



Species:	Ustilago nuda
Product Class(es):	SBI (DMI) and SDHI fungicides
Method type described:	Microtiter plate
Date of protocol:	2022-01
Version	1
comments	This work instruction establishes the procedure for Barley loose smut in-vitro testing of SBI (DMI) and SDHI, but it is suitable for covered smut (<i>U. hordei</i>) and wheat smut (<i>U. tritici</i>)

Sample Collection, Processing and Propagation

Teleutospores from infected ears were taken and solved in sterile H₂O by harvesting the spores with a cotton swab and moving the cotton swab in H₂O. The samples should be sent as soon as possible during the season. The biological material loose vitality the older the sample it is.

SD-Medium

- 6.7g Yeast Extract
- 20g Glucose
- 20g Agar
- 50mg Streptomycin (dissolve 100mg in 1ml sterile H₂O)
- 50mg Ampicillin (dissolve 100mg in 1ml sterile H₂O)

Weigh ingredients and adjust with purified H₂O to 1000ml. Autoclaved 20 min at 121°C. Cool down to 50°C Add 100mg Streptomycin and 100mg Ampicillin and mix gently

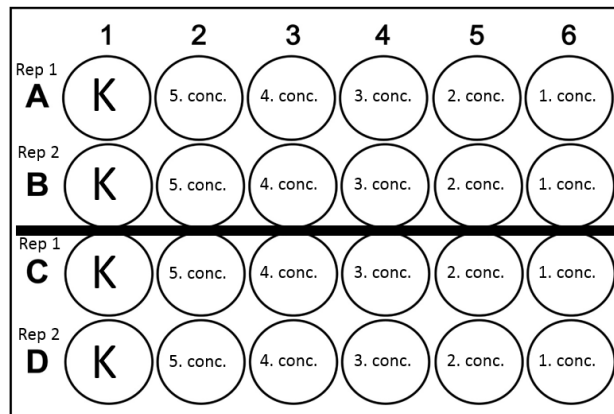
Sensitivity Tests

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*	10	1	0.1	0.01	0.001	0
Prothioconazole other SBI (DMI)	10	1	0.1	0.01	0.001	0

*A spore germination test is as well available

Pipette 100µl of each concentration in each well of a 24 well plate
Add 900µl of SD-Medium to each well and mix gently



The final concentration of spores per well should be 50'000 spores/ml so therefore the stock solution of the spore suspension should have a density of 250'000 spores/ml. Each well will be inoculated with 100µl suspension. Incubate the 24 well plates for 8 days at 20°C in the dark

Evaluation and Data Processing

Evaluation is performed after 8 days at 20°C after inoculation.

Percent growth is assessed visually and the EC50 value estimated for example using a statistic program (e.g. AGSTAT computer package).

authors

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