Original Article

SENSITIVITY OF *Phakopsora pachyrhizi* POPULATIONS TO DITHIOCARBAMATE, CHLORONITRILE, TRIAZOLE, STROBILURIN, AND CARBOXAMIDE FUNGICIDES

SENSIBILIDADE DE POPULAÇÕES DE *Phakopsora pachyrhizi* AOS FUNGICIDAS DITIOCARBAMATO, CLORONITRILA, TRIAZOL, ESTROBILURINA E CARBOXAMIDA

Fernando Cezar JULIATTI[1]; Lorraine Cristina POLLONI[2]; Tâmara Prado de MORAIS[3]; Nadia Romina Shneider ZACARIAS[4]; Erick Araújo SILVA[4]; Breno Cezar Marinho JULIATTI[5]

1. Professor Titular, Pesquisador 1D do CNPq. LAMIP, Instituto de Ciências Agrárias - ICIAG, Universidade Federal de Uberlândia - UFU, Uberlândia, MG, Brasil. juliatti@ufu.br; 2. Doutoranda Instituto de Genética e Bioquímica - UFU. LAMIP, Instituto de Ciências Agrárias - ICIAG, Universidade Federal de Uberlândia - UFU, Uberlândia, MG, Brasil; 3. PNPD, CAPES. LAMIP, Instituto de Ciências Agrárias - ICIAG, Universidade Federal de Uberlândia - UFU, Uberlândia, MG, Brasil; 4. Estudante de Graduação Agronomia. LAMIP, Instituto de Ciências Agrárias - ICIAG, Universidade Federal de Uberlândia - UFU, Uberlândia, MG, Brasil; 5. Doutorando em Fitopatologia da Universidade Federal de Lavras - UFLA, Lavras, MG, Brasil.

ABSTRACT: Asian soybean rust (ASR), caused by the phytopathogen *Phakopsora pachyrhizi* Sydow & Sydow, is a worldwide distributed fungal disease responsible for causing damages in soybean crops [*Glycine max* (L.) Merril] of up to 90% of its productive potential. So far, due to limited availability of resistant varieties, fungicide application is the most widely used strategy for ASR control, although some populations of the pathogen have shown reduced sensitivity to certain active ingredients. Several methods have been described to measure the sensitivity of a fungus to a given fungicide, or even the fungitoxicity of a chemical. The most used tests are spore germination in water-agar medium and evaluation of disease severity in detached soybean leaves. Experiments were carried out with Brazilian populations of the pathogen: one from Uberlândia - MG and the other from Chapadão do Sul - MS, following the mentioned methodologies. The results showed the reduction of benzovindiflupyr efficiency in relation to spore germination and disease severity for the MS population, as also did fluxapyroxad, cyproconazole, and tebuconazole. Multisite fungicides (chlorothalonil, copper oxychloride, and mancozeb) may be used in the management of ASR resistance in the fields of Brazil associated with strobilurins, triazoles, and carboxamides. The efficiency of the main active ingredient tested depends on the formulation and others molecules used in the commercial product.

KEYWORDS: Bioassays. Baseline. Asian soybean rust. Chemical control. Fungicides resistance.

INTRODUCTION

The Asian soybean rust (ASR), caused by the phytopathogen *Phakopsora pachyrhizi* (Sydow & Sydow), is a fungal disease distributed worldwide responsible for causing severe damage to soybean crops [*Glycine max* (L.) Merril], leading to yield reduction of up to 90% (YORINORI et al., 2005; HARTMAN et al., 2015; GODOY et al., 2016a). Since the introduction of this endemic fungus in Brazil, ASR has had negative economic impacts on soybean production, demanding approximately US$ 2 billion per year for its control (GODOY; BUENO; GAZZIERO, 2015).

This disease is characterized by necrotic lesions in the abaxial part of the host leaves with numerous uredia in each lesion. The spores are hyaline and the fungus penetrates directly through the cuticle (REIS; BRESOLIN; CARMONA, 2006). The damage caused by ASR is the reduction in the number of pods, number of grains, and grains weight, due to premature plant defoliation (OGLE; BYTH; McLEAN, 1979).

Until now, due to limited availability of soybean resistant varieties, fungicide application is the most used strategy for controlling ASR, although some populations of the pathogen have shown increased tolerance to certain active ingredients (GODOY et al., 2010). Currently, around 120 fungicides are registered in the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) for the control of ASR (GODOY et al., 2016b). The active ingredients registered to control this disease are in three major groups of systemic fungicides, formed by the triazoles (demethylation inhibitors - DMI), strobilurins (quinone outside inhibitors - QoI), and carboxamides (inhibition of succinate dehydrogenase - SDHI) (BUTZEN et al., 2005; FARIAS; ABDELNOOR; GODOY, 2014).

From the 2007/2008 crop season, it was observed that *P. pachyrhizi* populations in Brazilian fields were less sensitive to DMI fungicides, with a

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reduction in the disease control efficiency. On the other hand, the QoI fungicides always had lower control efficiency compared to DMIs, never being recommended alone. To avoid pathogen resistance and to maintain the chemical control of the disease, mixtures of DMI and QoI fungicides were performed added by multisite fungicides. However, since the 2013/2014 crop season, their efficiency reduced due to a decrease in the efficiency of the fungicide QoI in controlling the ASR fungus (FARIAS; ABDELNOOR; GODOY, 2014). Despite that scenario, it was observed that the performance of fungicides containing SDHI remained efficient. Nevertheless, for the first time, in the 2015/2016 crop season and particularly in the 2016/2017, in areas with an intensive use of SDHI fungicides and under high-pressure conditions, an efficiency reduction was detected (FRAC, 2017).

In order to verify the efficiency of the fungicides and to monitor the sensitivity behavior of the ASR to the control mechanisms in the market, great efforts have been invested in studies of the sensitivity of *P. pachyrhizi*. Several methods have been described to measure the sensitivity of a fungus to a given fungicide, or to monitor its sensitivity reduction or loss, or even the fungitoxicity of a chemical (FRAC, 1991; RUSSEL, 2004; BLUM, 2009; BLUM; REIS, 2013; REIS; DEUNER; ZANATTA, 2015).

