Species:	*Oculimacula yallundae, Oculimacula acuformis*
Product Class(es):	SDH-Inhibitors (SDHI)
Method type described:	Microtiter test
Date of protocol:	2009-02
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 ingredient listed above. Others not mentioned have to be evaluated carefully to ensure valid results

Method:

1. Sampling and isolation of strains: Dried wheat stems with *Oculimacula* symptoms are collected from the field and send to the laboratory between paper sheets. Strains are isolated from infected stem pieces after surface disinfection and incubation on 2% malt agar + 30 ppm streptomycin till mycelial growth is visible. Mycelium is harvested and transferred on PDA* + 30 ppm streptomycin.

2. Spore production: A portion of mycelium of *Oculimacula* spp. is then transferred onto PDA* and incubated at 12 h darkness / 12 h light for 14 days. The spores are scrubbed from the Petri dish surface with 3 ml of double concentrated YBG-medium** and filtered through 2 layers of cheese cloth. The suspension is adjusted to a spore density of $3x10^4$ / 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, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 ppm. For each isolate and fungicide concentration, four replicate wells are used. Four 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. Seven days after inoculation the growth is measured in a photometer at 405 nm. The values are corrected by comparison with the blanks. ED_{50} values are calculated by probit analysis and compared with those of sensitive standard isolates.

*Potato dextrose Agar (PDA)

39 g Difco Potato Dextrose Agar
10 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

*** Alternatively formulated product can be used, solved and diluted in sterile water

Authors	Simone Mießner, Dr. Gerd Stammler, BASF SE, 67117 Limburgerhof, Germany gerd.stammler@basf.com