Species:	Ustilago nuda
Product Class(es):	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 (U. hordei) and wheat smut (U. tritici)

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.

Sensitivity Tests

Prepare the fungicide dilution series at 100 times higher than the end concentration and dilute them directly in bottles of 2% water agar. One bottle for each fungicide concentration is prepared. Streptomycin is solved as a stock solution of 10'000 ppm (e.g. 250 mg in 25 ml H2O) after autoclaving the agar and cooling down to 50°C the antibiotic is added to the agar containing bottle. (1ml of the stock solution into 100ml of agar media). Greiner bio-one petridish (5ml) are used for the assay, because they have a grid which can give an orientation during counting the spores. One petri dish per concentration and isolate is prepared (consider inclusion of replicates).

Fungicide	**1. conc.**	**2. conc.**	**3. conc.**	**4. conc.**	**5. conc.**	**6. conc.**
Sedaxane	10	1	0.1	0.01	0.001	0

The final concentration of spores should be 100’000 spores/ml. Spores are counted with spore counting chamber (e.g. Neubauer haemocytometer or Thoma cell counting chamber). Each petri dish is inoculated with 30µL of the prepared spore suspension. The plates are incubated for 24 hours at 18°C in the dark.

Evaluation and Data Processing

Evaluation is performed after 1 day at 18°C after inoculation.
A total of 200 spores are counted for each tested concentration and rated as germinated when germ-tube reached at least spore diameter. Percentage of germination in relation to control (germ tube double the size of the diameter of the spore) is used to calculate EC50. EC50 value are estimated for example using a statistic program like AGSTAT computer package.

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