Species:	*Microdochium nivale* (Snow Mold)
Product Class(es):	SBI fungicides, also suited for other fungicide classes
Method type described	microtiter plate test
Date of protocol:	2006-05
Proven for	Prothioconazole, Tebuconazole
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 to ensure valid results

Method:

1. Sample wheat (or other cereal) ears infected with *Microdochium nivale*, selected at random from several plants from the field. To get an acceptable statistical result take not less than 20 ears 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 ears should be transported in paper bags.

2. Desinfection of the cereal grains with NaOCl (2 %; 4 min) followed by two wash steps (sterile distilled water, 5 min each) is neccessary to avoid the development of faster growing, secondary fungi located on the grain´s surface. Spread the infected grains in Petri dishes on potato dextrose agar (PDA, 5 grains per plate) and incubate them for 6 d at 10-15°C under permanent black light. Identify the developed *Microdochium* specie under the microscope

(shape/ type of conidia: majus has normally more than 2 cells) and propagate the mycelium for purification once again for 6 d under the same conditions.

3. After 6 days of incubation at 10-15°C and permanent black light, the spores originating from a single colony can be harvested and used for further testing.

4. Microtiter plate test: Prepare spore suspensions in potato dextrose broth (PDB, 10000 conidia per 3 ml medium) and 96 well microtiter plates by adding 140 µl of PDB per well amended with the fungicide to be evaluated.

   Examples (final test concentrations in 200 µl):

   - Tebuconazole, Prothioconazole: 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, 30 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 (200 conidia/well). Use one duplicate per isolate. Incubate microtiter plate for 5 days at 20°C, agitate gently. After incubation fungal growth is measured with a microtiter plate reader at 620 nm. Calculate EC_{50} values.

Additional comment:

Sampling of (young) plants showing typical symptoms on stems is also suitable to isolate *Microdochium* strains. Therefore, cut small discs (1 mm) out of the stems (after desinfection as described above for grains) and place them on PDA containing Petri dishes. Follow then the same procedure as described for grains (6 d incubation at 10-15°C under permanent black light etc.).

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