Species:	*Phytophthora infestans*
Product Class(es):	CAA fungicides
Method type described:	Detached leaf test (*in vivo)*
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 for 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. Fungicide application: Tomato plants were grown for two weeks in the greenhouse and the whole plants were treated in a spray cabinet with different concentrations of the fungicide product (for dimethomorph 0, 0.375, 0.75, 1.5, 6, 6, 12 ppm). 1 day after

application the upper two leaves are harvested and placed in a Petri dish containing water agar (0.6%)

4. Spore production and inoculation: Isolates are transferred and grown on rye agar** for 2 weeks at 16°C in the darkness. Spores from actively growing and sporulating cultures are washed from the surface of the agar plate with 5 ml cold water (4°C) per plate. The resulting suspension is filtered through 4 layers of cheese-cloth and the sporangia concentration is adjusted to $2x10^4$ sporangia/ml. Suspensions are incubated for 2 h in the fridge for release of zoospores. The zoospore suspension is applied with an airbrush (500 µl/Petri dish).

5. Evaluation: The Petri dishes are incubated for 5 days at 18°C in 12 h light /12 h darkness. The diseased leaf area is assessed and the inhibition of the different fungicide concentrations is calculated (% inhibition = [% diseased leaf area in untreated - diseased leaf area in treated / diseased leaf area in untreated] x 100%). The ED50 values are calculated and compared with sensitive standard isolates.

*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

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