Taking into account the various reports of the reduction of efficiency of fungicides against *P. pachyrhizi*, there is not enough information about which fungicides are still effective in controlling ASR. The aim of this study was to evaluate the efficiency of thirteen fungicides available in the market (Azoxystrobin-Priori Xtra®, Mancozeb-Unizeb Gold®, Prothioconazole-Fox®, Fluxapyroxad-Orkestra®, Tebuconazole-Folicur®, Benzovindiflupyr-Elatus®, Cyproconazole-Alto 100®, Trifloxystrobin-Sphere Max®, Pyraclostrobin-Comet®, Chlorothalonil-Previnil®, Epoxiconazole-Ativum®, Copper oxychloride-Difere® and a new carboxamide) in controlling ASR based on spore germination and evaluation of disease severity in detached leaves.

MATERIALS AND METHODS

Populations of *P. pachyrhizi* and active ingredients

P. pachyrhizi spores were obtained from soybean leaf samples from two different regions of Brazil: Minas Gerais (MG) and Mato Grosso do Sul (MS), in 2017. To collect the spores, naturally infected leaves were brushed onto the abaxial surface with a camel's hair brush. The spores collected were resuspended in deionized water and the concentration adjusted: Uberlândia 10^5 and Chapadão do Sul to 10^4 spores mL^{-1} and used soon after preparation.

In the experiments, the following 13 fungicides were tested at concentrations of 0.1; 1; 10; 50 and 100 ppm of active ingredient: Azoxystrobin-Priori Xtra®, Mancozeb-Unizeb Gold®, Prothioconazole-Fox®, Fluxapyroxad-Orkestra®, Tebuconazole-Folicur®, Benzovindiflupyr-Elatus®, Cyproconazole-Alto 100®, Trifloxystrobin-Sphere Max®, Pyraclostrobin-Comet®, Chlorothalonil-Previnil®, Epoxiconazole-Ativum®, Copper oxychloride-Difere® and a carboxamide standard (positive control). Calculus considered the main active ingredient cited above of each commercial fungicide and deionized water was used for dilutions and as negative control.

Detached leaf test

Considering the biotrophic nature of the fungus, this assay was conducted *in vivo* according to the methodology of detached leaves proposed by the Fungicide Resistance Action Committee (SCHERB; MEHL, 2006). Soybean cultivar 'Desafio' was cultivated in greenhouse for 20 days (maximum and minimum temperatures of 35±5°C and 22±2°C, respectively) to obtain the unifoliolate leaves. Detached leaves were immersed in different concentrations of the fungicides for approximately five seconds, and then placed on a filter paper saturated with deionized water, with the abaxial surface upwards, in acrylic boxes (Gerbox type). After 24 hours, spore suspensions of *P. pachyrhizi* from both populations were prepared with the respective concentration and sprayed with an airbrush to leaves. For each fungicide and concentration, four soybean leaves were inoculated (approximately 400 μL of spore suspension per leaf). Subsequently, the acrylic boxes were incubated in a humid B.O.D. chamber in the dark for 12 hours followed by 14 hours light at 22±2ºC. Three weeks after inoculation, infected leaf area was estimated by visual scoring (severity percentage - %) and by the number of lesions and sporulant pustules of *P. pachyrhizi* per cm^2. Control treatment consisted of soybean leaf samples treated only with deionized water. This experiment was carried out in a completely randomized design with four replicates.

In vitro spore germination test

The effects of the active ingredients on the germination of *P. pachyrhizi* spores were tested on

Petri dishes containing water-agar (2%) medium. The medium was autoclaved and cooled before the addition of the fungicides. Calculus considered the main active ingredient cited before of each commercial fungicide and used deionized water for dilutions. The spores suspensions of *P. pachyrhizi* were prepared in deionized water (at concentrations previously mentioned for each population) and 200 µL applied to each dish and spread with a Drigalski handle. Subsequently, the Petri dishes were incubated in the dark for 24h at 20ºC. After this incubation period, the percentage of germinated spores was quantified.

Statistical analysis

The experiments were conducted in a completely randomized factorial design with thirteen fungicides at five concentrations, for both MG and MS populations. Disease severity and spore germination were converted into percentage for each treatment, compared to control, and subjected to logarithmic and exponential regressions analysis using the statistical program R (R DEVELOPMENT CORE TEAM, 2017) with aid of the ExpDes for function implementation and calculus execution (FERREIRA; CAVALCANTI; NOGUEIRA, 2013). The concentrations capable of inhibiting 50 and 95% of spore germination and disease severity (EC_{50} and EC_{95}, respectively) for each population and fungicide were calculated based on the generated equations. Averages of fungicides treatments and their differences to the control (without chemical application) were compared by Tukey and Dunnett tests, respectively, at 0.05 significance level.

RESULTS AND DISCUSSION

Different sensitivity was observed between the Brazilian populations as fungicides conferred distinct efficiency in inhibiting spore germination and disease control (Tables 1 and 2). Even though, higher concentrations of active ingredients led to better response in reducing pathogen infection regardless of the site where spores were collected. Among tested fungicides, copper oxychloride exhibited lower efficiency for ASR control, providing average reduction of disease severity of 40.7 and 51.1% for MG and MS, respectively, compared to controls (without fungicide treatment). Those averages corresponded to 95 and 91% for prothioconazole, highlighting its efficacy.

A significant reduction in the sensitivity of the pathogen to the fungicide benzovindiflupyr was observed in Figure 1 regarding the spore germination test for Chapadão do Sul - MS population, which recorded EC_{50} value (effective concentration) above 6.68 ppm. In experiments previously carried out by Juliatti (2013) with sensitive populations of Uberlândia - MG and Rondonópolis - MT, the minimum inhibitory concentration to prevent spores germination was between 0.1 and 1.0 ppm for benzovindiflupyr, azoxystrobin and pyraclostrobin. At present, a minimum inhibitory concentration above 5 ppm for the two fungicides (azoxystrobin and benzovindiflupyr) is required.

The results demonstrate that there has been a reduction in sensitivity of the fungus to these fungicides in an order of magnitude of 5-50 folds. In the last four crop seasons, F129L mutation has been reported in relation to strobilurins (KLOSOWSKY et al., 2016) and mutations in the subunit C at position I86F, which reduces the sensitivity to SDHIs (FRAC, 2017).

