



Species:	Microdochium nivale var.nivale or majus
Product Class(es):	SBI and SDHI fungicides
Method type described:	Microtiter plate
Date of protocol:	2022-01
Version	1
comments	This work instruction establishes the procedure for cereal snow mold in-vitro testing of SBI (DMI) and SDHI, but it is suitable for other mode of actions

### Sample Collection, Processing and Propagation

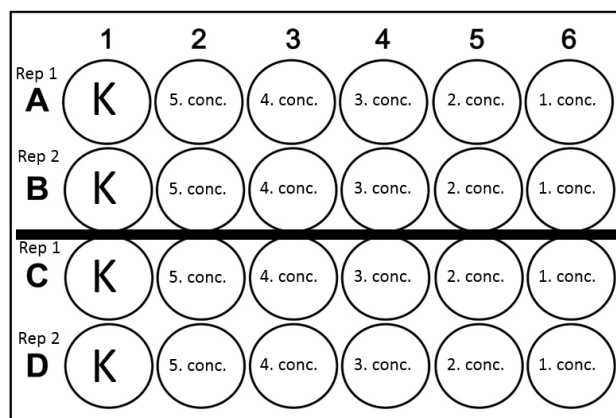
The cereal kernel must wash for 2 minutes with Natriumhypochlorit (3%) to disinfect them. After the disinfection the kernel must wash twice for 2 minutes with bidest. H<sub>2</sub>O. Disinfected kernel are taken on a tripartite Petridish with Saboraud maltose Agar (SMA) or Potato Dextrose Agar (PDA) with Streptomycin and Ampicillin and incubate at 20°C under UV light for 7 days. It is important to transfer an agar plug to a new SMA-Plate (mother plate) to get a pure culture for the test.

### Sensitivity Tests

Prepare the fungicide dilution series at 10 times higher than the end concentration

Fungicide	1. conc.	2. conc.	3. conc.	4. conc.	5. conc.	6. conc.
Sedaxane	100	10	1	0.1	0.01	0
Prochloraz	100	10	1	0.1	0.01	0

Pipette 100µL of the corresponding fungicide solution in each well of a 24 well plate and add 900µl medium to all wells.



Use a sterile object slide to scrape the fungus from the mother plate and taken into sterile deionised H<sub>2</sub>O and well mixed. The spore suspension should have a density of 10'000 spore/ mL (count with a Neubauer haemocytometer or a Thoma cell counting chamber). Each well will be inoculated with 50µL spore suspension. The plates are incubated for 6 days at 20°C in the dark.

### Evaluation and Data Processing

Evaluation is performed after 6 days at 20°C after inoculation.

Percent growth is assessed visually and EC<sub>50</sub> is calculate. EC<sub>50</sub> value is estimated for example using a statistic program (e.g. AGSTAT computer package).

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