



Species:	<i>Phakopsora pachyrhizi</i>
Product Class(es):	SBI fungicides, also suited for other fungicide classes
Method type described:	detached leaf test
Date of protocol:	2006-12
Proven for	Tebuconazole, Prothioconazole
Should be suitable for	other SBI-Fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	<ul style="list-style-type: none"> <li>• proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results.</li> <li>• validated routine method for labs equipped with climate chambers and/or greenhouses</li> </ul>

Method:

1. To obtain representative data from different soybean rust populations, samples of uredospores can be taken directly in the field by tapping the fully developed spores into a suitable dry tube or by random sampling of infected leaves. Tubes or leaves should be sent immediately after sampling within a dry plastic bag or paper bag to the testing facility. If available, samples should be despatched within an ice containing box to maintain the leaves in good condition.

Samples have to be taken during the peak phase of the epidemic whenever possible. Optimal sampling time seems to be after rain days, high air humidity in the beginning or end of the day.

Generally, in the laboratory, uredospores from sampled leaves should be tapped off short time before the sensitivity test.

If necessary, the spores can be propagated on 2-3 week old greenhouse plants grown under semi-standardized conditions (see below), or in Petri dishes on detached leaves. The sensitivity test described below mostly don't require a propagation step depending on the amount of leaves and spore viability of a given sample.

2. Soybean plants should be cultivated in the greenhouse under semi-standardized conditions in order to obtain leaves of similar age and size necessary for the detached leaf test. Therefore, a suitable or susceptible variety should be used (i.e. variety `CD 201, CD 205 or CD 208`). Unifoliated leaves of 3 weeks old plants have shown to be well suited for the test.

The semi-standardized cultivation allows a temperature range within the greenhouse from 18°C (minimum) up to 35°C (maximum) during the day or test period with a medium temperature of mostly around 23°C. The relative humidity in the greenhouse should not be lower than 40% but can also adjusted up to 100%.

A suitable photo period of around 12h per day can be controlled by greenhouse lights depending on the season or regional location of the testing facility at which the monitoring study is carried out.

3. Determine the SBI sensitivity of each rust population on a test set of detached leaves cut from plants grown under greenhouse conditions as described above.

For each fungicide concentration to be tested, dip four leaves into fungicide amended water (commercial available, single formulated product - no fungicide mixture) for around three seconds. Fungicide treatments should be graded logarithmically by a relatively small factor of two or three in order to obtain an optimal EC<sub>50</sub> evaluation for the sensitivity towards the active substance (depending on the compound).

Examples:

- Tebuconazole (Folicur EC200):  
0, 0.1, 0.3, 1, 3 mg/l ppm
- Prothioconazole (Proline EC250):  
0, 0.1, 0.3, 1, 3 mg/l ppm

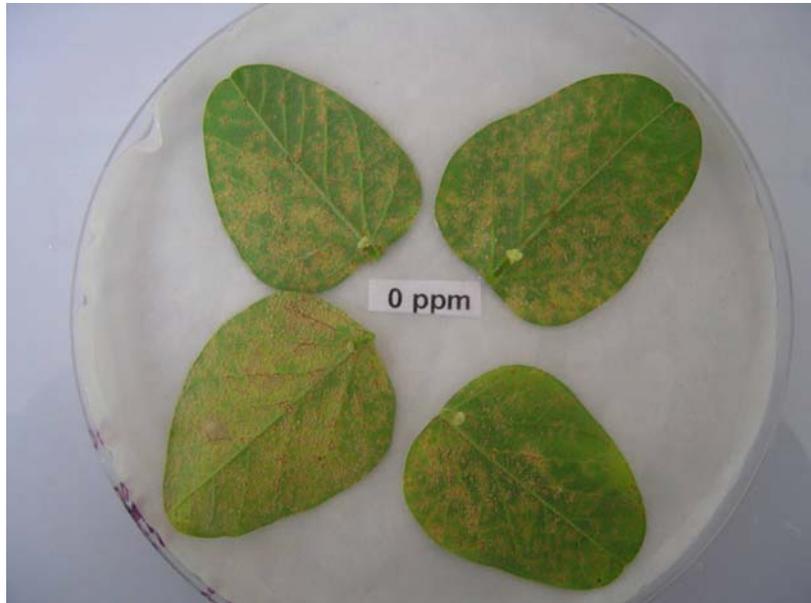
This type of fungicide application allows a comparison of obtained sensitivity values between different populations and years for the used single test compound, but not a direct sensitivity comparison between different products or SBI compounds depending on the characteristics of different formulation ingredients and their amounts.

Alternatively, depending on the available lab equipment, leaves can also be spray treated. This type of fungicide application is more exact and reflects better practical conditions but is more laborious and time consuming.

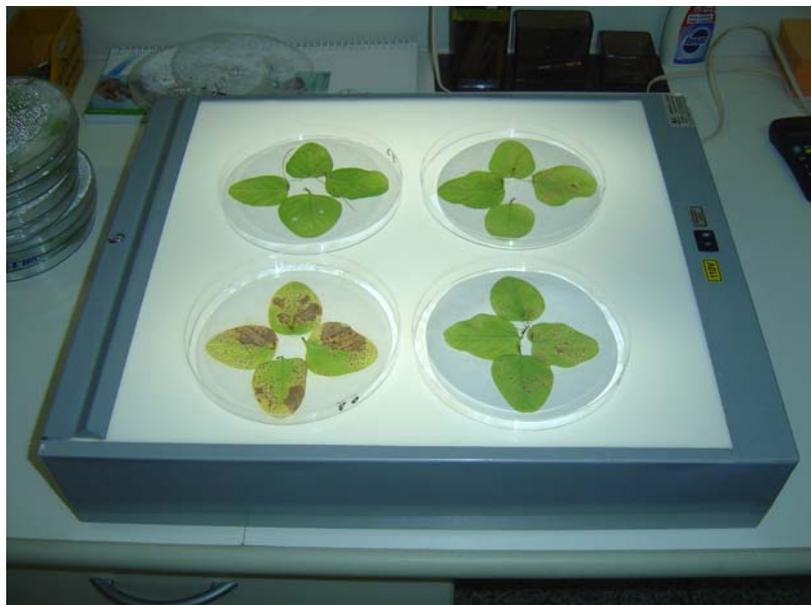
4. After the treated leaves have been dried, lay them top side down on wet filterpaper within a petri dish (Ø 15cm, Picture 1).
5. Prepare uredospore suspensions (100ml distilled water, 1 drop of Tween 20, 10<sup>5</sup> spores tapped off the sample leaves) just before inoculation of the leaves.
6. To avoid gas phase interactions between differently treated leaves in the test assortment, use separate disposable Petri dishes for each fungicide concentration in every single test set. As repetition, each Petri dish should contain several leaves (i.e. four leaves, see above) of different plants. A test assortment for a single population with four concentrations of the respective compound therefore consists of five Petri dishes including the untreated control.
7. The dishes of a test set should be preferably only placed next to each other during the inoculation phase. Inoculate the leaves with the spore suspension by air pressure (air brush - 1ml per Petri dish).
8. After an incubation period (lidded petri dishes in climate chamber: 20°C, 12h continuous light per day, minimum relative humidity: 60%) of fifteen days, score

each test assortment macroscopically regarding disease coverage/development in comparison to the untreated control (in percentage, see also Picture 2).  
Finally, calculate EC<sub>50</sub> values of each test population.

Picture 1: Example of a soybean rust detached leaf infection within a Petri dish  
- untreated control -



Picture 2: Macroscopically disease evaluation of a detached leaf test set



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