Species:	*Phytophthora infestans*
Product Class:	CAA fungicides, and also suited for other fungicide classes
Methods described:	Leaf segment microtiter plate test
Use	Standard method
Date of protocol:	2007-12
comments	validated routine method for labs equipped with microtiter plate technique

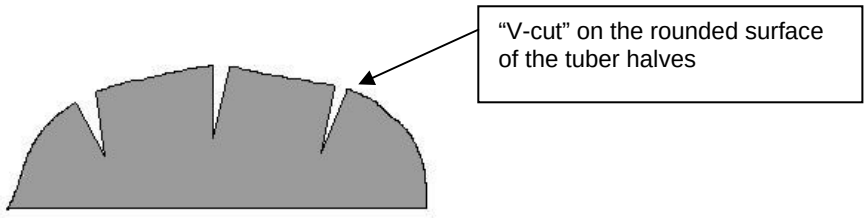
Method:

1) Sample collection

Potato leaves, with visible and sporulating *P. infestans* lesions, are collected from different plants and locations in the field. One leaf is sandwiched between two cut halves of a potato tuber (cv. “Bintje” or another highly susceptible variety). This method allows the pathogen to grow into the tuber during the shipment to the lab. One sample consists of at least 5 tubers. The tubers are placed into a sending card box. The card boxes are immediately sent to the lab.

2) Sample incubation

The leaves must remain 3 days (including the shipment time) between the tuber halves to allow the fungus to grow into the tuber. When the tubers arrive in the lab, the halves are separated from each other and the leaf is removed. One to three “V-cuts” are made on the rounded surface. The tuber halves are then placed with the cut surface down in a glass Petri dish on a dry paper filter and incubated at 19°C in the dark.



After 5 to 7 days, fresh mycelium is growing in the "v-cuts" and picked up with a sterile needle to be transferred on RDA+A+PCNB (Rye decoction agar + Antibiotics + Pentachloronitrobenzene) and incubated at 19°C in the dark.

RDA + A + PCNB

- 200g rye seeds
- 5g D-glucose
- 20g Bacto-Agar from Difco

1. autoclave the rye- seeds with approx. 800ml bi-dest.H2O for 30min at 121°C
2. Sieve the decoction, throw seeds away, and adjust the decoction to 1L with bi-dest. H_2O and add the glucose and Bacto-Agar
3. autoclave the second time for 30min at 121°C
4. cool down to approx. 50°C and add:
    - pimaricin 10ppm
    - Ampicilin 125ppm (diluted in 2ml bi-dest.H2O)
    - Rifampicin 10ppm (diluted in 2ml Ethanol)
    - Pentachloronitrobenzene (PCNB) 100ppm (diluted in 2ml Acetone)

The isolates are transferred as often as necessary until the isolates are free of contaminations. When done, the isolates are transferred on RDA-plates, without antibiotics and PCNB. After incubation at 19°C in the dark for 15-21 days, the isolates are ready for sensitivity tests.

3) Leaf disc assay

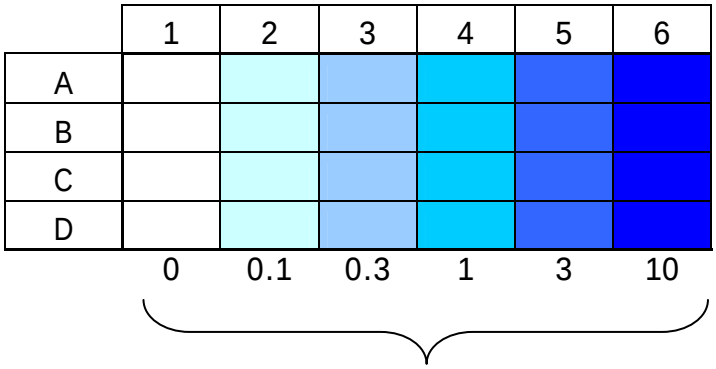
The sensitivity test is carried out in 24-well plates.

Fungicides: Mandipropamid: 10'000g ai/l in DMSO: 0 – 0.1 – 0.3 – 1 – 3 – 10ppm

Mandipropamid a spraying-method using the Tecan-Genesis-automated equipment is applied. At first, 1.5ml of 0.2% water-agar from Difco is distributed in each well.

Fresh leaves (the 3rd to the 5th from the top) from 4 weeks old potato plants cv."Bintje" are collected in the greenhouse, and leaf- pieces (diam.15mm) are cut with a cork borer. The leaf-pieces are placed with the upper leaf disc face down in each well onto the water agar.

10µl of the fungicides solutions are sprayed on each well like following:



Repartition of the concentrations of Mandipropamid (in ppm) per 24-well plate for 1 isolate

4) Inoculum preparation

A sporangial suspension from 15-21 days old plates of each isolate is prepared by spraying about 7ml bi-dest.H_2O in the petri dish, the sporangium suspension is filtered through a sieve to remove mycelium and adjusted to 2.10^4 spores/ml. One 30µl droplet is pipetted in the middle of each leaf disc. The plates are incubated in a Sanyo Incubator at 19°C and 85% humidity for 6 to 8 days.

For each test, a sensitive strain (#9, Syngenta internal) was tested in order to be used as reference.

5) Evaluation

The test is assessed visually by estimating the percent infection on each leaf disc. The results are converted in EC-50 value using suitable computer programme.

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