Populations collected in 2015/2016 were monitored indicating a reduction in sensitivity to benzovindiflupyr, partially also observed in the 2016/2017 samples. Intensive monitoring programs are being conducted to investigate the magnitude and relevance of these findings (FRAC, 2017). However, as can be seen in Figures 1 to 5, the change occurred only in the carboxamide benzovindiflupyr, which does not result in the presence of a cross mutation in the genome of the pathogen.

The significance of this result is the difference in sensitivity between carboxamide fungicides. Further analysis of these alterations with sequencing of the genome in the affected regions may support the evidence obtained in this study. Juliatti, Bortolin and Bauti (2015) evaluated the sensitivity of *P. pachyrhizi* in *in vitro* and *in vivo* bioassays and pointed out the need for the use of multisite fungicides (chlorothalonil, mancozeb, cuprics, etc.) associated to penetrant mobile fungicides (carboxamides, strobilurins, and triazoles) to reduce the directional selection, and to establish strategies to stabilize control efficiency, and thus, slow down the process in progress. The authors pointed out that many triazole and strobilurin fungicides have ASR control below 30%.

Table 1. *In vitro* germination of *Phakopsora pachyrhizi* spores and severity of soybean rust disease in detached leaves due to increasing concentrations of fungicides. Fungus population from Uberlândia-MG.

Fungicide (a.i.)	Concentration (ppm)[1]				
	0.1	**1**	**10**	**50**	**100**
	GERMINATION (%)				
	Control (0 ppm): 51.25				
Cyproconazole	18.75 ab	15.00 cde	10.00 bc	0.00 b	0.00 b
Epoxiconazole	10.00 b	16.25 cd	0.00 c	0.00 b	0.00 b
Pyraclostrobin	17.50 ab	10.00 cde	11.25 b	0.00 b	0.00 b
Copper oxychloride	27.50 a	52.50^{ns} a	30.00 a	10.00 ab	25.00 a
Benzovindiflupyr	22.50 a	20.00 bc	5.00 bc	5.00 ab	0.00 b
Tebuconazole	17.50 ab	8.75 de	5.00 bc	0.00 b	0.00 b
Prothioconazole	21.25 a	5.00 e	5.00 bc	0.00 b	0.00 b
Fluxapyroxad	22.50 a	18.75 bcd	15.00 b	10.87 a	10.00 b
Chlorothalonil	17.50 ab	27.50 b	0.00 c	0.00 b	0.00 b
Azoxystrobin	22.50 a	10.00 cde	10.00 bc	6.25 ab	0.00 b
Trifloxystrobin	17.50 ab	10.00 cde	5.00 bc	0.00 b	0.00 b
Standard	10.00 b	10.00 cde	5.00 bc	0.00 b	0.00 b
Mancozeb	22.50 a	5.00 e	0.00 c	0.00 b	0.00 b
	SEVERITY (%)				
	Control (0 ppm): 67.50				
Cyproconazole	47.50 ab	12.50 cd	0.00 e	0.00 b	0.00 a
Epoxiconazole	35.00 b	6.25 cd	0.00 e	0.00 b	0.00 a
Pyraclostrobin	45.00 ab	42.50 ab	32.50 bc	35.00 a	0.00 a
Copper oxychloride	50.00 a	50.00 a	50.00 a	40.00 a	10.00 a
Benzovindiflupyr	10.00 cd	6.25 cd	0.00 e	0.00 b	0.00 a
Tebuconazole	35.00 b	12.50 cd	0.00 e	0.00 b	0.00 a
Prothioconazole	10.00 cd	5.00 cd	0.00 e	0.00 b	0.00 a
Fluxapyroxad	42.50 ab	35.00 b	12.50 de	1.25 b	0.00 a
Chlorothalonil	47.50 ab	47.50 ab	35.00 bc	47.50 a	0.00 a
Azoxystrobin	42.50 ab	47.50 ab	25.00 cd	0.00 b	0.00 a
Trifloxystrobin	15.00 c	15.00 c	0.00 e	0.00 b	0.00 a
Standard	0.00 d	0.00 d	0.00 e	0.00 b	0.00 a
Mancozeb	45.00 ab	42.50 ab	40.00 ab	35.00 a	5.00 a

a.i.: active ingredient; ^{ns}: non-significant according to the Dunnett test at 0.05 significance level; [1]averages followed by different letters, in each column, are statistically different by the Tukey test at 0.05 significance level.

Table 2. *In vitro* germination of *Phakopsora pachyrhizi* spores and severity of soybean rust disease in detached leaves due to increasing concentrations of fungicides. Fungus population from Chapadão do Sul-MS.

Fungicide (a.i.)	Concentration (ppm)[1]				
	0.1	**1**	**10**	**50**	**100**
	GERMINATION (%)				
	Control (0 ppm): 15.00				
Cyproconazole	3.75 cd	6.25^{ns} ab	0.00 b	0.00 b	0.00 a
Epoxiconazole	5.00^{ns} bcd	0.00 b	0.00 b	0.00 b	0.00 a
Pyraclostrobin	0.00 d	2.50 ab	0.00 b	1.25 ab	0.00 a
Copper oxychloride	12.50^{ns} abc	12.50^{ns} a	7.50^{ns} b	12.50^{ns} a	2.50 a
Benzovindiflupyr	10.00^{ns} abcd	3.75 ab	10.00^{ns} ab	7.50^{ns} ab	0.00 a
Tebuconazole	20.19^{ns} a	2.50 ab	0.00 b	2.50 ab	0.00 a
Prothioconazole	0.00 d	2.50 ab	0.00 b	1.25 ab	1.25 a
Fluxapyroxad	16.25^{ns} ab	0.00 b	2.50 b	0.00 b	0.00 a
Chlorothalonil	7.50^{ns} bcd	0.00 b	0.00 b	0.00 b	0.00 a
Azoxystrobin	10.00^{ns} abcd	7.50^{ns} ab	5.00^{ns} b	2.50 a	3.75 a
Trifloxystrobin	0.00 d	3.75 ab	20.00^{ns} a	0.00 b	0.00 a
Standard	15.00^{ns} abc	0.00 b	0.00 b	0.00 b	0.00 a
Mancozeb	0.00 d	0.00 b	0.00 b	2.50 a	0.00 a
	SEVERITY (%)				
	Control (0 ppm): 56.00				
Cyproconazole	25.00 c	42.50 a	50.00 a	0.00 b	0.00 b
Epoxiconazole	5.00 d	5.00 c	0.00 d	0.00 b	0.00 b

