



<b>Species:</b>	<i>Botrytis cinerea</i>
<b>Product Class(es):</b>	Anilinopyrimidines and QoI fungicides, and also suited for other fungicide classes
<b>Method type described:</b>	Whole plant
<b>Date of protocol:</b>	2006-05
<b>Proven for:</b>	Pyrimethanil
<b>Should be suitable for:</b>	Other anilinopyrimidines and other classes of fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
<b>Version:</b>	1
<b>Comments:</b>	<ul style="list-style-type: none"> <li>• Validated routine method for labs</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:** Samples of *Botrytis cinerea* are collected by lightly touching sporulating lesions of infested fruits with a sterile cotton swab (one berry or fruit per swabs) which are sealed in sterile plastic tubes for transportation to the test laboratory. It is recommended to take 10 to 20 samples per site.
2. **Inoculum production:** Testing is carried out using the freshly prepared spore suspension from the cotton swabs. This is prepared by rinsing under agitation each swab with 2.5 ml of Sabouraud Malt Broth containing 1% mycological peptone plus 2% maltose. 50 µl of the resulting suspension is then used to inoculate each apple.

3. Sensitivity test: Each *Botrytis cinerea* isolates sampled is inoculated on two apples, variety Golden Delicious, one treated with pyrimethanil, one non-treated. In each apple, a hole of 8 mm diameter and 3-4 mm depth is made. This hole should not be too deep other that symptoms could develop under the apple skin and this assessment would be difficult.

The concentration of the pyrimethanil solution used for apple treatment is 20ppm (0.1 mg of pyrimethanil technical grade are dissolved in 2 ml of DMSO, then this solution is dissolved in water in order to obtain the treatment concentration).

The control apple is treated with 50 µl sterile water; the test apple is treated with 50 µl of the 20 ppm pyrimethanil solution.

Inoculation is carried out 2 hours after treatment at the minimum in order to let the product penetrated in the apples. Then apples are put in dark condition at 18-20°C.

4. Evaluation: symptom observed is a necrosis of the apple around the inoculation point. 5 days after inoculation, the diameter of the necrosis is measured (mean of the 2 diameters taken at right angle). Then the percentage of efficacy is calculated according to the following formula:

$$[(\Phi \text{ control} - 8) - (\Phi \text{ treated} - 8)] / (\Phi \text{ control} - 8) \times 100$$

Each Isolate showing a percentage of efficacy inferior to 50% are retested at the same concentration, but using 3 replicates.

If the percentage of efficacy inferior to 50% is confirmed, the isolate is considered as less sensitive.

## Reference

B Forster & E Müller (1996). In vivo method for monitoring cyprodinil sensitivity in populations of *Botrytis cinerea*. EPPO Bulletin 26, 191-194

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