

First report of black Sigatoka disease in banana caused by *Mycosphaerella fijiensis* on Reunion Island

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Pseudocercospora fijiensis, the causal agent of black Sigatoka disease of banana (*Musa* spp.) (BLSD), is considered to be the major economic threat for banana cultivated for export (de Lapeyre de Bellaire *et al.*, 2010). The disease has a worldwide distribution throughout the humid tropical regions and has been previously reported in the southwest Indian Ocean area: in 1993 in Mayotte and Comoros islands (Jones & Mourichon, 1993), in Mauritius in 1996 (Soomary & Benimadhu, 1997) and in Madagascar in 2000 (Jones, 2003). In December 2017, typical BLSD symptoms were observed in a Cavendish cv. Petite Naine crop located in Grand Etang municipality, St. Benoit (Site S1, Fig. 1) in the east-central area of Reunion Island. Typical early symptoms (stage 2) were 1-4 mm long brown streaks on the abaxial leaf surface, and typical older streaks (stages 3 and 4) were also observed (Figs. 2-3).

In March 2018, banana leaves bearing leaf spot symptoms were collected in the four following sites (Fig. 1): Grand Etang (S1), where potential BLSD symptoms were observed in December 2017, and Piton St Rose (S2), Anse des Cascades (S3) and Langevin (S4). At each site, four leaf fragments (A, B, C & D) cut from different banana plants were sampled resulting in 16 distinct samples.

All leaf fragments were sent to the ANSES laboratory where molecular diagnostics was performed following the protocol developed by Arzanlou *et al.* (2007). Genomic DNA was extracted from ground leaf fragments displaying symptoms using the DNeasy Plant Mini Kit (Qiagen, Germany). The DNA extracts were used as templates for a real-time PCR assay designed to specifically detect the presence of *P. fijiensis* DNA. The presence of DNA from *Pseudocercospora eumusae* and *P. musicola*, two related ascomycetous fungi causing other banana leaf spot diseases, were also assessed using the protocol of Arzanlou *et al.* (2007). Both positive and negative controls were included in the assay and every sample reaction was duplicated.

Pseudocercospora fijiensis was detected in three of the four samples from Grand Etang (site S1, see Table 1). The three positive PCR products were sequenced and deposited in GenBank (Accession Nos. MK729105-

MK729107). No positive signal for *P. fijiensis* was found in the other three sites. To our knowledge, this is the first report of BLSD in Reunion Island. Interestingly, our results also showed co-infection with *P. eumusae* (Table 1). This observation is in accordance with the literature since *P. eumusae*, the fungus causing eumusae leaf spot disease was identified on Reunion Island in 2000 (Jones, 2003). Further investigation will be required to monitor the spread of BLSD on the island and to identify the origin of the populations responsible for the introduction of the disease from the first reports of BLSD in the southwest Indian Ocean area in the 1990's.

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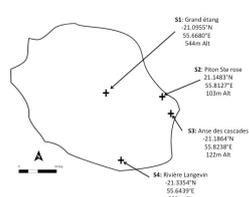


Figure 1
Table 1. Results of real-time PCR assays for samples from Grand Etang (S1).

Samples	qPCR mean (Ct value ±SD)		
	<i>Pseudocercospora fijiensis</i>	<i>Pseudocercospora eumusae</i>	<i>Pseudocercospora musicola</i>
S1-A	Negative	Negative	Negative
S1-B	30.12 ±0.06	28.45 ±0.31	Negative
S1-C	30.71 ±0.23	30.43 ±0.05	Negative
S1-D	27.59 ±0.67	32.91 ±0.07	Negative

Figure 4



Figure 2



Figure 3

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