



Species:	<i>Blumeria graminis</i> f.sp. <i>tritici</i>
Product Class(es):	SBI- and QoI-fungicides
Method type described:	<i>In vivo</i> test
Date of protocol:	2006-05
Proven for	Kresoxim-methyl, Fenpropimorph, Epoxiconazole
Should be suitable for	other SBI- and QoI-Fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	<ul style="list-style-type: none"> • proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results • Validated routine method for labs

Method:

1. Sampling: Wheat plants with young fresh sporulating pustules of powdery mildew are collected. If these are to be sent to the laboratory the whole plants including roots are bundled together and the roots are covered with a plastic bag containing moist paper towels. This is to prevent the leaves and mildew pustules from drying out.
2. Plant material: Wheat seedlings (var. Kanzler) are grown in the greenhouse to an age of 8-10 days. Segments (5.5 cm long) are then cut and laid in Petri dishes (3-5 per dish) onto the surface of 0.4% water agar + 40 ppm benzimidazole + 30 ppm streptomycin. Both cut ends of the segments are pushed under the surface of the agar.
3. Isolation and propagation of *Blumeria graminis* from field samples: Segments of leaves containing fresh sporulating pustules are cut from the samples and laid in Petri dishes onto the surface of 0.4% water agar + 40 ppm benzimidazole + 30 ppm streptomycin. The cut ends of the segments are

pushed under the surface of the agar. The dishes are incubated at 18°C for 24-48 h with 12 h light / 12 h darkness to promote further sporulation of the pustules. From these pustules conidia are removed with a fine brush and inoculated onto leaf segments in Petri dishes prepared as described above. The dishes are then incubated at 18°C for 8-10 days with 12 h light/darkness to allow infection and sporulation of the isolates. The isolates are propagated by sucking conidia from the segments into a 10 ml pipette and ejecting into the top of a small settling tower at the bottom of which is a Petri dish (without lid) containing 5 leaf segments on water agar. Several holes are made in the lid of the Petri dishes, which are then incubated for 10-11 days at 18°C with 12 h light / 12 h darkness (Figure 1).

4. Fungicide treatment of test plants: 8-10 day old potted wheat seedlings are sprayed in a spray cabin with different concentrations of the fungicide (e.g. 0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 ppm for kresoxim-methyl and epoxiconazole and 0, 1, 2, 4, 8, 16, 32, 63, 125 ppm for fenpropimorph). One day after spraying the seedlings, 5 leaf segments per fungicide concentration are cut and laid in a Petri dish on 0.4% water agar + 40 ppm benzimidazole + 30 ppm streptomycin as described above (Figure 2).
5. Inoculation of leaf segments: Petri dishes representing all of the fungicide concentrations are placed open in a settling tower. As inoculum 8 leaf segments with abundant sporulating pustules, following the propagation procedure described above, are taken and held in a unit at the top of the settling tower. A gentle flow of compressed air is then used to blow the conidia into the settling tower where they fall onto the leaf segments below. The conidia density can be checked by examining the surface of the water agar under a microscope. The dishes are then closed and incubated at 18°C with 12 h light / 12 h darkness (Figure 2).
6. Evaluation: 7 days after inoculation the leaf segments are examined under the microscope and the number of pustules counted. The inhibition is calculated ($\% \text{ inhibition} = [\text{No. pustules in untreated} - \text{No. pustules in treated} / \text{No. pustules in Untreated}] \times 100\%$). ED50 values are calculated and compared with sensitive standard isolates and less sensitive or resistant isolates (Figure 2).

