Species:	*Pyrenophora graminea* (aka Helminthosporium graminearum, Drechslera graminea)
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 stripe in-vitro testing of SBI (DMI) and SDHI, but it is suitable for other mode of actions

Sample Collection, Processing and Propagation

<u>Isolation from kernels</u>

Kerels must wash with Natriumhypochlorit (2min 3%) to disinfect them. After the disinfection the kernel have to wash twice for 2min with sterile H2O. Disinfected kernels are taken on a tripartite petri dish with PDA + antibiotics and incubate at 20°C under UV-light for 7 days. Transfer an agar plug with Pyrenophora graminea to a new PDA-Plate and incubate for 7-10 days at 20°C under UV-light to get a pure culture. Pipette 5ml sterile H2O per petri dish. Scrape the fungus with a sterile object slide and transfer the suspension to a sterile tube. Take a CDV8 petri dish and put a Cellophane- or paper filter inside. Pipette 200µl of Spore suspension onto filter and spread them with a Cell spreader (Triangle Shape Cell Spreader). Incubate plates for 7 days at 20°C under UV-light.

<u>Isolation from leaves</u>

Cut leaf or stems of samples with symptoms into 2 cm long pieces and surface sterilise for 2 min in 2% Natriumhypochlorit. Rinse samples with H2O bidest in order to remove the Natriumhypochlorit. Leaf pieces (upper side of the leaves upwards) or stems were placed in petri dishes with very wet filter paper (use 3 ml H20 for a 8cm dish) and incubate dishes at 20°C under black Light (NUV) 2-4 days. On the leaf pieces conidiophores emerge and eventually spores are produced, predominantly at the edges of the leaves. On the stems the pseudothecia ripe and become “softer”. Under the binocular single spores are picked with a fine needle and transferred to a malt agar plate (& Chloramphenicol). The pseudothecia from the stems are picked

with a pincer, opened and also transferred to malt agar. The plates are incubated for 4 to 5 days at 20°C under black light (NUV). To reisolate, a small part of the growing mycelium is transferred to new PDA plate and incubated under black light (NUV) for another 7 to 10 days at 20°C. Pipette 5ml sterile H2O per petri dish. Scrape the fungus with a sterile object slide and transfer the suspension to a sterile tube. ake a CDV8 petri dish and put a Cellophane- or paper filter inside. Pipette 200µl of Spore suspension onto filter and spread them with a Cell spreader (Triangle Shape Cell Spreader). Incubate plates for 7 days at 20°C under UV-light.

PDA for 1000ml

- 39g Potato dextrose Agar

Weigh PDA and adjust with purified H_2O to 1000ml. Autoclaved 20 min at 121°C. Cool down to 50°C

PDA with antibiotics for 1000ml

- 39g Potato dextrose Agar
- 100mg Streptomycin (dissolve 100mg in 2ml sterile H_2O)
- 100mg Ampicillin (dissolve 100mg in 2ml sterile H_2O)

Weigh PDA and adjust with purified H_2O to 1000ml. Autoclaved 20 min at 121°C. Cool down to 50°C
Add 100mg Streptomycin and 100mg Ampicillin

CDV8 for 1000ml

- 200ml V8 Juice
- 3g $CaCO_3$
- 45.4g Capek Dox Solution Agar
- 10g Bacto Agar

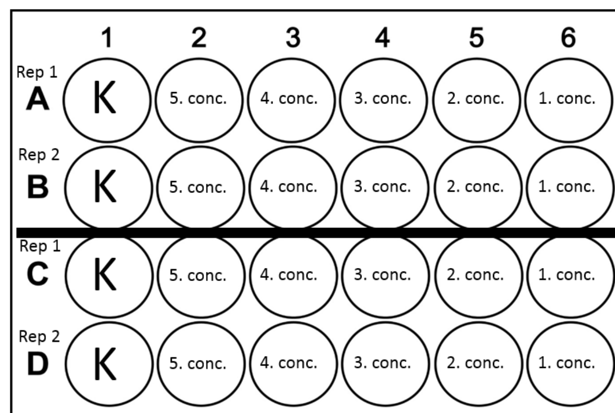
Weigh ingredients and solute them in ~800ml purified H_2O. Adjust pH of medium to 6.3 and adjust with purified H_2O to 1000ml. Autoclaved 30min at 121°C. Cool down to 50°C.

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
Prothioconazole	100	10	1	0.1	0.01	0

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



For each isolate 1 petri dish is needed (Ø 8cm) as starting material. With a sterile object slide is scraped the fungus and taken into sterile deionized H2O and well mixed. The spore suspension should have a density of 100'000 spore/ ml (count with a Neubauer hemocytometer or similar counting chamber). Filter the suspension if necessary. Each well will be inoculated with 100µl suspension is sprayed in each well. Leave Plates 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 the EC50 value estimated for example using a statistic program (e.g. AGSTAT computer package).

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