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NEW DISEASE REPORT

First report of banana freckle disease caused by *Phyllosticta cavendishii* in Mauritius

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Banana (*Musa* sp.) is among the most important fruit crops grown in Mauritius with significant socioeconomic and cultural considerations. The annual production of banana approximates to around 8000 tonnes (Statistics Mauritius, 2018). Dwarf Cavendish is the main variety grown commercially in Mauritius. During the hot and humid month of February 2014, symptoms consisting of dark black spots were observed on unripe banana of Dwarf Cavendish from the region of Nouvelle Découverte (super humid zone). A disease incidence of more than 50% with a moderate to severe level of severity across the field was reported by the extension services of the Food and Agricultural Research and Extension Institute. On the fruits, the initial symptom was tiny reddish-brown spots, bordered by dark green water-soaked margins (Figure 1). As the spots enlarged and coalesced, the surface became rough and turned black. These spots had a rough texture like sand-paper, similar to the description described by Wong et al. (2012). Comparable symptoms were observed on the leaves (Figure 2). Results from a rapid island-wide survey showed that only the Cavendish-type banana varieties displayed symptoms of infection. Out of 98 banana fields surveyed (50.9 ha), 65% were affected.

Morphological identification of the suspected fungal pathogen was done using a compound light microscope. A sterile needle was used to tease pycnidia on leaves in a drop of sterile water to release conidia, before being mounted in clear lactic acid for microscopic examination. Conidia (Figure 3) formed singly at the apex of the conidiogenous cell were hyaline, aseptate, oblong to ellipsoidal, coarsely guttulate and measured 7.1-15.6 × 4.4-8.3 μm. The apical mucilaginous appendage arising from the conidium was straight to curved and was 5.1-12.9 μm long. Spermagonia were 70–125 μm in diameter and 50–85 μm high. Dumbbell-shaped spermatia (Figure 4) were hyaline, aseptate, biguttulate and measured 7.5-9.5 × 1.3-1.8 μm. These morphological characters are consistent with the species description of *Phyllosticta cavendishii* (Wong et al., 2012).

FIGURE 1 Reddish-brown freckle spots on banana fruit (Dwarf Cavendish variety)

FIGURE 2 Freckle spots clustering in lines across banana leaf

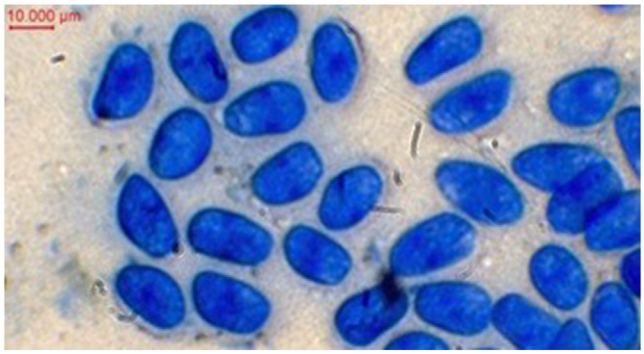

FIGURE 3 Conidia of *Phyllosticta cavendishii*

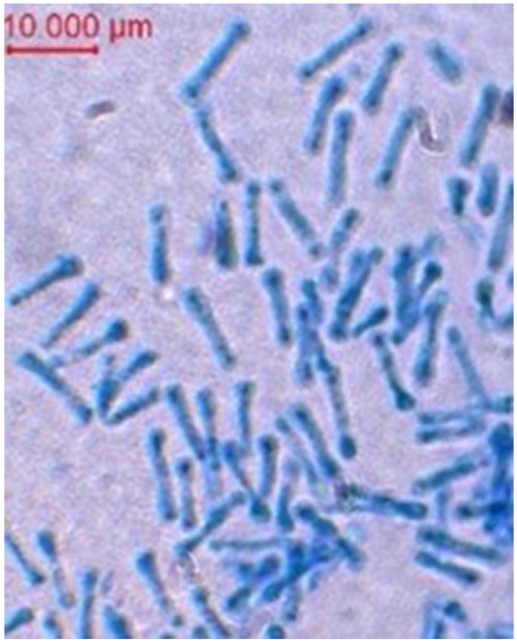

FIGURE 4 Spermatia of *Phyllosticta cavendishii*

Lesions from 11 samples of leaf and fruit with fruiting bodies were ground in liquid nitrogen followed by genomic DNA extraction. Extraction of DNA was done on c. 50 mg fresh plant tissue using the Biosprint 15 DNA Plant Kit (Qiagen, Germany). Tissues were first homogenized using a Tissuelyser (Qiagen, Germany) in a 2 ml microcentrifuge tube containing a 3 mm metal bead for two 30 second bursts at 30Hz. Two microliters of the eluted DNA was used as template in an ITS PCR amplification using AmpliTaq Gold 360 Mastermix (Applied Biosystems, USA) and 0.5 μM each of primers GMF1 and GMR2 (Wong et al., 2012). Thermal cycling conditions were 95°C for 10 min followed by 40 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 1 min. Final extension of PCR products was done at 72°C for 10 min. Amplicons (550bp) were directly sequenced using the same primer pair. Resulting sequences were analysed using BlastN as well as by manual comparison to known sequences of *Phyllosticta* isolates which cause freckle disease of banana. The causal agent from all 11 samples tested was found to be *Phyllosticta cavendishii*. The ITS sequences were deposited in GenBank (Accession Nos. MW520341-MW520351) and at the Queensland Plant Pathology Herbarium (BRIP 72048 to 72058).

Pathogenicity was tested using twenty mature green healthy fruits of banana variety Dwarf Cavendish. Fruits were surface sterilised using 0.2% sodium hypochlorite for 2 mins, rinsed with sterilised distilled water and allowed to dry before inoculation. Ten fruits were artificially inoculated on the outer surface, clearly marked, with a conidial suspension of 10^6 conidia/ml, prepared from pycnidia extracted from field infected tissue. Control fruits were treated with sterile distilled water. All the fruits were covered with plastic bags for 48 hours and incubated at room temperature, 80% relative humidity and with a 12-hour photoperiod. After 7 days, typical minute black spots identical to those observed in the field, appeared on inoculated fruits, whereas controls remained healthy. The morphological characteristics of the fungus were consistent with those previously observed, hence fulfilling Koch's postulates.

To our knowledge, this is the first report of *Phyllosticta cavendishii* causing banana freckle disease in Mauritius.

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