



Species:	<i>Mycosphaerella fijiensis</i> (Banana Black Sigatoka)
Product Class(es):	MBC's, SBI's, QoI's, AP's
Method type described:	Ascospore germ tube elongation
Date of protocol:	03.10.2008
Proven for	MBC's, SBI's, QoI's, AP's
Version	1
Comments	In vitro method

Method:

Leaf sampling procedure

Leaf samples are collected from the different farm areas. Each sample consists of 10 composite Sigatoka-infested leaves collected from different areas within the sampling site and delivered to laboratory as soon as possible, but not later than 24 hours after collection.

Inoculum production

The leaf samples are air-dried upon arrival for a day to avoid moisture retention. Leaf sections showing massive Sigatoka infection is located. The mass-infected leaf sections are then cut into leaf pieces of about 25 x 20 cm. Incubation of ascospore bearing leaf tissue is then done by placing the leaves in a plastic bag lined with moist paper towel. The paper towel is moistened using warm distilled water. The plastic bag is then inflated and sealed with rubber band. This is then placed in the growth room maintained at approximately 26°C for 48 hours. This ensures that perithecia mature to release ascospores during bio-assay.

After incubation, the leaf samples are then cut into small pieces of about 2 x 2 cm. Five (5) pieces of these leaf sections are attached with staples to 9.0 cm filter papers so that the leaf sections are equally spaced from each other. The filter papers with leaf sections are then soaked in distilled water for 5 minutes.

Sensitivity tests

Tests are performed on different concentrations contained in 2.0 % water agar (1000 ml of distilled water and 20 grams of agar for different fungicides).

- ❖ Calixin (using technical grade tridemorph)
- ❖ Opal (using technical grade epoxiconazole)
- ❖ Cabrio (using technical grade pyraclostrobin)
- ❖ Baycor (using technical grade bitertanol)
- ❖ Folicur (using technical grade tebuconazole)
- ❖ Impulse 500 EC – Product Form
- ❖ Siganex (using technical grade pyrimethanil)
- ❖ Twist (using technical grade trifloxystrobin)
- ❖ Bankit (using technical grade azoxystrobin)
- ❖ Sico (using technical grade difenoconazole)
- ❖ Tilt 250 EC – Product Form

Each treatment is replicated 3 times.

Discharge of ascospores is done by placing the filter papers with the attached leaf samples inside the top of a Petri dish plate containing 2.0 % water agar medium and amended with various concentrations of fungicide for one (1) hour under high relative humidity. This enables ostioles to open up and ascospores to discharge.

After ascospore discharge, the Petri dish plates are turned upside down and the leaf areas of each leaf piece is marked on the bottom of the dish using permanent ink pens. The filter papers are removed and the plates are placed bottom-side up to allow the germination of the discharged ascospores. The plates are then placed under continuous fluorescent light, ambient humidity at room temperature for 48 hours.

After 48 hours of ascospore germination, all amended plates are refrigerated at 4°C. Germ tube length is then measured starting with the plates amended with the lowest concentration. A total of 90 spores (depending on the chemical being tested) are measured per concentration.

Percent inhibition in comparison to the average length on the control plates is calculated for each of the remaining test concentrations. Data is then recorded as frequency distribution with the following inhibition ranges:

- 0.0-10.0
- 10.1-30.0
- 30.1-50.0
- 50.1-70.0
- 70.1-90.0
- 90.1-100%

for Opal, Cabrio, Baycor, Folicur, Impulse, Siganax, Twist, Sico and Tilt

0.0-10.0
10.1-30.0
30.1-50.0
50.1-70.0
>70.0%

for Calixin and Bankit

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