Species:	*Mycosphaerella graminicola (Septoria tritici)*
Product Class(es):	SDH-inhibitors (SDHI), demethylation inhibitors (DMI)
Method type described:	Microtiter test
Date of protocol:	2009-01
Proven for	Boscalid, pyraclostrobin, epoxiconazole, metconazole, prochloraz
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 *Septoria* symptoms are collected from the field and send to the laboratory between paper sheets. Strains are isolated from single pycnidia after surface disinfection and incubation on 2% malt agar overnight. Pycnidiospores are harvested and transferred on MYA* + 30 ppm streptomycin.

2. Spore production A portion of a colony of *Septoria tritici* is then transferred onto MYA* and incubated at 12 h darkness / 12 h light for 6-10 days. The spores are harvested with a cotton swab and this is dipped in 3 ml of double concentrated YBG-medium** and the suspension is adjusted to a spore density of $1.6x10^4$ / 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 and of epoxiconazole, metconazole, prochloraz 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3 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. 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.

4. References:

G. Stammler, L. Kern, M. Semar, A. Glättli and U. Schöfl (2008). Sensitivity of *Mycosphaerella graminicola* to DMI fungicides related to mutations in the target gene cyp51 (14α-demethylase). In: *Modern Fungicides and Antifungal Compounds V.* pp. 137-142.

G. Stammler, M. Carstensen, A. Koch, M. Semar, D. Strobel and S. Schlehuber (2008). Frequency of different CYP51-haplotypes of *Mycosphaerella graminicola* and their impact on epoxiconazole-sensitivity and -field efficacy. *Crop Protection* **27**, 1448-1456.

*Malt Yeast Agar (MYA)

10 g malt
4 g yeast extract
4 g glucose
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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