Species:	*Uncinula* / *Erysiphe necator*
Product Class(es):	SBI fungicides, also suited for other fungicide classes
Method type described:	leaf disc test (detached leaf method)
Date of protocol:	2006-05
Proven for	Tebuconazole, Spiroxamine, Triadimenol
Should be suitable for	other SBI and also QoI's. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	• proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results • validated routine method for labs equipped with climate chambers and/or greenhouses • the method was developed and kindly provided by the company EpiLogic, Germany (Dr. F.G. Felsenstein)

Method:

1. Sampling:
   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 fresh leaf material from a highly susceptible variety placed in Petri dishes on water agar (0.6% agar, 35 mg/l benzimidazole). Samples can be taken up to the last stage of the growing season, when the peak phase of spore concentration occurs in the air as the effect of previous fungicide applications is declining. Let the sampled spores grow to single colony isolates (climate chamber: 22°C, 25

µmol/m²s, 12/12 h light/darkness period, 70 % RH) and transfer them onto fresh leaf material on water agar for maintenance and propagation before testing.

b) Conidio-spores from sampled leaves should be propagated on fresh leaf material placed in Petri dishes on water agar as mentioned in a).

2. Analysis:
   Multiply isolates for the SBI sensitivity bio-assay tests by transferring several single conidia chains per isolate onto two to three disinfected fresh leaves. Spray vine plants (whole plants cultured under cellophane bags) with the respective fungicide solutions to run-off conditions one day before cutting the leaf discs (14 mm diameter) from young leaves and before inoculation of the test sets. The fungicide treatments should be graded logarithmically by a factor of two to three in order to obtain an optimal EC_{50} evaluation for the sensitivity towards the active compound (depending on the compound).

   Examples:

   - Tebuconazole:
     0, 0.3, 1.0, 3.0, 10.0, 30.0 mg/l
   - Spiroxamine:
     0, 3, 10, 30, 100, 300 mg/l
   - Triadimenol:
     0, 0.05, 0.25, 1,25, 6.25, 31.25, 156.25 mg/l a.i.

   Keep differently treated plants strictly separated to avoid gas phase interactions. Use separate disposable Petri dishes for each concentration to avoid gas phase interactions between differently treated leaf discs. Each Petri dish should contain four leaf discs from four different leaves treated with the same fungicid concentration. A test set for analysing one isolate involving, for example, six fungicide concentrations (including the untreated control) consists therefore of six Petri dishes.

3. Inoculation and Incubation:
   The dishes of a test set should be only placed next to each other under a settling tower for about 60 seconds throughout the inoculation phase (during the period of time where the leaf discs are exposed to conidia). Inoculate each test set from above by dispersing the conidia spores of the test isolate from well sporulating leaves by means of air pressure. Adjust the inoculation density generally of about 200-300 conidia per cm².
   Incubate test Petri dishes in a climate chamber for 14-16 days (22°C, 25 µmol/m²s, 12/12 h light/darkness period, 70 % RH). Petri-dishes with differently treated leaf discs should be separated from each other to avoid gas phase interactions.

4. After the incubation period, score the disease coverage relative to the untreated control. Calculate EC_{50} values of the bio-assayed isolates by Probit analysis.

References:

HASYN S, FELSENSTEIN FG, KUCK K-H, 2000. Untersuchungen zur Sensitivität des Echten Mehltaus an Reben (*Uncinula necator*) gegenüber Spiroxamine. Mitteilungen a. d. Biologischen Bundesanstalt f. Land- und Forstwirtschaft 376, 478.

HASYN S, FELSENSTEIN FG, 2002. Untersuchungen zur Sensitivität des Rebenmehltaus (*Uncinula necator*) gegenüber Trifloxystrobin. Mitteilungen a. d. Biologischen Bundesanstalt f. Land- und Forstwirtschaft 390, 357.

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