

NOTAS CIENTÍFICAS

***In vitro* Determination of Fungicide Inhibitory Concentration for *Phakopsora pachyrhizi* isolates**

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ABSTRACT

Moura, B; Boller, W.; Deuner, C.C. *In vitro* Determination of Fungicide Inhibitory Concentration for *Phakopsora pachyrhizi* isolates. *Summa Phytopathologica*, v.42, n.2, p.170-171, 2016.

In vitro assays were performed to obtain the IC₅₀ of eight fungicides against *Phakopsora pachyrhizi* isolates from Passo Fundo, RS, Ponta Grossa, PR, and Primavera do Leste, MT. Different concentrations of the fungicides were added to Petri dishes containing soybean leaf extract agar medium. One milliliter of *P. pachyrhizi* uredospore suspension at the concentration

of 3.0 x 10⁴ uredospores/mL was added to each dish for subsequent viability quantification. Only pyraclostrobin and the mixture trifloxystrobin + prothioconazole showed IC₅₀ values inferior to 1.0 mg/L for all tested isolates, demonstrating high fungitoxicity. There was not loss of sensitivity to any of the tested fungicides.

Keywords: IC₅₀, Asian soybean rust, fungitoxicity, sensitivity loss.

RESUMO

Moura, B; Boller, W.; Deuner, C.C. Concentração inibitória *in vitro* de fungicidas a isolados de *Phakopsora pachyrhizi*. *Summa Phytopathologica*, v.42, n.2, p.170-171, 2016.

Em ensaios *in vitro*, foram obtidos os valores da CI₅₀ de oito fungicidas para o controle de *Phakopsora pachyrhizi* provenientes de Passo Fundo, RS, Ponta Grossa, PR e Primavera do Leste, MT. Diferentes concentrações dos fungicidas foram incorporadas ao meio ágar-extrato de folhas de soja em placas de Petri. Um mL da suspensão de uredosporos de *P. pachyrhizi* na

concentração de 3,0 x 10⁴ uredosporos/mL foi acrescentada a cada placa, para posterior quantificação da viabilidade. Somente a piraclostrobina e a mistura trifloxistrobina + protriocanazol apresentaram valores de CI₅₀ inferiores a 1,0 mg/L para todos os isolados testados, demonstrando alta fungitoxicidade. Não houve perda de sensibilidade a nenhum dos fungicidas testados.

Palavras-chave: CI₅₀, ferrugem-asiática-da-soja, fungitoxicidade, perda de sensibilidade.

Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi* Sydow & P. Sydow, is one of the most destructive plant diseases in Brazil and a great threat to the country's soybean production (8). Chemical management of ASR started in Brazil during the 2002-2003 season (6) and since then it has been the main disease management strategy.

Pathogen sensitivity to a fungicide is measured based on the IC₅₀, which is the fungicide concentration (mg/L) that inhibits 50% of spore germination or mycelial growth (5).

According to Edgington & Klew (3), for a chemical to be considered a fungicide, its IC₅₀ for a specific pathogen should not be greater than 50 mg/L. Fungicides that are considered highly toxic have an IC₅₀ that does not exceed 1 mg/L. An IC₅₀ value between 1 and 50 mg/L would be defined as moderately toxic. We hypothesized that *P. pachyrhizi* sensitivity to fungicides has reduced along the seasons they were used.

The aim of this study was to determine the *in vitro* IC₅₀ values of eight fungicides against *P. pachyrhizi* isolates and to determine the sensitivity reduction factor (SRF) by comparing the new IC₅₀ values to those already reported in the literature.

Isolates of *P. pachyrhizi* were obtained from naturally infected plants during the 2013-2014 season from the cities Passo Fundo, Ponta

Grossa and Primavera do Leste.

