Species:	*Botrytis cinerea*
Product Class(es):	SBI fungicides (Classes I and III)
Method type described:	microtiter plate test
Date of protocol:	2006-05
Proven for	Fenhexamid
Should be suitable for	other SBI-Fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	• validated routine method for labs equipped with microtiter plate technique and a climate chamber • proven methodology for the active ingredient listed above. Others not mentioned have to be evaluated carefully to ensure valid results

Method:

1. Sample grapes or strawberries infected with *Botrytis cinerea*, selected at random from several plants from the orchard or field. To get an acceptable statistical result take not less than 20 berries ideally taken diagonally from the field. Don't sample immediately after rainfall. The fruits should be placed in small plastic boxes which have to have small holes at the upper part of the side walls (for ventilation, otherwise berries gets moldy quite quickly). The berries in plastic boxes should be dispatched by express mail.

2. To obtain fresh spores developing out of the fruits, place plastic boxes containing the berries of about one week at 10°C and permanent black light in a climate chamber. Take a single fruit with clearly visible spores carefully out of a plastic box with a pair of tweezers and blow the conidia slightly with your mouth onto a Petri dish containing water agar. Let the spores germinate for 24

h (18°C, black light). Prepare single spore isolates by cutting pieces of water agar containing the germinated spore and by transfering them into glass tubes filled with potato dextrose broth (PDB). Incubate glass tubes at 20°C and 150 rpm.

3. After 3 days of incubation, prepare mycelial suspensions by homogenisation (mixer) for further testing.

4. Microtiter plate test: Prepare 96 well microtiter plates by adding 140 µl of PDB per well containing e.g. 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, and 3 µg a.i. / ml of the fungicide to be evaluated (e.g. fenhexamid). The concentration range has to be adapted to a) the intrinsic activity of the fungicide to be tested and b) to the expected sensitivity variation in the fungal population.

5. Inoculate each well with 60 µl of the prepared mycelial suspension. Use one duplicate per isolate. Incubate microtiter plate for 3 days at 20°C, agitate gently. After incubation fungal growth is measured with a microtiter plate reader at 620 nm. Calculate EC_{50} values.

Further comment:
Sampling can alternatively be carried out by using cotton buds. For this, sterile cotton buds should be carefully rubbed at infected fruits (one bud per fruit) and dispatched seperated to the laboratory. In the lab, spores from these buds can be transferred onto agar to obtain *Botrytis* populations (further working steps as described before).

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