Pyraclostrobin	50.00^{ns} a	25.00 b	31.00 bc	31.00 a	0.00 b
Copper oxychloride	25.00 c	25.00 b	25.00 c	31.00 a	31.00 a
Benzovindiflupyr	37.00 b	25.00 b	25.00 c	0.00 b	0.00 b
Tebuconazole	31.00 bc	25.00 b	6.00 d	0.00 b	0.00 b
Prothioconazole	25.00 c	0.00 c	0.00 d	0.00 b	0.00 b
Fluxapyroxad	31.00 bc	25.00 b	0.00 d	0.00 b	0.00 b
Chlorothalonil	25.00 c	25.00 b	25.00 c	0.00 b	0.00 b
Azoxystrobin	37.00 b	31.00 b	0.00 d	0.00 b	0.00 b
Trifloxystrobin	25.00 c	6.00 c	0.00 d	0.00 b	0.00 b
Standard	0.00 d	0.00 c	0.00 d	0.00 b	0.00 b
Mancozeb	50.00 ^{ns} a	50.00 ^{ns} a	37.00 b	25.00 a	0.00 b

a.i.: active ingredient; ^{ns}: non-significant according to the Dunnett test at 0.05 significance level; [1]averages followed by different letters, in each column, are statistically different by the Tukey test at 0.05 significance level.

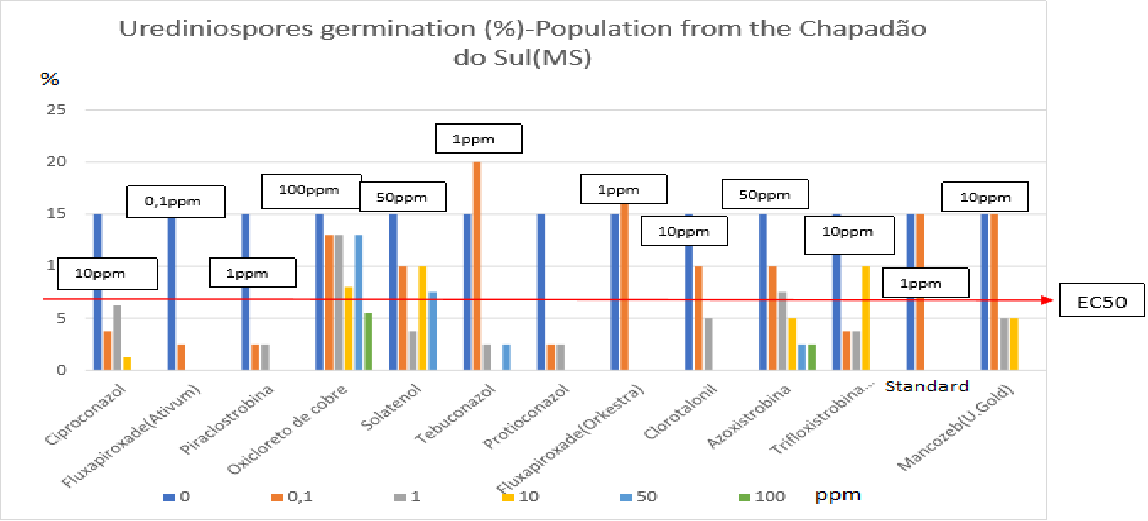

Figure 1. Sensitivity of *Phakopsora pachyrhizi* urediniospores from Chapadão do Sul - MS to fungicides in different concentrations, 2016/2017 crop season.

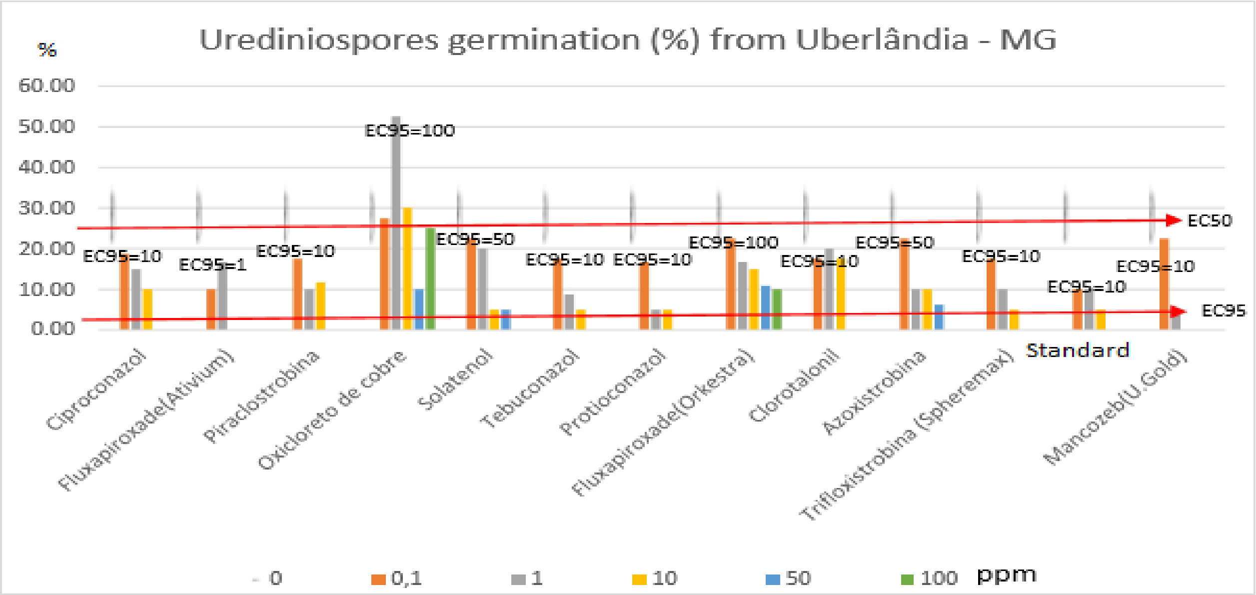

Figure 2. Sensitivity of *Phakopsora pachyrhizi* urediniospores from Uberlândia - MG to fungicides in different concentrations, crop season 2016/2017.

