Species:	*Pyrenophora teres*
Product Class(es):	SDH-inhibitors (SDHI)
Method type described:	Microtiter test
Date of protocol:	2009-01
Proven for	Boscalid
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 and isolation of strains: Dried leaves with net blotch symptoms are collected from the field and send to the laboratory between paper sheets. Strains are isolated from single lesions after incubation on wet filter paper in Petri dishes for 3 to 4 days at 12 h darkness at 18°C and 12 h light at 22°C. Single spores are harvested and transferred on 2% malt agar + 30 ppm streptomycin. Plates are incubated at 24°C in the darkness for 3-4 days and then transferred ob POA*.

2. Spore production: For spore production, isolates are cultivated on POA* and incubated at 12 h darkness at 18°C / 12 h light at 22°C for 10 days. The spores are harvested with a Drigalski and 3-4 ml double concentrated YBG** medium. The suspension is filtered through two layers of cheesecloth and the suspension is adjusted to a spore density of $4x10^3$ / ml.

3. 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. Fifty µl fungicide solution and 50 µl spore suspension are mixed in 96-well microtiter plates. Final concentrations of boscalid are 0, 0.0025, 0.01, 0.04, 0.156, 0.625, 2.5 and 10 ppm. For each isolate and fungicide concentration, three replicate wells are used. Three replicate wells are also used per fungicide concentration as blanks (fungicide solution + YBG 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_{50} values are calculated by probit analysis and compared with sensitive standard isolates.

*Peanut oakmeal agar (POA)

50 g peanut leaf extract
15 g Schmelzflocken (Kölln)
20 g agar

fill up 1000 ml with bidest water

**Yeast Bacto Glycerol medium (YBG), 2 x concentrated

20 g yeast extract
20 g Bacto peptone
40 ml glycerol

fill up to 1000 ml with bidest water

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