Species:	*Phytophthora infestans*
Product Class(es):	CAA fungicides
Method type described:	Microtiter test with sporangia
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. Spore production: Isolates are transferred and grown on pea 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 nutrient solution (double concentrated pea broth***) at room temperature. The resulting suspension is filtered

through cheese-cloth and the sporangia concentration is adjusted to 2 x 10^4 sporangia/ml.

4. Sensitivity tests: Pure technical fungicide ingredient is dissolved in dimethylsulfoxide (DMSO) and the dilutions are prepared in sterile deionised water immediately before mixing with the spore suspension. 50 µl fungicide solution and 50 µl spore suspension are mixed in each well of 96 well microtiter plates. For dimethomorph the following final concentrations are recommended: 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 ppm. For each isolate and fungicide concentration 3 replicate wells are used. Three replicate wells are also used per fungicide concentration as blanks (fungicide solution + pea broth). The microtiter plates are put into plastic bags to avoid evaporation and incubated at 18°C in darkness. Five days after inoculation the growth is measured in a photometer at 405 nm. The values are corrected by comparison with the blanks and ED50 values are calculated. The ED values are 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

**Pea agar

150 g frozen peas
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 water

***Pea broth, double concentrated:

300 g frozen peas
boiled for 1 h in 1000 ml bidest water and filtered through a sieve
10 g glucose
fill up to 1000 ml with bidest water

author	Dr. Gerd Stammler, BASF-AG, 67117 Limburgerhof, Germany