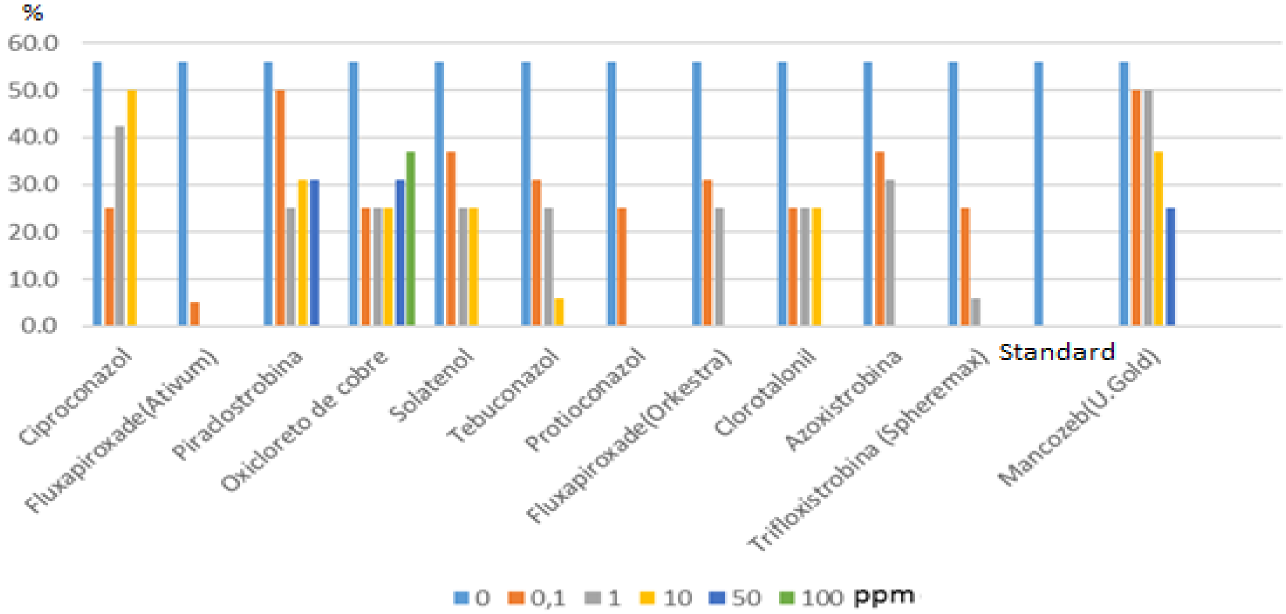

Figure 3. Soybean rust (*P. pachyrhizi*) appraised severity (%) after treatment of soybean leaves with multisite, triazole, strobilurin, and carboxamide fungicides in different concentrations (0 to 100 ppm). Population of spores from Chapadão do Sul - MS, crop season 2016/2017.

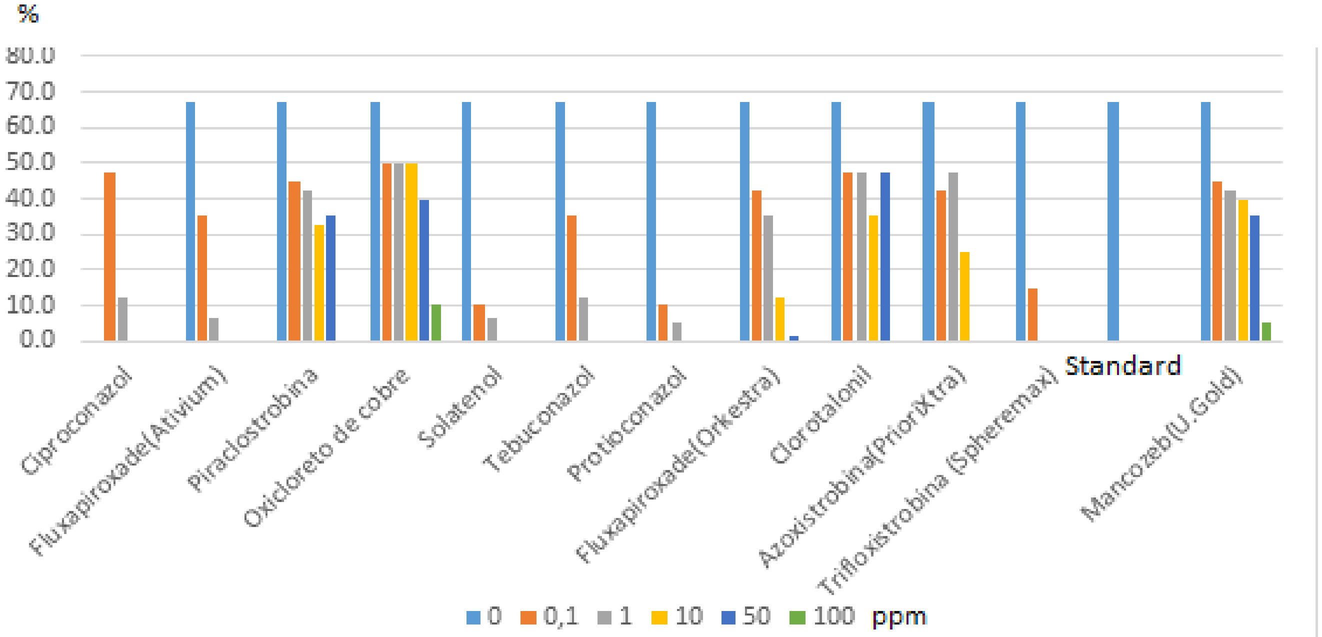

Figure 4. Rust severity (%) (*P. pachyrhizi*), on leaves of soybean (cultivar 'Desafio'), after 22 days of incubation at 22^{o}C, and under different concentrations of multisite, carboxamide, strobilurin, and triazole fungicides. Population from Uberlândia - MG, crop season 2016/2017.

