



Species:	<i>Phytophthora infestans</i>
Product Class(es):	Piperidinyl-Thiazole-Isoxazoline fungicides, also suited for other fungicide classes
Method type described:	Leaf disc assay
Date of protocol:	2017-06
Version	1
comments	Proven for Oxathiapiprolin. Protocol adjustments may be needed for other fungicide classes depending on the individual compound characteristics.

### Sample Collection

Field samples are collected by the CP company technicians in the untreated checks of field trials or in commercial fields in different potato or tomato growing regions of Europe. Samples are collected at dry weather conditions. In case of a commercial field, samples should not be taken too soon after a fungicide application (1 week minimum). One sample consists of at least 30 leaves with young, single late blight lesions. The leaves are put loosely in a plastic bag (without anything else!!) and are dispatched in a parcel, not in an envelope. Samples are taken only on Monday or Tuesday and sent to the analytical lab the day of sampling by an express carrier (24-48 hours). In order to avoid cross-contamination, different samples are packed separately. All field samples have to be accompanied by a sample information sheet containing the information necessary for sample identification. Four single colony isolates per sample are tested regarding their sensitivity towards the active substance.

### Sample Processing and Propagation

At analytical lab, infected leaf material of each sample is placed in Petri dishes with wet filter paper at the bottom and incubated in the climate chamber (24-72 h period, 18 °C, 100 µmol/m<sup>2</sup>s, 16/8 h light/darkness, 80 % RH) in order to obtain freshly sporulating colonies. Then, for maintenance and propagation purpose, the sporangia of single colony isolates are transferred with a hair pencil onto fresh leaf material placed inside the water agar Petri dishes. After a dark and cold (2-4°C) period of 2 h, the isolates are incubated in the climate chamber for 6-7 days (18 °C, 100 µmol/m<sup>2</sup>s, 16/8 h light/darkness period, 80 % RH).

### **Sensitivity Tests**

The following test concentrations of oxathiapiprolin are prepared with a 0.05 % Uniperol-solution: 0, 0.000064, 0.00032, 0.0016, 0.008, 0.040, 0.200, 1 ppm, in order to obtain an EC<sub>50</sub> evaluation. For the test, whole potato plants cv. Bintje are sprayed with the respective fungicide solutions to run-off conditions. One day after treatment, leaf disc tests are prepared and inoculated. Separate disposable Petri dishes of 6 cm diameter are used for each concentration. Each Petri dish contains four leaf discs (14 mm diameter) considered as replicates. They are originating from different leaves from plants treated with the same fungicide concentration. A test set for one isolate with eight fungicide concentrations (including untreated control) consists therefore of eight Petri dishes. Each test set is inoculated with sporangia suspensions by equally spraying the suspension onto the leaf discs. After a dark and cold (2-4°C) period of 2 h, the test is incubated in the climate chamber for 6 days (18 °C, 100 µmol/m<sup>2</sup>s, 16/8 h light/darkness period, 80 % RH).

### **Evaluation and Data Processing**

The visual assessment of the test is done by estimating the percentage of sporulating area of each leaf disc relative to the untreated check. Then, Probit analysis is used to calculate the EC<sub>50</sub> values of the bio-assayed isolates, based on the mean percentage of sporulation for each concentration.

These values are compared to EC<sub>50</sub>s of sensitive reference strains and to the mean EC<sub>50</sub> value of the baseline.

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