Species:	*Plasmopara halstedii*
Product Class(es):	Piperidinyl Thiazole Isoxazoline fungicides
Method type described:	Whole plant – Germinated seeds inoculation
Date of protocol:	2017-06
Version	1
comments	Suitable for Oxathiapiprolin May be suitable for other PTI- and Phenylamides. Protocol adjustments may be needed due to the individual compound characteristics.

Sample Collection

6 leaves with fresh downy mildew symptoms are collected at dry weather conditions in the early growing season. It is important to collect only contaminated leaves with recent white sporulation.

Once collected, leaves are placed in pairs, with the lower faces against each other, and wrapped into paper towels. Three wraps (so 6 leaves) are placed into a craft envelope or rolled inside several layers of newspapers.

Only paper, no plastic material should be used to wrap the leaves. In order to avoid cross-contamination, different samples are packed in different craft envelopes.

Samples can be stored in the fridge in a paper bag until shipment, but no more than 2 weeks after sampling. Avoid condensation, but keep leaves fresh to feed the pathogen: sporangia remain vital but do not release zoospores. Once collected or taken out of the fridge, the samples are sent to the lab as quickly as possible.

All field samples have to be accompanied by a sampling sheet describing sampling date, cultivar, seed treatment used, efficiency of product, and address of sampler.

Sample Processing and Propagation

The isolates from the field can be tested directly after their arrival at the lab, when a sufficient number of sporangia can be obtained from the 6 infected leaves per sample. The leaves are first washed using a wash bottle containing fresh distilled water and the sporangia solution is collected into a 500mL beaker. A reasonable amount of water needs to be used to not dilute too much the sporangia in the collected solution. After the 6 leaves are washed, the rinse solution is slightly stirred to remove the last spores from mycelium fragments, filtered through hydrophilic gauze, and slightly stirred again to obtain a homogeneous spores concentration. The

sporangia concentration is assessed twice using a Malassez cell, and the average concentration is calculated based on these 2 counts.
The goal is to obtain at least 180mL of spore solution containing 100'000 – 250'000 sporangia/mL. This concentration can be adjusted by adding fresh distilled water or by decantation (and supernatant removing), if the initial solution is too, or not concentrated enough.
If not enough spores can be obtained, the fungus can be propagated by letting around 100 sunflower (Peredovick variety) pre-germinated seeds in the spores solution during 5h at 20°C in dark conditions. Pre-germinated seeds are then sown in miniature greenhouses in a commercial potting mix. After irrigation, the trays are covered with plastic lids and placed in an incubator (20°C, 75% HR, 14/10h D/N). 24H after sowing, plastic lids are removed. Irrigation is made in average every two days by putting 300mL of distilled water / tray. This ensures the substrate stays wet in order to maximize the development of the fungus in the roots. 11 days after sowing, trays are irrigated, water is sprayed on cotyledons and plastic lids are put again over each tray to induce sporulation. Freshly sporulating seedlings are obtained 3 days later (14 days after sowing), and can be washed to produce a new inoculum solution.

Sensitivity Tests

In order to obtain an even infection from seeds to seeds in this test, sunflower pre-germinated seeds (Peredovik variety) used in this test need to have a radicle length ranging from 0,5 to 1cm maximum. Seeds that are not meeting this requirement are discarded from the experiment (20% more seeds are added at the beginning as a safety margin). Pre-germinated seeds are soaked during 5H at 20°C in dark conditions in petri dishes. Each petri dish contains 42 pre-germinated sunflower seeds, 29mL of spores solution (1-2.5 x 10^5 sporangia/mL), and 1 mL of Oxathiapiprolin solution at different concentration levels (0pp, 0.003ppm, 0.03ppm, 0.3ppm, 3ppm and 30ppm). The final concentrations of Oxathiapiprolin in each of the 6 petri dish to be obtained are 0ppm, 0.001ppm, 0.01ppm, 0.1ppm, 1ppm and 10ppm.
Seeds are then sowed in miniature greenhouses to allow seedlings and fungus development for 2 weeks. A test unit consists of one miniature greenhouse where 60 seeds are sowed as following: 5 rows of 10 seeds corresponding to the 5 Oxathiapiprolin rates be tested + 1 row of 10 untreated seeds. 4 replicates are tested per sample (= 4 miniature greenhouses / sample = 40 seedlings tested per product rate per sample).
The trays are incubated in the same conditions as mentioned in "Sample Processing and Propagation".14 days after sowing and after sporulation occurred, the assessment is made by counting the number of seedlings per treatment that show sporulation on their cotyledons.

Evaluation and Data Processing
The visual assessment of the test is done by counting the number of seedlings per treatment that show sporulation on the cotyledons. EC50 values are calculated using Dose-Response v2.2 (DuPont internal software).

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