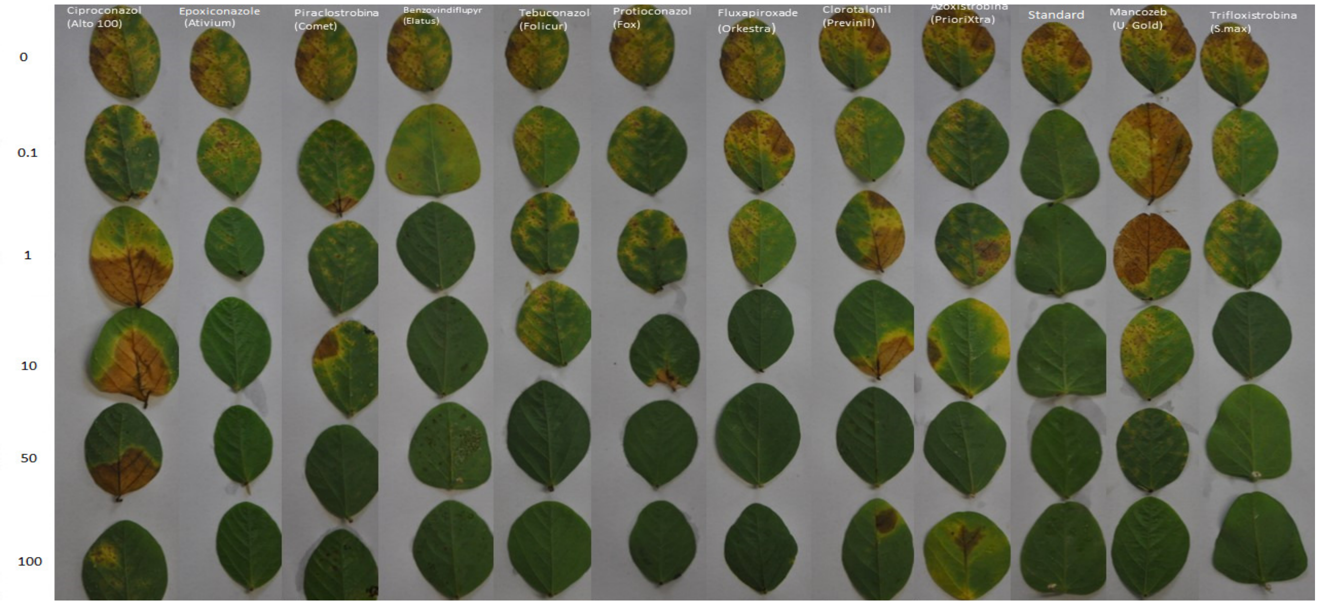

Figure 5. Soybean rust (Uberlândia population) in detached leaves, after incubation at 22°C and 22 days in different concentrations (0 to 100 ppm) of fungicides. Above (Left to right direction): Cyproconazole, Epoxiconazole, Pyraclostrobin, Benzovindiflupyr, Tebuconazole, Prothioconazole, Fluxapyroxad, Chlorothalonil, Azoxystrobin, Standard-Control, Mancozeb, and Trifloxystrobin.

When comparing Figures 1 and 2 regarding the fluxapyroxad formulation, in relation to the two sites, a differentiated sensitivity between the two *P. pachyrhizi* populations is observed (from Chapadão do Sul - MS and Uberlândia - MG). For this fungicide, basing the calculations in ppm on the carboxamide and neglecting the other active ingredient, an EC_{50} = 1 ppm is observed for the population of Chapadão do Sul - MS and 100 ppm for Uberlândia - MG. For the fungicide epoxiconazole, the minimum inhibitory concentration was between the values of 0.01 to 0.03 ppm for the two sites and, therefore, did not undergo significant change in relation to the germination of urediniospores. It is noteworthy that for the multisite fungicides chlorothalonil, mancozeb, and copper oxychloride the inhibitory concentrations to kill 50% of the population (EC_{50}) reached values between 10-100 ppm for both populations of spores. These results demonstrate the care that must be taken when reducing the dose of multisite fungicides in the field and the loss or low effectiveness of the field strategy to reduce the action of directional pressure in relation to the use of systemic fungicides. Fixed or insoluble copper compounds such as oxychloride showed higher efficiency at concentrations equal to or above 100 ppm and therefore should be used in higher concentrations when compared to organic multisite fungicides such as chlorothalonil and mancozeb, which are more efficient in the antimicrobial strategy-resistance when evaluated through the germination of the pathogen spores and disease severity in detached leaves ((Tables 1 and 2 and Figures 1-4). Figure 5 shows the behavior of the fungicides tested in order of increasing concentration of 0 to 100 ppm in relation to the spores collected in Uberlândia - MG.

In Figures 3 and 4, epoxiconazole continued to be highly effective in reducing pathogen severity (0.1 to 1 ppm) for both populations (MG and MS), as well as the prothioconazole active ingredient. Benzovindiflupyr exhibited high effectiveness only for the population of Uberlândia - MG. In relation to multisite fungicides, they showed their greatest effectiveness in concentrations equal to or above 100 ppm. Similar to the spore germination assay, in the severity evaluation the results maintained the same behavior or trends previously observed.

Other important information observed in this work was that since the concentrations were calculated from non-isolated samples of each active ingredient, but using the commercial product, which was formulated with another molecule, it is possible that the formulation and the active substances in each commercial product cause a positive or negative interference in the studies carried out. It is suggested and recommended from these results that the baseline or resistance monitoring studies be carried out with the commercial products since in the field no more isolated fungicides are used.

Tables 3, 4, 5 and 6 show the adjusted regression equations for all fungicides to reduce urediniospores germination (%) and disease severity

