



Species:	<i>Venturia inaequalis</i>
Product Class(es):	Anilinopyrimidines and SBI fungicides, and also suited for other fungicide classes
Method type described:	Whole plant
Date of protocol:	2006-03
Proven for	Difenoconazole, Cyprodinyl
Should be suitable for	other SBI- and Anilinopyrimidines. 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 with access to seed plants</li> </ul>

## Method:

### Sample Collection

30-50 leaves with apple scab symptoms, i.e. sporulative lesions, are collected at dry weather conditions. It is essential to have a break of at least 5 days between fungicide application and sampling process.

The samples are sent to the lab as quickly as possible. Only paper, no plastic material should be used to wrap the leaves. In order to avoid cross-contamination, different samples are packed separately.

All field samples have to be accompanied by a sampling sheet describing sampling date, cultivar, fungicide spray sequence, amount of fungicide, efficiency of product, and address of sampler.

In the order of their arrival in the lab, the sample and the sample sheet will be marked with a sample ID consisting of one or two letters, a number and a date, e.g. CH12.05 being the 12<sup>th</sup> sample from Switzerland in the year 2005.

### **Sample Processing and Propagation**

All the work has to be done in the clean bench. Between the processing of two samples, all instruments used have to be cleaned with 70% EtOH.

The isolates from the field are first propagated on untreated apple seedlings to obtain sufficient starting material for the sensitivity tests. Typical scab lesions with visible conidia are cut out from the leaves (appendix, photo 1) and collected in clean Petri dishes. An appropriate amount of these leaf fragments is soaked in deionised water and slightly stirred with a spatula. The suspension is filtered through hydrophilic gauze and conidia are counted using a Neubauer counting chamber. The density of the spore suspension should be within the range of 100'000 – 350'000 conidia/ml. Ready suspensions have to be processed within one hour; meanwhile they are stirred at regular intervals to prevent the conidia from precipitating.

The spore suspension is sprayed onto the whole apple seedlings with a chromatography sprayer. A thin layer of vaporized spore suspension should be visible. The plants are thereafter covered with a grid and a nylon cloth and incubated in a climate chamber for 48h (no light, 18°C, 90% relative humidity, i.e. 8 seconds intermittent misting every 2 minutes). Then, the plants are taken to the greenhouse and incubated for another 13 days or until enough conidia are visible (20°C in the day, 18°C in the night, 14 hours of light, 60% relative humidity). The leaves are harvested, dried and stored in Petri dishes at room temperature.

### **Sensitivity Tests**

The field isolates are tested on apple seedlings (3-5 leaf stage) treated either with cyprodinil (Chorus WG50) or difenoconazole (Score WP10). The concentrations are as follows: For CDL 0ppm, 0.3ppm, 3ppm, 10ppm, 30ppm, 100ppm, 300ppm. For DFZ 0ppm, 0.1ppm, 1ppm, 3ppm, 10ppm, 30ppm, 100ppm.

The tests with CDL and DFZ can be carried out at the same time.

Per concentration step mentioned above, 6 apple seedlings (7 for the 0ppm control) are treated with fungicide. For the application, a turntable spray cabinet is used. The control plants (0ppm) are sprayed with water only.

The day after fungicide treatment, the plants are inoculated with *Venturia inaequalis* spore suspension (200'000 – 300'000 conidia/ml) which is to be sprayed onto the upper surface of the two youngest, still shiny, leaves of apple seedlings (3-5 leaf stage). The plants are thereafter incubated on the same conditions as mentioned in "Sample Processing and Propagation". After 2 days in the climate chamber, the plants are taken to the greenhouse and incubated for another 13-14 days. The test is then evaluated by visual assessment.

After the test, all plant material has to be autoclaved.

### **Evaluation and Data Processing**

The visual assessment of the test is done by estimating the percentage of leaf area which is attacked by *Venturia inaequalis*. These values are fed into an excel sheet. The numbers in each concentration step are sorted by size. In order to decrease the effect of the biological variation, the EC50 is calculated with only the three biggest values of each fungicide concentration. EC50 values are calculated using Agstat 1.59

(Syngenta internal). To obtain the sensitivity distribution, the EC50 values are compared to EC50 values of sensitive and less sensitive reference strains.

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