Species:	*Mycosphaerella graminicola, Septoria tritici*
Product Class(es):	SBI fungicides, also suited for other fungicide classes
Method type described:	microtiter plate test
Date of protocol:	2006-05
Proven for	Tebuconazole, Prothioconazole, Epoxyconazole
Should be suitable for	other SBI and also QoI's. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	• validated routine method for labs equipped with microtiter plate technique • proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully and methods may have to be adjusted accordingly (e.g. solvents, concentrations) to ensure valid results

Method:

1. Sample *Septoria tritici* infected leaves with visible developed pycnidia, selected at random from several plants from the field. To get an acceptable statistical result take not less than 20 leaves ideally taken diagonally from the field. Don't sample immediately after rainfall. The samples should be air-dried for 24h before dispatching not to become mouldy. The leaves should be transported in paper bags.

2. Spread the infected leaves in Petri dishes on water agar and incubate them for 24h at room temperature. Isolate freshly developed spores from the pycnidia by removing them with a sterilized needle. Put the spores from a single pycnidium onto Czapek-Dox vegetable juice agar plates, each.

3. After 6 days of incubation at 18°C, the spores originating from a single colony can be harvested and used for further testing.

4. Microtiter plate test: Incubate harvested spores (3) in 3 ml sterile glucose peptone medium (glucose 14.3 g/l; peptone 7.1 g/l) for 24h at 18°C for germination. Prepare 96 well microtiter plates by adding 140 µl of glucose peptone medium per well amended with the fungicide to be evaluated.

   Examples (final test concentrations in 200 µl):

   - Tebuconazole, Prothioconazole, Epoxyconazole:
     0, 0.0064, 0.032, 0.16, 0.8, 4.0, 20.0, 100.0 mg/l

   The concentration range has to be adapted to a) the intrinsic activity of the fungicide to be tested and b) to the expected sensitivity variation in the fungal population.

5. Inoculate each well with 60 µl of the prepared spore suspension. Use one duplicate per isolate. Incubate microtiter plate for 6 days at 18°C, agitate gently. After incubation fungal growth is measured with a microtiter plate reader at 620 nm. Calculate EC_{50} values.

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