Species:	Parastagonospora nodorum (aka Leptosphaeria nodorum)
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 Septoria nodorum blotch of wheat in-vitro testing of SBI (DMI) and SDHI, but it is suitable for other mode of actions

Sample Collection, Processing and Propagation

Isolation from wheat kernels

Wheat kernel must wash with Natriumhypochlorit (2min 3%) to disinfect them. After the disinfection the kernel have to wash twice for 2min with sterile H_2O. Disinfected kernels are taken on a tripartite petri dish with PDA amended with antibiotics and incubate at 20°C under UV-light for 4-7 days. Transfer an agar plug with P. nodorum 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.

Isolation from wheat leaves

Cut leaf of stem samples with symptoms into 2 cm long pieces and surface sterilize for 2 min in 2% Natriumhypochlorit. Rinse samples with H_2O bidest 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 H_2O for an 8 cm 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 transferred to malt agar. The plates are incubated for 4 to 5 days at 20°C under black light (NUV). 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. 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.

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

Malt Agar + Chloramphenicol for 1000ml

- 20g Malt Extract
- 20g Bacto Agar
- 100mg Chloramphenicol (dissolve 100mg in 1ml EtOH 100%)

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

CDV8 for 1000ml

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

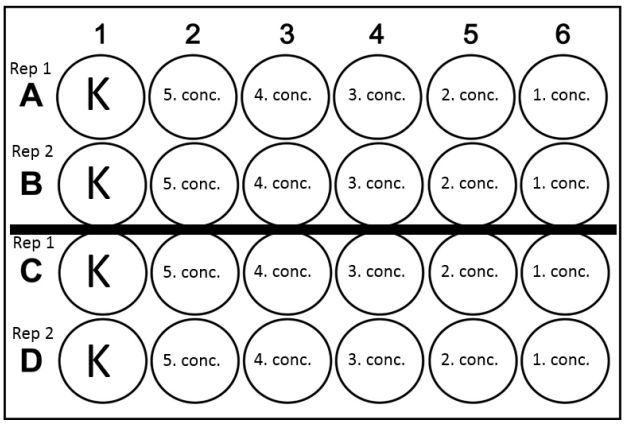
Weight ingredients and solute them in ~800ml purified H_2O. Adjust pH of medium to 6.3 and adjust with purified H_2O to 1000ml. Autoclaved 30ml at 121°C. Cool down to 50°C.

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	100	10	1	0.1	0.01	0
Benzovindiflupyr	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 and mix gently



For each isolate 1 petri dish (mother plate) is needed (Ø 8 cm). With a sterile object slide scrap the fungus, transfer it into sterile deionized H_2O and mix well. The spore suspension should have a density of 100'000 spore / ml. Spore counting can be done with a Neubauer hemocytometer or any other spore counting chambers. Filter the suspension if necessary. Each well is inoculated with 100µl suspension. Incubate plates for 4 days at 20°C in the dark.

Evaluation and Data Processing

Evaluation is performed after 4 days at 20°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).

authors	Stefano Torriani, Reto Kühn - Syngenta Crop Protection AG, CH-4332 Stein Switzerland