



Species:	<i>Mycosphaerella fijiensis</i> (Banana Black Sigatoka)
Product Class(es):	SBI's
Method type described:	Ascospore germ tube elongation
Date of protocol:	03.10.2008
Proven for	All SBI's used in bananas
Version	1
comments	Also adaptable to MBC's and Qol's

Method:

Sampling

Collect dry necrotic tissue in grade 6 (Stover's international scale method). Select one cable or block per farm. Sample the same areas whenever possible throughout the program.

Inoculum production

Cut the necrotic tissue into small pieces (1-2 cm²). Incubate in a plastic bag for 48 hours at 26°C using a moist paper towel or in a humidity chambers.

Propagation

Monitoring is done mainly with ascospores therefore it is not necessary to propagate the pathogen. Ascospore will be obtained directly from the perithecia.

Sensitivity tests

Staple the leaf pieces on a paper (any type of white paper will work) The paper with the attached leaf pieces is submerged for 5 minutes in distilled water and then immediately, placed inside the top of a Petri plate and over 2% water agar (Bacto Agar Difco® 2%, 20g/L) amended with the different SBI's concentrations.

Prepare the concentration ranges:

Propiconazole, bitertanol and tebuconazole:

In untreated populations	0	0.01	0.03	0.1	1.0 ppm
In commercial areas	0	0.03	0.10	0.3	1.0 ppm

Difenoconazole and epoxiconazole:

In untreated populations	0.0001	0.001	0.01	0.1 ppm
In commercial areas	0.001	0.01	0.1	1.0 ppm

As a minimum you need to include 0, 0.1 and 1.0 ppm and incubate 48 hours at 26°C. Two replicates per concentration are included.

Allow 1 hour for ascospore discharge, any extra time will increase the amount of contaminants. When the discharge period is completed, identify areas where ascospores have been released by turning the Petri dishes upside down and marking the leaf area of each leaf piece with a wax pencil on the bottom of the dish. The filter paper with the attached leaf pieces is then removed and plates will be incubated for 48 hours at 26°C.

Evaluation

Measure germ-tube length of 15 ascospores per dish (30 germ-tubes per concentration) over different areas within the dish. At 0 ppm and 0.1 ppm, the number of readings is increased to 60 ascospores per concentration. If there are two germ tubes per ascospore, measure the longer one. Determine EC50-values using a semi-logarithmic scale graphically. Determine the frequency distribution at 0.1 ppm.

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