(%) based on the fifty and ninety inhibitory concentrations (EC_{50} and EC_{95}). The equations showed high accuracy and precision ($R^2 > 80\%$) and some equations obtained a little QMR (Medium Residual Square) for the proper models' adjustment. These reveal that these equations have high similarity between observed values and proposed values. The models varied between logarithmic and exponential, and these models were presented in all variables for both populations as demonstrated on tables 3, 4, 5, and 6. There is significant difference between fungicide sensibilities between both populations ($p < 0.05$). The amount of a.i. (active ingredient) to obtain the EC_{50} in detached leaves for the fungicides cyproconazole (0.004 mg L^{-1}), pyraclostrobin (4.79 mg L^{-1}), benzovindiflupyr (1.83 mg L^{-1}), tebuconazole (0.004 mg L^{-1}), fluxapyroxad (0.05 mg L^{-1}) and mancozeb (19.88 mg L^{-1}), were lower at the MG population in comparison with the MS population. On the other hand, the opposite was observed for epoxiconazole (1.54 mg L^{-1}), copper oxychloride (2.93 mg L^{-1}), prothioconazole (0.056 mg L^{-1}), chlorothalonil (1.97 mg L^{-1}), azoxystrobin (0.6 mg L^{-1}), and trifloxystrobin (0.0001 mg L^{-1}). Therefore, the EC_{50} in detached leaves for the fungicides with protection properties, as for mancozeb, was lower in MG and the copper oxychloride and chlorothalonil were lower for MS. The population of MG at the EC_{50} for detached leaves was more sensible to the carboxamides benzovindiflupyr and fluxapyroxad. The lower EC_{50} that obtained lesser germination for the MG population, was for the following fungicides cyproconazole (0.15 mg L^{-1}), epoxiconazole (0.01 mg L^{-1}), benzovindiflupyr (0.1 mg L^{-1}), tebuconazole (0.002 mg L^{-1}), prothioconazole (0.0001 mg L^{-1}), fluxapyroxad (0.006 mg L^{-1}), azoxystrobin (0.48 mg L^{-1}), trifloxystrobin (0.003 mg L^{-1}) and control (Standard) (0.002 mg L^{-1}). For the population of MS, the fungicides with lower EC_{50} were pyraclostrobin (0.009 mg L^{-1}), copper oxychloride (10.36 mg L^{-1}), chlorothalonil (0.009 mg L^{-1}) and mancozeb (0.0009 mg L^{-1}). Therefore, the EC_{50} for all three fungicides with protection properties, mancozeb, copper oxychloride and chlorothalonil, were lower for MS. The population of MG was more sensible at the EC_{50} for spore germination with the use of the carboxamides benzovindiflupyr and fluxapyroxad. For the variables EC_{50} and EC_{95} at spore germination and efficacy in detached leaves, the population at MG was more sensible for the carboxamide benzovindiflupyr. The fungicides with protection properties, copper oxychloride and chlorothalonil, have lower EC_{50} and EC_{95} at the population of Chapadão do Sul in the state of Mato Grosso do Sul. The fungicide mancozeb, showed a more mixed data for lesser EC_{50} and EC_{95}, with variability at MG population and MS population.

The data for EC_{95} percent of inhibition were overall more reliable than the EC_{50}, because of higher similarity of efficacy observed in the field, so the data for EC_{50} were usually overestimated. We can infer the occurrence of these phenomena for the great variability between repetitions. In future experiments, more replicates should be used to guarantee an improvement in the evaluation for these variables. The carboxamides loss of efficacy showed by field trials, and data from FRAC about the screening mutations, starting from this information we can infer there is difference in efficacy between both regions, for fluxapyroxad and benzovindiflupyr.

Table 3. Rust disease control in detached soybean leaves. Effective concentration (EC_{50}) of different active ingredients ranged from 0.004 mg L^{-1} (cyproconazole) to 69.31 mg L^{-1} (copper oxychloride). Fungus population from Uberlândia-MG.

Fungicide (a.i.)	Equation	R^2 (%)	EC_{50}	EC_{95}	QMR
Cyproconazole	f = 9.9937*exp(-0.1535*x)	99.72	0.004	4.63	0.39
Epoxiconazole	f = 9.9938*exp(-0.3072*x)	99.72	2.13	2.15	0.39
Pyraclostrobin	f = 100.0394/(1+exp(-(x- 39.9693)/ -3.3555))	99.55	4.79	ND	74.95
Copper oxychloride	f = 100.0056/(1+exp(-(x- 53.3329)/ -4.0904))	99.28	69.31	ND	119.90
Benzovindiflupyr	f = 9.9948*exp(-0.3102*x)	99.73	1.83	2.11	0.38
Tebuconazole	f = 9.9936*exp(-0.1534*x)	99.72	0.004	4.64	0.38
Prothioconazole	f = 10.0000*exp(-0.3454*x)	100	3.16	3.77	0
Fluxapyroxad	f = 83.1045*exp(-0.1456*x)	89.71	0.05	40.12	1703.56
Chlorothalonil	f = 100.8181/(1+exp(-(x- 40.0598)/ -8.4424))	91.24	23.74	99.25	1450.06
Azoxystrobin	f = 50.0065*exp(-0.0565*x)	99.07	2.96	37.69	34.60
Trifloxystrobin	f = 10.0000*exp(-0.1450*x)	99.42	0.007	4.84	0.81
Standard	NA	-	-	-	-
Mancozeb	f = 100.8170/(1+exp(-(x- 40.0859)/ -7.0620))	89.60	19.88	ND	1720.61

a.i.: active ingredient; NA: not adjusted to standard mathematical models; ND: not determined (values above 100 ppm); QMR: Medium Residual Square for the adjusted model.

Table 4. Sensitivity of *Phakopsora pachyrhizi* (germination of spores) towards different active ingredients. EC$_{50}$-values from all tested fungicides ranged from 0.0001 mg L^{-1} (prothioconazole) to 12.23 mg L^{-1} (copper oxychloride). Fungus population from Uberlândia-MG.

Fungicide (a.i.)	Equation	R^2 (%)	EC$_{50}$	EC$_{95}$	QMR
Cyproconazole	f = 50.0287*exp(-0.1167*x)	98.38	0.15	27.91	59.95
Epoxiconazole	f = 10.0018*exp(-0.1356*x)	99.97	0.011	5.01	0.04
Pyraclostrobin	f = 50.0471*exp(-0.1221*x)	98.17	0.11	27.17	67.70
Copper oxychloride	f = 104.9945/(1+exp(-(x- 36.8461)/ -6.4937))	87.77	12.23	ND	2024.04
Benzovindiflupyr	f = 100.0047*exp(-0.1385*x)	90.33	0.1	50.03	1600.59
Tebuconazole	f = 50.0636*exp(-0.1974*x)	99.72	0.002	18.65	10.32
Prothioconazole	f = 50.0000*exp(-0.2538*x)	97.82	0.0001	14.05	81.01
Fluxapyroxad	f = 12641.59*exp(-0.2883*x)	86.77	0.006	ND	2190.42
Chlorothalonil	f = 9.9991*exp(-0.0859*x)	99.70	0.13	6.50	0.41
Azoxystrobin	f = 101.7755*exp(-0.1070*x)	92.55	0.48	59.60	1232.41
Trifloxystrobin	f = 50.0339*exp(-0.1892*x)	99.95	0.003	19.42	1.79
Standard	f = 50.0835*exp(-0.2016*x)	99.55	0.002	18.27	16.62
Mancozeb	f = 10.0000*exp(-0.2648*x)	99.99	1.77	2.66	0.02

a.i.: active ingredient; ND: not determined (values above 100 ppm); QMR: Medium Residual Square for the adjusted model.

