



Species:	<i>Monilinia laxa</i> , <i>Monilinia fructigena</i>
Product Class(es):	SDH-inhibitors (SDHI), Qo-inhibitors (QoI)
Method type described:	Germ tube elongation test
Date of protocol:	2009-01
Proven for	Boscalid, pyraclostrobin
Should be suitable for	Other fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
Comments	<ul style="list-style-type: none"><li>• Validated routine method</li><li>• Proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results</li></ul>

Method:

1. Sampling and isolation of *Monilinia* spp. from field samples: Samples of *Monilinia laxa* and/or *Monilinia fructigena* may be collected from infected fruits, blossoms or twigs by transferring conidia from sporulating pustules on the surface of Petri dishes containing 2% malt agar and 200 ppm streptomycin. Clean cultures are produced by transferring conidia or mycelium of *Monilinia* spp. to new agar plates.
2. Spore production: For spore production, *M. laxa* is cultivated on V8 agar and *M. fructigena* on 2% malt agar. The dishes are incubated at 18°C for 10-12 days under 12 h dark and 12 h white light to promote dense sporulation. Spores are harvested by washing the agar plates with 5 ml double concentrated YBA medium\*. The resulting suspension is filtered through 2

layers of cheesecloth and the suspension is adjusted to a spore density of  $2 \times 10^4$  / ml.

3. Sensitivity tests: Pure technical active ingredient of boscalid or pyraclostrobin is solved in dimethylsulfoxide and the dilutions are prepared in sterile deionised water immediately before mixing with the spore suspension. Fifty  $\mu$ l fungicide solution and 50  $\mu$ l spore suspension are mixed in 96-well microtiter plates. Final concentrations of boscalid or pyraclostrobin are 0, 0.0025, 0.01, 0.039, 0.156, 0.625, 2.5, 10 ppm. For each isolate and fungicide concentration, three replicate wells were used. The microtiter plates are put into plastic bags to avoid evaporation and incubated at 18°C in darkness. Five days after inoculation the growth is assessed visually in five classes: 0, no growth; 1, less than 50 % of control; 2, ~50% of control; 3, > 50% of control; 4, same growth as control. The minimum inhibitory concentrations (MIC, lowest concentration which totally inhibits growth) are determined for each isolate. Growth at different concentrations and MIC values are compared with sensitive standard isolates. Please note that some isolates may germinate and that the germ tube might elongate to some extent in the presence of SDH-inhibitors, but that further mycelial development is inhibited.
4. References: J. Spiegel and G. Stammler (2006). Baseline sensitivity of *Monilinia laxa* and *M. fructigena* to pyraclostrobin and boscalid. *Journal of Plant Diseases and Protection*, **113**, 199-206.

\*Yeast Bacto Acetate medium, 2 x concentrated

20 g yeast extract  
20 g Bacto peptone  
40 g sodium acetate  
fill up to 1000 ml with bidest water

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