



Species:	<i>Venturia inaequalis</i>
Product Class(es):	Anilinopyrimidines and QoI fungicides, and also suited for other fungicide classes
Method type described:	Whole plant
Date of protocol:	2006-05
Proven for	Pyrimethanil
Should be suitable for	other Anilinopyrimidines, QoI and other classes of fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	<ul style="list-style-type: none"> <li>Validated routine method for labs with greenhouses</li> <li>proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results</li> </ul>

#### Method:

1. **Sampling:** A sample consists of ~20 apple leaves with sporulating scab lesions per location. The leaves are placed between layers of paper, wrapped in newspaper and then taken as quickly as possible to the laboratory, preferably in a cool box. If the sample is to be sent to the laboratory, the leaves should be air-dried and then placed between sheets of newspaper.
2. **Inoculum production:** Lesions from scab-infected leaves are cut out and conidia are harvested by rinsing ca. 40 - 60 lesions with deionised water and filtering the conidia suspension through four layers of cheese-cloth to remove debris. The concentration of conidia is determined and adjusted to approximately  $5 \times 10^5$  spores/ml.
3. **Plant production:** Apple seeds ('Golden Delicious') are vernalized in sand for 2-3 months at 3-6°C. The seeds are then grown for 3 weeks. The germinated apple seedlings are transferred and incubated at 20-24°C, 70-75% RH and an 18 h photoperiod for 3 weeks in the greenhouse. For powdery mildew control, the seedlings are exposed to sulphur vapour for 3-5 h at night.

4. Propagation: Conidia suspensions are sprayed with an airbrush onto the upper leaf surface of the youngest developed leaves on untreated plants. The plants are incubated in a darkened moist chamber at 99% RH and 18°C for 24 h. They are then kept in the greenhouse at ca. 70% RH, 22°C and 12 h light / 12 h dark. 13-20 days after inoculation the infected leaves with abundant sporulation of the fungus are harvested, dried and can be stored until required.
5. Sensitivity tests: The fungicide is suspended and diluted in water immediately before application. Different dilutions of the field rate should be used (e.g. 0, 100, 300 ppm for Scala, 40% pyrimethanil). The fungicides are applied in a spray-cabin onto both leaf surfaces until just before run off. Foliar application is made protectively, 24 h prior to inoculation. Three to five replicate plants are used for each fungicide concentration. Inoculation is carried out 24 h after the fungicide application and is made to the youngest fully open leaf (this is marked for future identification by cutting off the leaf tip). The method of inoculation and incubation are the same as described above.
6. Evaluation: 13-15 days after inoculation the percentage leaf area covered with sporulating scab lesions is estimated. For pyrimethanil, sporulating lesions at 300 ppm indicate the presence of less sensitive spores in the population of the sample.

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