Species:	Phoma lingam (aka Leptosphaeria maculans)
Product Class(es):	SBI
Method type described:	Microtiter plate
Date of protocol:	2022-01
Version	1
comments	This work instruction establishes the procedure for blackleg disease on Brassica crops in-vitro testing of SBI (DMI), but it is suitable for other mode of actions

Sample Collection, Processing and Propagation

Cut plant material (leaf or stem) samples with symptoms into 2 cm long pieces and surface sterilize for 2 min in 2% Natriumhypochlorit. Rinse samples with H2O bidest to remove the Natriumhypochlorit. Leaf pieces (upper side of the leaves upwards) or stems are placed in petri dishes with very wet filter paper (use 3 ml H_20 for an 8 cm petri dish) and incubate at 20°C under black Light (NUV) for 2 to 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 PDA plate amended with antibiotics. The pseudothecia from the stems are picked with a pincer, opened, and transferred to PDA. The plates are incubated for 3 to 6 days at 20°C under black light (NUV). Transfer an agar plug with Leptosphaeria maculans to a new PDA petri dish and incubate it for 7-10 days at 20°C under black light (NUV) to get a pure culture. To use the obtained strains in the bio test, transfer a plug to a CDV8 petri dish (mother plate) and incubate them for 7-10 days at 20°C under black light (NUV).

PDA for 1000ml

- 39g Potatodextrose Agar

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

CDV8 for 1000ml

- 200ml V8 Juice
- 3g CaCO3

- 45.4g Capek Dox Solution Agar
- 10g Bacto Agar

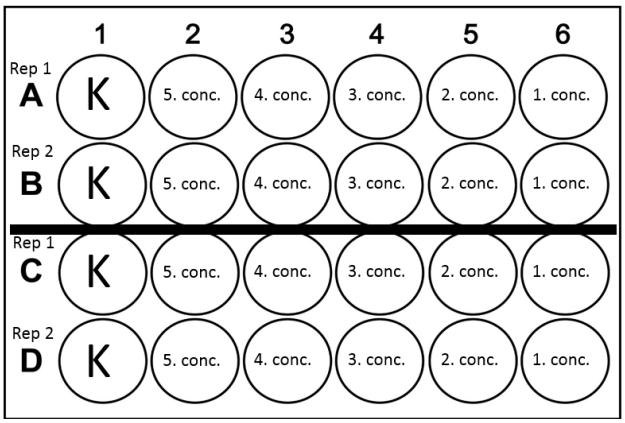
Weigh ingredients and solute them in ~800ml purified H_2O. Adjust pH of medium to 6.3 and adjust with purified H_2O to 1000ml. Autoclaved 30min at 121°C. Cool down to 50°C.

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.**
Difenoconazole	10	1	0.1	0.01	0.001	0

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



For each isolate 1 petri dish (mother plate) is needed (Ø 8 cm). With a sterile object slide scrap the fungus from the petri dish, transfer it into sterile deionized H2O and mix well. The spore suspension should have a density of 50'000 spore/ml. Spore suspension can be prepared using a Neubauer hemocytometer or a Thoma spore counting cell. Filter the suspension if necessary. Each well is inoculated with 100µl suspension sprayed in each well. Incubate 24 well plates for 7 days at 18°C in the dark

Evaluation and Data Processing

Evaluation is performed after 7 days at 18°C after inoculation.
Percent growth is assessed visually and EC50 is calculated. EC50 value is estimated for example using a statistic program (e.g. AGSTAT computer package).

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