Species:	*Venturia inaequalis*
Product Class(es):	QoI fungicides, and other fungicide classes inhibiting spore germination (not for BCM, SBI and anilinopyrimidines)
Method type described:	Spore germination tests
Date of protocol:	2006-05
Proven for	Kresoxim-methyl, Pyraclostrobin
Should be suitable for	other QoI's. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	• 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: A sample consists of ~20 apple leaves with sporulating scab lesions per location. The leaves are placed between layers of paper, wrapped in newspaper and then taken as quickly as possible to the laboratory, preferably in a cool box. If the sample is to be sent to the laboratory, the leaves should be air-dried and then placed between sheets of newspaper.

2. Inoculum production: Lesions from scab-infected leaves are cut out and conidia are harvested by rinsing ca. 40 - 60 lesions with deionised water and filtering the conidia suspension through four layers of cheese-cloth to remove debris. The concentration of conidia is determined and adjusted to approximately 5×10^5 spores/ml.

3. Agar plates: The QoI-fungicide is suspended and diluted in sterile deionised water immediately before the agar plates are prepared. The fungicide is applied to sterile 2% water-agar, which should not be warmer than ~50°C. Homogenise well before pouring about 20 ml agar in each Petri dish. Final

concentrations are 0 and 2 ppm a.i. (e.g. for QoI-fungicides). Let cool for at least 24 hours before use. These agar plates may be stored up to 4 weeks at 6-8°C.

4. Inoculation: Conidia suspensions are placed as 10 µl drops onto the agar. The areas of the droplets are marked with a pen. The plates are incubated at 18-20°C in the dark for 24 h hours.

5. Evaluation: 24 hours after inoculation the percentage of germinated spores out of 100 spores in each droplet is counted. The percentage of viable spores is determined at 0 ppm (No. of germinated spores / total No. of spores] x 100%). The percentage of QoI-resistant spores in the sample is determined by dividing the % of germinated spores at 2 ppm with the % of germinated spores at 0 ppm and multiplied with 100% (% QoI-res. Spores = [% germinated spores at 2 ppm / % germinated spores at 0 ppm] x 100%)

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