Species:	*Plasmopara viticola*
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

In France, Italy, Germany, Austria and Switzerland, samples are taken by the analytical lab employee directly from different field sites while passing through the regions of interest. To obtain representative data from the regional populations of *Plasmopara viticola*, the sampling distance within a vine growing area is approximately 100 km on average. Within this area, 4-6 samples are taken. Per sample, 2-3 isolates are analysed for their fungicide sensitivity. Thus 10-15 isolates per area are bio-assayed. Samples are taken in the last stage of the growing season when the effect of previous fungicide applications is declining, and when a peak phase of pathogen attack in the field is present.

In other vine growing countries such as Spain, Portugal, Romania, Bulgaria, Czech Republic and Hungary, field samples are collected by the CP company technicians in the untreated checks of field trials or in commercial fields and sent to the analytical lab. Samples are collected at dry weather conditions. In case of a commercial vineyard, samples should not be taken too soon after a fungicide application (1 week minimum). One sample consists of at least 30 leaves with young downy mildew symptoms. 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 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 (22 °C, 18-24 h darkness period, 70 % 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 wet period of 18-24 h, the isolates are incubated in the climate chamber for 7 days (22 °C, 10 µmol/m²s, 12/12 h light/darkness period, 70 % RH).

Sensitivity Tests
The following test concentrations of oxathiapiprolin are prepared with a 0.05 % Uniperol-solution: 0; 0.00024; 0.00098; 0.0039; 0.0156; 0.0625; 0.25; 1 ppm, in order to obtain an EC_{50} evaluation. For the test, whole vine plants cv. Kerner (cultured under cellophane bags) 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 wet period of 18-24 h, the test is incubated in the climate chamber for 8 days (22 °C, 10 µmol/m²s, 12/12 h light/darkness period, 70 % 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 EC50 values of the bio-assayed isolates, based on the mean percentage of sporulation for each concentration.
These values are compared to EC50s of sensitive reference strains and to the mean EC50 value of the baseline.

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