



Species:	<i>Phakopsora pachyrhizi</i>
Product Class(es):	Strobilurin fungicides
Method type described:	In vitro agar plate test
Date of protocol:	2006-12
Proven for	Azoxystrobin
Should be suitable for	Other strobilurin / QoI Fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	<ul style="list-style-type: none">• validated routine method for labs equipped to carry out agar plate tests• proven methodology for the active ingredient listed above. Others not mentioned have to be evaluated carefully to ensure valid results

Introduction

The sensitivity of *Phakopsora pachyrhizi* isolates to strobilurins is determined by comparing the germination of uredospores of each isolate on water agar with soybean leaves extract (WASLE) amended (or not) with fungicide.

Method:

1. PREPARATION OF 2% WATER AGAR WITH SOYBEAN LEAVES EXTRACT¹ (WASLE) AMENDED OR NOT WITH THE FUNGICIDE

1) Collect 5 g of soybean leaves and grind them with 1 litre of distilled water (deionised water can also be used)

2) Sift the extract with a tissue

¹ Personal communication Dr. Erlei Melo Reis Universidade Passo Fundo-RS 9 (erleireis@tpo.com.br)

- 3) For each fungicide concentration, prepare 150 mL of 2% water agar with soybean leaves extract (WASLE), adding 3 g of Agar-Agar²;
- 4) Autoclave the agar medium for 20 minutes at 121 C;
- 5) Prepare the fungicide solutions using commercial product (it should also be possible to use technical grade product, diluted in acetone or other solvent).
- 6) For azoxystrobin the range of concentration could be between 0.005 - 0.34 mg/L (=ppm).
- 7) When the flasks with the agar media are cooled to a temperature at which they can be held with comfort, add the fungicide;
- 8) Dispense at least 10 mL of agar with or without fungicide into Petri Plates of 3.5 - 9 cm.
- 9) Keep the plates at room temperature for a maximum of 3 days;

2. PRODUCTION OF UREDOSPORES

The uredospores can be produced on whole plants or detached leaves. For this, use seeds of a susceptible soybean variety.

When using whole plants, use plants at V2-V3 stage. Prepare a suspension of the uredospores (100,000/mL) with distilled water and spray the leaves. It is important that during the inoculation the leaves do not become too wet. Put the plants on hermetic chambers to avoid cross contamination. Incubate at 25°C, 12 hours of dark/ 12h of light.

When using detached leaves, use leaves at V2-V3 stage; prepare Petri plates (15 cm) with 2 humid paper filters. Detach the healthy leaves and put them into the Petri plate. Put humid cotton pieces around the petiole leaves. Prepare a suspension of the uredospores (100,000/mL) with distilled water and spray the leaves. It is important that in the inoculation the leaves do not become too wet. Incubate at 25°C, 12 hours of dark/ 12h of light.

For both methods, it is possible after 10 days to see some yellow lesions and after 15 days it is already possible to observe sporulation of the lesions ("pustules").

3. SENSITIVITY TEST

The sensitivity of *Phakopsora pachyrhizi* isolates is determined as follows:

1. Add 100 µl of the uredospores suspension (1×10^4 uredospores/mL) to the control plates and to plates containing the fungicide tested;
2. Spread the uredospore suspension onto the agar with a glass rod

² Agar-Agar Granulated Merck 1.01614

3. For each treatment use 5 plates (5 replications);
4. Let the uredospore suspension dry on the agar before closing the plate (do not seal the plates with parafilm);
5. Incubate plates under continuous dark, at 25°C.

4. TEST EVALUATION

1. The number of germinated uredospores out of 100 (%) is determined after 6 hours;
2. A uredospore is rated as germinated if a normally developing germ tube is at least the length of the uredospore;
3. The effective dose for 50% control of the uredospore germination (ED50 values) is calculated by regressing the uredospore germination data against the log of the fungicide concentration.



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Uredospore germinated



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Uredospore not germinated

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