Species:	*Botrytis cinerea*
Product Class(es):	SDH-inhibitors (SDHI), Qo-inhibitors (QoI)
Method type described:	Microtiter test
Date of protocol:	2009-01
Proven for	Boscalid, pyraclostrobin
Should be suitable for	Other fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	2
comments	• Validated routine method • Proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results

Method:

1. Sampling: Samples of *Botrytis cinerea* are collected by lightly touching sporulating lesions with a cotton swab. These swabs can be placed in 2 ml reaction tubes and sent to laboratory.

2. Isolation of *Botrytis cinerea* from field samples: Spores from these cotton swabs are transferred to the surface of Petri dishes containing 2% malt agar + 200 ppm streptomycin. The Petri dishes are then incubated at 18°C until sporulating colonies of *B. cinerea* had grown, and these are then checked under the microscope for contamination.

3. Spore production: A portion of a sporulating colony of *B. cinerea* is then transferred onto 2% malt agar. The dishes are incubated at 18°C for 5-7 days under UV-light to promote dense sporulation. The spores are harvested with a

cotton swab and this is dipped in 3 ml of double concentrated YBA-medium*. The resulting suspension is filtered through 2 layers of cheesecloth and the suspension is adjusted to a spore density of $2x10^4$ / ml.

4. Sensitivity tests: Pure technical active ingredient is solved in dimethylsulfoxide and the dilutions are prepared in sterile deionised water immediately before mixing with the spore suspension. 50 µl fungicide solution and 50 µl spore suspension are mixed in 96-well microtiter plates. The following final concentrations of e.g. boscalid or pyraclostrobin are used in the microtiter assays: 0, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 ppm active ingredient. For each isolate and fungicide concentration, three replicate wells were used. Three replicate wells were also used per fungicide concentration as blanks (fungicide solution + YBA medium). The microtiter plates are put into plastic bags to avoid evaporation and incubated at 18°C in darkness. Five days after inoculation the growth is measured in a photometer at 405 nm. The values are corrected by comparison with the blanks. The ED values are compared with sensitive standard isolates.

5. References: G. Stammler and J. Speakman (2006). Microtiter method to test the sensitivity of *Botrytis cinerea* to boscalid. *Journal of Phytopathology* **154**, 508-510.

*Yeast Bacto Acetate medium, 2 x concentrated

20 g yeast extract
20 g Bacto peptone
40 g sodium acetate
fill up to 1000 ml with bidest water

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