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| Species: | <i>Puccinia triticina</i> (<i>P. recondita</i>) |
| Product Class(es): | SBI fungicides, also suited for other fungicide classes |
| Method type described: | leaf segment test (detached leaf method) |
| Date of protocol: | 2006-05 |
| Proven for | Tebuconazole, Prothioconazole |
| Should be suitable for | other SBI-fungicides and active ingredients from other classes. Protocol adjustments may be needed due to the individual compound characteristics. |
| Version | 1 |
| comments | <ul style="list-style-type: none"> • validated routine method for labs equipped with climate chambers and/or greenhouses • proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results. • the method was developed and kindly provided by the company EpiLogic, Germany (Dr. F.G. Felsenstein) |

Method:

1. To obtain representative data from different rust populations, samples of uredo-/teleuto-spores can be taken by spore traps (mobile or stationary) (a) or by random sampling of infected leaves (b).
 - a) Uredo-/teleuto-spores are collected (trapped) onto segments of primary leaves from a highly susceptible variety placed in Petri dishes on water agar (0.6 % agar, 35 mg/l benzimidazole). Samples have to be taken during the peak phase of the epidemic whenever possible. Let the sampled spores grow to single colony isolates (climate chamber: 18 °C, 10-20 µmol/m²s continuous light) and transfer them onto fresh leaf segments on water agar for maintenance and for propagation before testing.
 - b) Uredo-/teleuto-spores from sampled leaves should be propagated on fresh leaf segments placed in Petri dishes on water agar as mentioned in a).

2. Determine the SBI sensitivity of each single spore progeny on a test set of leaf segments which are placed on benzimidazole agar (6 mg/l agar-agar, 35 mg/l benzimidazole).

One day before cutting the leaf segments, treat ten day old plants (first to second leaf stage) with the compound to run off conditions. Fungicide treatments should be graded logarithmically by a relatively small factor of two or three in order to obtain an optimal EC₅₀ evaluation for the sensitivity towards the active substance (depending on the compound).

Examples:

- Tebuconazole:
0, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, 20.48 mg/l
- Prothioconazole:
0, 0.64, 1.28, 2.56, 5.12, 10.24, 20.48, 40.96, 81.92, 163.84 mg/l

Keep differently treated plants strictly separated to avoid gas phase interactions. A day after the spray treatment of the plants, cut out the leaf segments. To avoid gas phase interactions between differently treated leaf segments in the test assortment, use separate disposable Petri dishes for each fungicide concentration in every single test set. As repetition, each Petri dish should contain several segments of different plants treated with the same concentration. A test assortment for a single isolate with ten concentrations of the respective compound therefore consists of ten Petri dishes including the untreated control.

3. The dishes of a test set should be only placed next to each other during the inoculation phase. Inoculate the leaf segments with the single spore progeny by air pressure (dry conditions - no aqueous spore solution).
4. After an incubation period (climate chamber: 18 °C, 10-20 µmol/m²s continuous light) of ten days, score each test assortment macroscopically regarding disease coverage/development in comparison to the untreated control (percentage). Then, calculate EC₅₀ of each test isolate by Probit analysis.

References:

FELSENSTEIN FG, 1996. Response of *Puccinia recondita* f.sp. *tritici* to the recent selection pressure of DMI fungicides within western Europe. Proc. Of the 9th European and Mediterranean Cereal Rusts & Powdery Mildews Conference, 2-6 September 1996, Lunteren, The Netherlands, P2.27, 155.

FELSENSTEIN FG, 1997. Brown rust of wheat – a European wide monitoring programme to study its fungicide sensitivity. Resistance '97: Integrated Approach to Combating Resistance, IACR-Rothamsted, Harpenden, UK, 70.

FELSENSTEIN FG, PARK RF, ZELLER FJ, 1998. The use of detached seedling leaves of *Triticum aestivum* to study pathogenicity in *Puccinia recondita* f.sp. *tritici*. J. Phytopathology 146, 115-121.

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