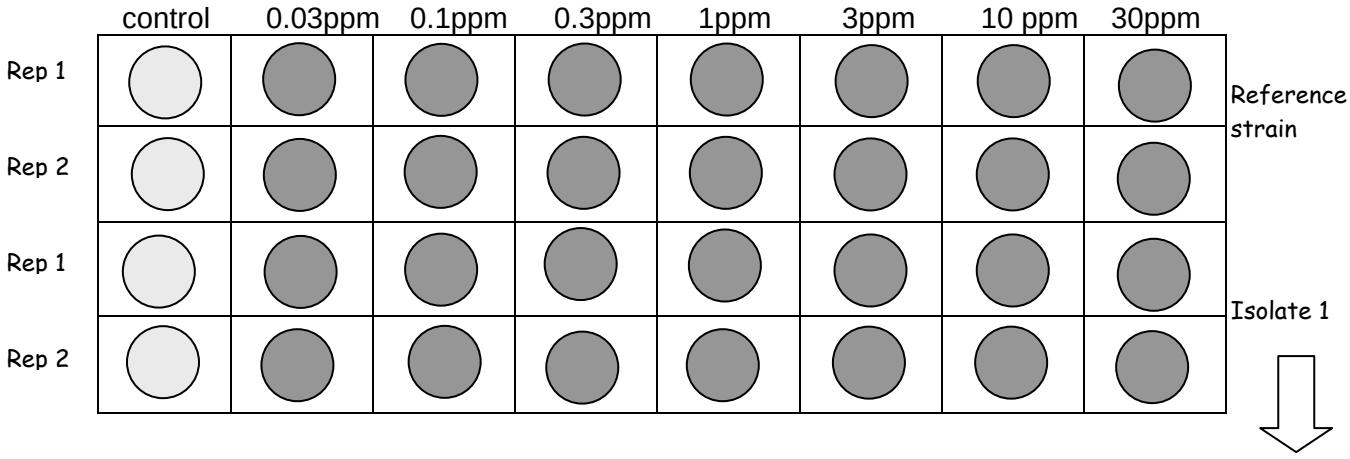
Species:	*Phytophthora infestans*
Product Class(es):	QoI fungicides
Method type described:	Assay plate with 96 well flat bottom
Date of protocol:	2005
Proven for	Famoxadone
Should be suitable for	other QoI fungicides. Protocol adjustments may be needed due to the individual compound characteristics
Version	1
Version	Method validated for labs equipped with a plate reader (wavelength 405 nm)

Method:

1. Sampling: To obtain a representative sample, 10-15 infected potato or tomato leaves are processed. At least 5-6 single-spot strains are isolated using the Rye-B agar medium amended with antibiotics. Then *Phytophthora infestans* isolates are cultivated on Rye-B agar medium at 19-20°C in the dark. The isolates of *P.infestans* can be stored in mycelium plug form in sterile water.

2. Pathogen preparation: After about 3 weeks of culture sporangia of each isolate are harvested by rinsing the Petri dish with 6 mL of cold sterile Pea Extract-Glucose-Broth. The resulting suspension is filtered through 2 layers of gauze and adjusted to 20'000 spores/mL.

3. Assay plate preparation: Accomplish the sensitivity assay in 96-well plates with flat bottom. Fill 50 µL of DMSO solution at 0.1% v:v into control wells (2 reps) and 50 µL of fungicide solutions at 0.06, 0.2, 0.6, 2.0, 6.0, 20.0 and 60.0 µg/mL into remaining wells (2 reps). The final DMSO concentration is the same in all the wells. Fill 50 µL of sporangia suspension in all the wells. Include a sensitive reference strain into each test.



4. Incubation: Cover the plate with a low evaporation lid and put the plate at 19-20°C in the darkness.

5. Assessment: The assessment of the optical density of each well is done at day 0 and after 7 days of incubation using the Biotech plate reader at 405 nm wavelength. A Grafit 5.0 computer software (Erythacus Ltd) is used to capture and process the data. Then the EC50 for each strain is determined.

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