Table 5. Rust disease control in detached soybean leaves. Effective concentration (EC$_{50}$) of different active ingredients ranged from 0.0001 mg L^{-1} (trifloxystrobin) to 22.78 mg L^{-1} (mancozeb). Fungus population from Chapadão do Sul-MS.

Fungicide (a.i.)	Equation	R^2 (%)	EC$_{50}$	EC$_{95}$	QMR
Cyproconazole	f = 50.1097*exp(-0.0448*x)	99.44	5.33	40.05	20.67
Epoxiconazole	f = 10.0000*exp(-0.3597*x)	99.42	1.54	1.65	0.81
Pyraclostrobin	f = 100.0956/(1+exp(-(x- 35.9362)/ -5.1686))	90.33	6.18	99.84	1600.01
Copper oxychloride	f = 50.0290*exp(-0.0567*x)	97.26	2.93	37.67	87.08
Benzovindiflupyr	f = 50.0290*exp(-0.0567*x)	97.65	2.93	37.67	87.08
Tebuconazole	f = 49.9962*exp(-0.1660*x)	99.95	0.012	21.80	1.77
Prothioconazole	f = 1.0001*exp(-0.0574*x)	99.99	0.056	0.75	0.0001
Fluxapyroxad	f = 10.0005*exp(-0.0665*x)	99.61	0.35	7.17	0.55
Chlorothalonil	f = 50.0008*exp(-0.0646*x)	96.77	1.97	36.19	119.96
Azoxystrobin	f = 9.9995*exp(-0.0560*x)	99.60	0.60	7.55	0.56
Trifloxystrobin	f = 10.0000*exp(-0.2379*x)	99.99	0.0001	3.04	0.02
Standard	NA	-	-	-	-
Mancozeb	f = 102.6292*exp(-0.0301*x)	92.32	22.78	88.28	1271.86

a.i.: active ingredient; NA: not adjusted to standard mathematical models; QMR: Medium Residual Square for the adjusted model.

Table 6. Sensitivity of *Phakopsora pachyrhizi* (germination of spores) towards different active ingredients. EC$_{50}$-values from all tested fungicides ranged from 0.0009 mg L^{-1} (mancozeb) to 10.36 mg L^{-1} (copper oxychloride). Fungus population from Chapadão do Sul-MS.

Fungicide (a.i.)	Equation	R^2 (%)	EC$_{50}$	EC$_{95}$	QMR
Cyproconazole	f = 10.0000*exp(-0.6906*x)	99.27	1.00	5.01	1.01
Epoxiconazole	f = 1.0000*exp(-0.0691*x)	100	0.031	0.70	0.0001
Pyraclostrobin	f = 10.0000*exp(-0.1382*x)	100	0.009	5.01	0.0001
Copper oxychloride	f = 96.4741/(1+exp(-(x- 23.2995)/ -12.6140))	84.70	10.36	78.15	2532.18
Benzovindiflupyr	f = 102.0414*exp(-0.0545*x)	98.00	6.68	77.70	330.64
Tebuconazole	f = 10.0000*exp(-0.0695*x)	100	0.30	7.06	0.0005
Prothioconazole	f = 10.0000*exp(-0.1740*x)	99.42	0.0016	4.18	0.81
Fluxapyroxad	f = 10.0015*exp(-0.0705*x)	99.96	0.29	7.03	0.05
Chlorothalonil	f = 10.0000*exp(-0.1382*x)	99.99	0.009	5.01	0.02
Azoxystrobin	f = 332.6498*exp(-0.0828*x)	83.00	5.29	ND	2813.27
Trifloxystrobin	f = 10.0000*exp(-0.0921*x)	100	0.10	6.30	0
Standard	f = 1.0001*exp(-0.0464*x)	99.98	0.098	0.79	0.0002
Mancozeb	f = 1.0000*exp(-0.1382*x)	100	0.0009	0.50	0

a.i.: active ingredient; ND: not determined (value above 100 ppm); QMR: Medium Residual Square for the adjusted model.

CONCLUSIONS

It was observed a reduction of benzovindiflupyr efficiency in relation to spore germination and disease severity for the population of Chapadão do Sul - MS. The same behavior was found for fluxapyroxad, cyproconazole, and tebuconazole. Multisite fungicides (chlorothalonil, copper oxychloride, and mancozeb) may be used in the management of ASR resistance in the fields of Brazil associated with strobilurins, triazoles, and carboxamides. The efficiency of the main active ingredient tested depends on the formulation and others molecules used in the commercial product.

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RESUMO: A ferrugem asiática da soja (FAS), causada pelo fitopatógeno *Phakopsora pachyrhizi* Sydow & Sydow, é uma doença fúngica distribuída mundialmente e responsável por causar danos na cultura da soja [*Glycine max* (L.) Merril], em até 90% do potencial produtivo. Até agora, devido à limitada disponibilidade de variedades resistentes, a aplicação de fungicida é a estratégia mais utilizada para controlar a FAS, embora algumas populações do patógeno tenham demonstrado menor sensibilidade a determinados ingredientes ativos. Vários métodos foram descritos para medir a sensibilidade de um fungo a um dado fungicida, ou para monitorar sua redução ou perda de eficácia, ou mesmo a fungitoxicidade de um produto químico. Os testes mais utilizados são a germinação de esporos *in vitro* e a severidade da doença (%) em folhas de soja destacadas. Baseando-se nessas metodologias, foram realizados ensaios com populações do patógeno oriundas de Uberlândia - MG e do Chapadão do Sul - MS. Os resultados mostraram a redução da eficiência de benzovindiflupyr em relação à germinação e à severidade de doença para a população de Chapadão do Sul - MS. O mesmo comportamento foi obtido para os ingredientes ativos fluxapyroxad, cyproconazole e tebuconazole. Os fungicidas multissítios (clorotalonil, oxicloreto de cobre e mancozeb) podem ser utilizados no manejo da resistência à FAS nas lavouras do Brasil associados às estrobilurinas, triazóis e carboxamidas. A eficiência do principal ingrediente ativo estudado depende dos outros ingredientes ativos ou fungicidas associados na formulação comercial.

PALAVRAS-CHAVE: Bioensaio. Baseline. Ferrugem asiática da soja. Controle químico. Resistência a fungicidas.

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