI, M. G. Escala diagramática para avaliação da severidade da ferrugem da soja. *Fitopatologia Brasileira*, v. 31, n. 1, p. 63-68, 2006.

GULLINO, M. L. et al. Mancozeb: past, present and future. *Plant Disease*, v. 94, n. 9, p. 1076-1087, 2010.

HARTMAN, G. L. et al. *Compendium of soybean diseases and pests*. 5. ed. Saint Paul: APS Press, 2015.

HIRANO, M. et al. Validação de escala diagramática para estimativa de desfolha provocada pela ferrugem asiática em soja. *Summa Phytopathologica*, v. 36, n. 3, p. 248-250, 2010.

JAMES, C. *Manual of assessment keys for plant diseases*. Saint Paul: APS Press, 1971.

JULIATTI, J. C. et al. Sensitivity of *Phakopsora pachyrhizi* populations to dithiocarbamate, chloronitrile, triazole, strobilurin, and carboxamide fungicides. *Bioscience Journal*, v. 33, n. 4, p. 933-943, 2017.

KOWATA, L. S. et al. Escala diagramática para avaliar severidade de míldio na soja. *Scientia Agraria*, v. 9, n. 1, p. 105-110, 2008.

KUMAGAI, H. et al. Absorption, translocation and metabolism of polycarbamate, a dithiocarbamate fungicide, in kidney bean seedlings. *Journal of Pesticide Science*, v. 16, n. 4, p. 641-649, 1991.

KUMAR, U.; AGARWAL, H. C. Fate of [C-14] mancozeb in egg plants (*Solanum-Melongena* L.) during summer under subtropical conditions. *Pesticide Science*, v. 36, n. 2, p. 121-125, 1992.

MCKENZIE, T. R.; WYLLIE, T. D. The effect of temperature and lesions size on the sporulation of *Peronospora manshurica*. *Phytopathology*, v. 71, n. 4, p. 321-326, 1971.

ROSSMAN, A. Y.; PALM, M. E. Why are *Phytophthora* and other *Oomycota* not true fungi? *Journal of Biofuels*, v. 17, n. 5, p. 217-219, 2006.

SARAIVA, O.; LEITE, R.; CASTRO, C. *Reunião de pesquisa de soja da região central do Brasil*. Londrina: Embrapa Soja, 2009.

SCHMITZ, H. K. et al. Sensitivity of *Phakopsora pachyrhizi* towards quinone-outside-inhibitors and demethylation-inhibitors, and corresponding resistance mechanisms. *Pest Management Science*, v. 70, n. 3, p. 378-388, 2014.

SHANER, G.; FINNEY, R. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology*, v. 67, n. 8, p. 1051-1056, 1977.

SILVA, O. C. et al. Fontes de fosfito e acibenzolar-S-metílico associados a fungicidas para o controle de doenças foliares na cultura da soja. *Tropical Plant Pathology*, v. 38, n. 1, p. 72-77, 2013.

ZAMBOLIN, L. Tipos de fungicidas empregados no controle de doenças de plantas. In: ZAMBOLIN, L. et al. *Produtos fitossanitários*: fungicidas, inseticidas, acaricidas e herbicidas. Viçosa: Ed. UFV, 2008. p. 263-348.