The assays were conducted in disposable plastic Petri dishes containing 15 mL of 2% agar water + 0.5% fresh soybean leaf extract mixed with fungicide suspensions at the following concentrations: 0; 0.01; 0.1; 1; 10; 20; 40 and 50 mg/L. The tested fungicides were: epoxiconazole, pyraclostrobin, azoxystrobin, trifloxystrobin, pyraclostrobin + epoxiconazole, trifloxystrobin + prothioconazole, pyraclostrobin + fluxapyroxad and azoxystrobin + benzovindiflupyr.

Dishes containing the culture medium received 1 mL of uredospore suspension at a concentration of 3.0 x 10⁴ uredospores/mL. The dishes were incubated in a Biochemical Oxygen Demand (BOD) chamber, at 22°C, for six hours, in the dark. For each dish, 100 uredospores were counted and the percentage of germinating uredospores was calculated. These values were used in Probit regression procedure to estimate the IC₅₀ values. The sensitivity reduction factor (SRF) was calculated by dividing the estimated IC₅₀ values by the baseline IC₅₀ values obtained from the literature (2, 7).

Results show that pyraclostrobin and trifloxystrobin + prothioconazole were fungicides of high toxicity for all *P. pachyrhizi* isolates (Table 1).

Epoxiconazole was highly toxic to Primavera do Leste isolate but

Table 1. *In vitro* inhibitory concentration (IC₅₀) of fungicides to inhibit uredospore germination of *Phakopsora pachyrhizi* isolates, and sensitivity reduction factors (SRF) based on new IC₅₀ and baselines.

Active Ingredient	Location*	IC ₅₀	SRF ¹	SRF ²
Epoiconazole (125 g/L SC)	A	11.42	25.37	10.01
	B	9.77	21.71	8.57
	C	0.86	1.91	0.75
Pyraclostrobin (133 g/L SE) + Epoiconazole (50 g/L SE)	A	0.13	-	-
	B	0.13	-	-
	C	3.15	-	-
Pyraclostrobin (250 g/L EC)	A	0.69	9.20	6.27
	B	0.36	4.80	3.27
	C	0.14	1.87	1.27
Trifloxystrobin (150 g/L SC) + Prothioconazole (175 g/L SC)	A	0.29	-	-
	B	0.27	-	-
	C	0.37	-	-
Pyraclostrobin (333 g/L SC) + Fluxapyroxad (167 g/L SC)	A	12.86	-	-
	B	0.73	-	-
	C	17.69	-	-
Azoxystrobin (300 g/L WDG) + Benzovindiflupyr (150 g/L WDG)	A	1.38	-	-
	B	0.16	-	-
	C	1.39	-	-
Azoxystrobin (250 g/L WDG)	A	32.98	471.14	-
	B	1.77	25.28	-
	C	9.83	140.42	-
Trifloxystrobin (500 g/L WDG)	A	15.63	1042	-
	B	0.41	27.33	-
	C	5.69	379.33	-

*A - Passo Fundo; B - Ponta Grossa; C - Primavera do Leste. ¹Baseline IC₅₀ obtained from Blum et al. (2). ²Baseline IC₅₀ values obtained from Reis et al (7).

only moderately toxic to Passo Fundo and Ponta Grossa isolates. This difference may be due to a fungicide resistance strategy used in Mato Grosso State since the 2007-2008 season, which replaced DMIs with mixtures of QoI + QoI (4). On the other hand, the mixture of QoI + DMI (pyraclostrobin + epoxiconazole) was moderately toxic to Primavera do Leste isolate but highly toxic to Passo Fundo and Ponta Grossa isolates.

The IC₅₀ compared to previously reported values, confirm an increase in the sensitivity reduction factor (SRF) (2) for epoxiconazole, azoxystrobin, pyraclostrobin and trifloxystrobin. The QoIs azoxystrobin and trifloxystrobin showed high SRF values. However, in comparison with the data from Reis et al. (7), the SRF of epoxiconazol (DMI) decreased for Primavera do Leste isolate.

Overall, the data show that no fungicide had sensitivity loss, but only reduction. IC₅₀ values varied among fungicides and regions.

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