Species:	*Sclerotinia sclerotiorum*
Product Class(es):	SDH-inhibitors (SDHI), QoI
Method type described:	Tissue culture plate assay with mycelial plugs
Date of protocol:	2008-12
Proven for	Boscalid, Dimoxystrobin
Should be suitable for	Other fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
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 *Sclerotinia sclerotiorum* are collected by harvesting sclerotia from infected plant tissue. These sclerotia can be placed in paper envelopes and sent to laboratory.

2. Isolation of *Sclerotinia sclerotiorum* from sclerotia: Three to five isolates are made from each sample by transferring surface disinfected sclerotia (with 70% ethanol) in Petri dishes containing 2% malt extract agar + 30 mg/l streptomycin. Pure cultures are grown for 5-7 days on 2% malt extract agar. Mycelial plugs (6 mm diameter) taken from the margin of the colonies are used as inoculum for the sensitivity test.

3. Sensitivity tests: Approximately 500 µl fungicide-amended YBA medium (10 g yeast, 10 g Bacto peptone, 20 g sodium acetate in 1 l deionized water) are added to each well of 24-well tissue culture plates with 16.2 mm well diameter. The final fungicide concentrations are 0, 0.01, 0.03, 0.1, 0.3 and 1.0 mg

boscalid per litre or 0, 0.03, 0.1, 0.3, 1 and 3 mg dimoxystrobin per litre. Each well is then inoculated with a mycelial plug, with the mycelium surface up. Two replicate wells are used for each isolate and fungicide concentration. The tissue culture plates are put into plastic bags to reduce evaporation, and incubated at 18°C in darkness.

4. Evaluation: Growth is assessed visually 9 days after inoculation: 0 = no growth, 1 = slight growth, 3 = mycelium covered up to 30% of the well, 6 = up to 60%, 10 = up to 100%. ED_{50} values (concentration at which growth was inhibited by 50% relative to the untreated control) are calculated for each isolate by probit analysis. Additionally, MIC values (lowest concentration at which growth was completely inhibited) can be determined. A baseline analysis showed that all isolates were completely inhibited at the highest concentration used. For more detailed description of the method and fungicide sensitivity please refer to Stammler *et al*. 2007.

5. References: G. Stammler, G. Benzinger and J. Speakman (2007). A rapid and reliable method for monitoring the sensitivity of *Sclerotinia sclerotiorum* to boscalid. *Journal of Phytopathology* **155**, 746-748.

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