Species:	*Phytophthora infestans*
Product Class(es):	CAA fungicides
Method type described:	Tissue culture plate assay with mycelial plugs
Date of protocol:	2006-05
Proven for	Dimethomorph
Should be suitable for	other CAA compounds. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	• Validated routine method • proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results

Method:

1. Sampling: A sample consists of ~10-20 potato or tomato leaves with sporulating lesions per location. The leaves are placed between layers of paper, wrapped in newspaper and then taken as quickly as possible to the laboratory, preferably in a cool box.

2. Isolation of *Phytophthora infestans* from field samples: Potato tubers from varieties susceptible to late blight (e.g. “Bintje”) are peeled and surface sterilized with ethanol. The tubers are washed with sterile water and cut into two halves. Leaves from potato or tomato with late blight infections are placed between the two halves of the tuber and the halves are fixed with tape. They are incubated in a moist chamber for two days at 16°C. When mycelium emerges on the tuber surface, it is transferred on potato dextrose agar containing antibiotics*.

3. Inoculum production: Isolates are transferred and grown on rye agar** for 2 weeks at 16°C in the darkness. Mycelial plugs from actively growing cultures with 5 mm diameter were used as inoculum for the microtiter test.

4. Sensitivity tests: The test is carried out in 24 well tissue culture plates. Pure technical fungicide ingredient is dissolved in dimethylsulfoxide (DMSO) and the final concentrations are prepared by dilution with V8 medium***. 6 concentrations of the fungicide are used (e.g. for dimethomorph 0, 0.1, 0.3, 1, 3, 10 ppm). Mycelial plugs are transferred in each well containing 1 ml of the nutrient solution containing the different fungicide concentrations. For each isolate and fungicide concentration 2 replicate wells are used. The microtiter plates are incubated at 18°C in darkness. After 7 days the mycelial growth is visually assessed. The lowest concentration without mycelial growth is defined to be the MIC (minimum inhibitory concentration) value. The MIC-values are compared with values from sensitive strains.

*Potato dextrose agar

39 g Potato dextrose agar
10 g agar
fill up to 1000 ml with bidest water
after autoclave add
200 mg ampicillin
20 mg rifampicin
10 mg pimaricin
at 45°C

**Rye agar

200 g rye grains,
boiled for 1 h in 1000 ml bidest water and filtered through a sieve
5 g glucose
20 g agar
fill up to 1000 ml with bidest H_2O

***V8 medium:

300 ml V8 juice
4,5 g $CaCO_3$
mix and centrifuge dilute supernatant
1:5 dilution with bidest Water

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