Figure 1: Isolation and propagation of *Blumeria graminis* from field samples

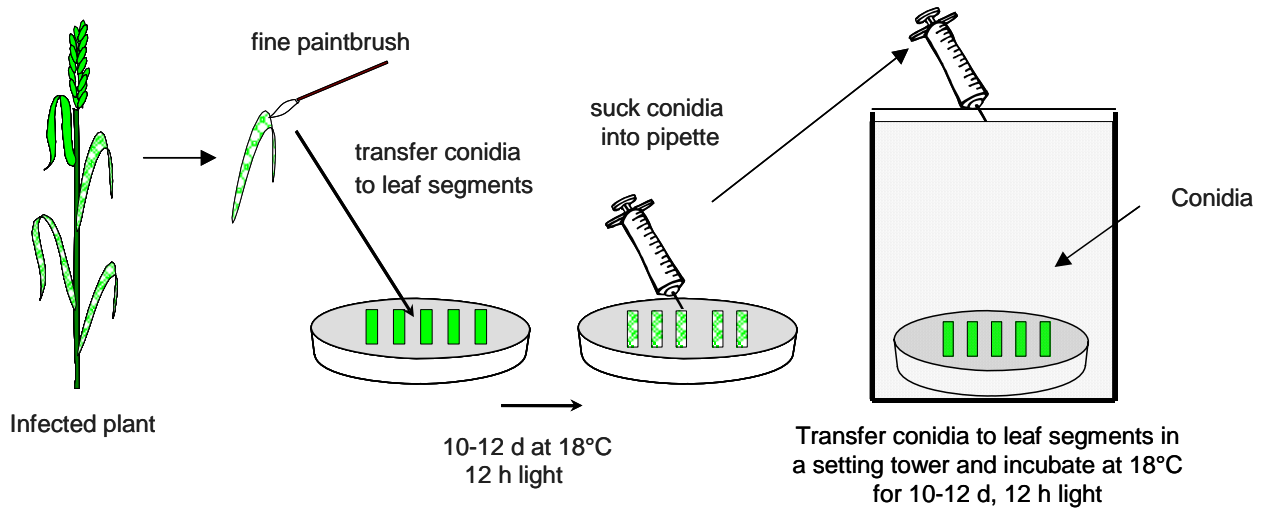
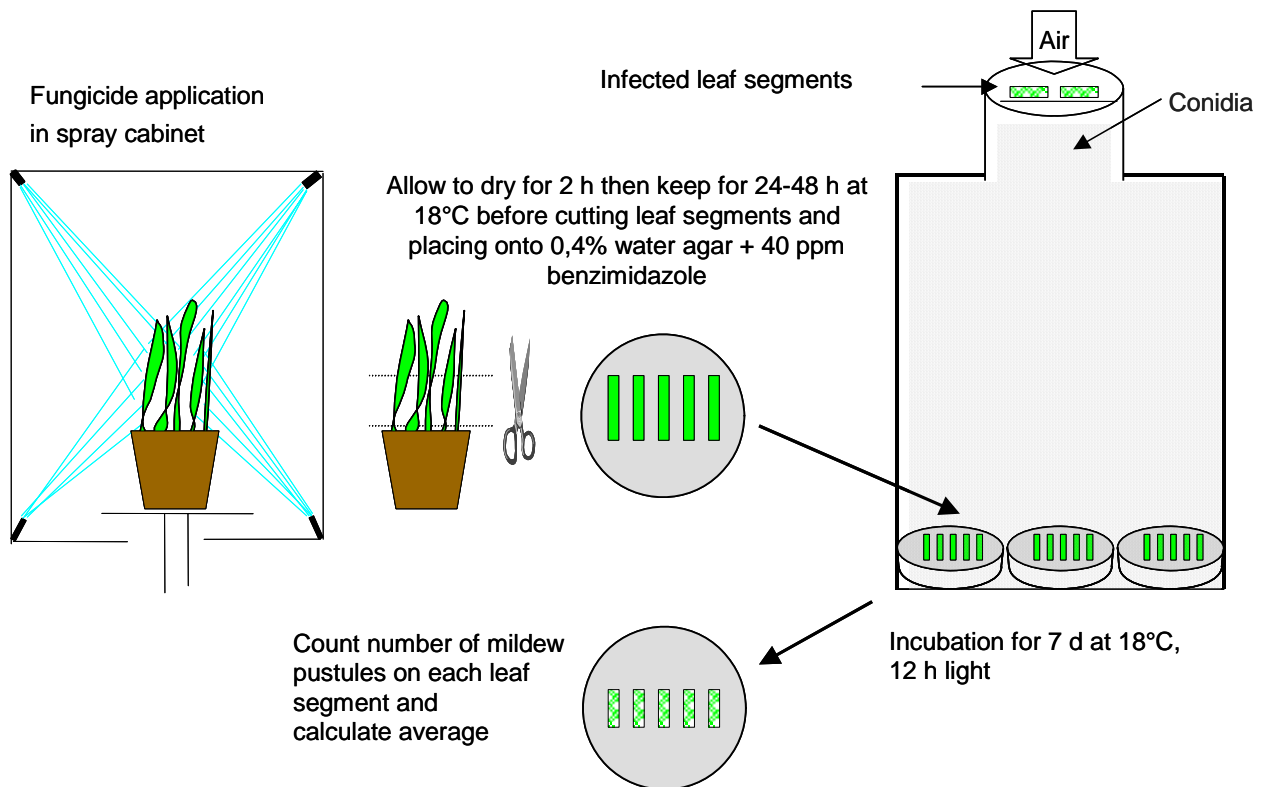


Figure 2: Sensitivity test



author	Dr. Gerd Stammler, Dr. Kristin Klappach, BASF-AG, 67117 Limburgerhof, Germany
--------	---