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

Bianca Moura1,2, Walter Boller1, Carolina Cardoso Deuner1

1Universidade de Passo Fundo, FAMV/UPF, Passo Fundo, 99052-290, RS. 2Bolsista da Capes.

ABSTRACT

In vitro assays were preformed 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 \times 10^4 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, fungitoxicidade, sensitivity loss.

RESUMO


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 \times 10^4 uredosporos/mL foi acrescentada a cada placa, para posterior quantificação da viabilidade. Somente a piraclostrobin e a mistura trifloxystrobin + prothioconazole apresentaram valores de CI50 inferiores a 1,0 mg/L para todos os isolados testados, demonstrando alta fungitoxidade. Não houve perda de sensibilidade a nenhum dos fungicidas testados.


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 uredospor suspension at a concentration of 3.0 \times 10^4 uredosporos/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 uredosporos were counted and the percentage of germinating uredosporos 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...
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 IC\textsubscript{50} 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. IC\textsubscript{50} values varied among fungicides and regions.

**ACKNOWLEDGEMENTS**

We are thankful for the support from PPGAgro/UPF, CAPES and Dr. Erlei M. Reis. We thank José de Freitas from Fundação ABC and Tatiane Zambiasi for supplying the isolates.

**REFERENCES**