



Species:	<i>Blumeria / (Erysiphe) graminis f.sp. tritici</i>
Product Class(es):	SBI fungicides, also suited for other fungicide classes
Method described:	leaf segment test (detached leaf method)
Date of protocol:	2006-05
a.i.'s proven	Tebuconazole, Prothioconazole, Spiroxamine
Should be suitable for	other SBI's and also QoI. 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> <li>• the method was developed and provided by the company EpiLogic, Germany (Dr. F.G. Felsenstein)</li> </ul>

## Method:

1. To obtain representative data from different mildew populations, samples of conidio-spores can be taken by spore traps (mobile or stationary) (a) or by random sampling of infected leaves (b).
  - a) Conidio-spores are collected (trapped) onto segments of primary leaves from a highly susceptible variety placed in Petri dishes on water agar (0.6 % agar, 35 mg/l benzimidazole). Samples have to be taken during the peak phase of the epidemic whenever possible. Let the sampled spores grow to single colony isolates (climate chamber: 18 °C, 10-20 µmol/m<sup>2</sup>s continuous light) and transfer them onto fresh leaf segments on water agar for maintenance and for propagation before testing.
  - b) Conidio-spores from sampled leaves should be propagated on fresh leaf segments placed in Petri dishes on water agar as mentioned in a).

2. Determine the SBI sensitivity of each single spore progeny on a test set of leaf segments which are placed on benzimidazole agar (6 mg/l agar-agar, 35 mg/l benzimidazole).

One day before cutting the leaf segments, treat 10 day old plants (first to second leaf stage) with the compound to run off conditions. Use technical active ingredient. 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:  
0, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24 mg/l
- Prothioconazole:  
0, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, 20.48, 40.96, 81.92 mg/l
- Spiroxamine:  
0, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80, 160 mg/l

Keep differently treated plants strictly separated to avoid gas phase interactions. A day after the spray treatment of the plants, cut out the leaf segments. To avoid gas phase interactions between differently treated leaf segments 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 segments of different plants treated with the same concentration. A test assortment for a single isolate with ten concentrations of the respective compound therefore consists of ten Petri dishes including the untreated control.

3. The dishes of a test set should be only placed next to each other during the inoculation phase. Inoculate the leaf segments with the single spore progeny by air pressure (dry conditions - no aqueous spore solution).
4. After an incubation period (climate chamber: 18 °C, 10-20 µmol/m<sup>2</sup>s continuous light) of ten days, score each test assortment macroscopically regarding disease coverage/development in comparison to the untreated control (percentage). Then, calculate EC<sub>50</sub> of each test isolate by Probit analysis.

References:

FELSENSTEIN FG, 1991. Virulenz und Fungizidsensitivität des Weizenmehltaus, *Erysiphe graminis* DC f.sp. *tritici* Marchal, in Europa. Dissertation (PhD Thesis), Technische Universität München, Freising-Weihenstephan, 168 S (pp).

FELSENSTEIN FG, 1994. Sensitivity of *Erysiphe graminis* f.sp. *tritici* to demethylation inhibiting fungicides in Europe. Fungicide Resistance, BSPC Monograph 60, 35-42.

FELSENSTEIN FG, KUCK K-H, 1998. Spiroxamine-Empfindlichkeit des Weizen- und Gerstenmehltaus: Bestimmung der Ausgangssituation und Einschätzung des Resistenzrisikos. Pflanzenschutz-Nachrichten Bayer 51/1998, 1, 5-24.

English version: Spiroxamine sensitivity of wheat and barley powdery mildew:  
Determination of baseline sensitivity and assessment of the risk of resistance.  
Pflanzenschutz-Nachrichten Bayer 51/1998, 1, 5-24.

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