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NEW DISEASE REPORT

First report of *Fusarium acacia-mearnsii* causing leaf blight on pumpkin in Mauritius

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Cucurbita maxima, fungal plant disease, phylogeny

Pumpkin (*Cucurbita maxima*) is an important crop in Mauritius that is cultivated throughout the island. The annual production was 7,412 tonnes in 2019 and pumpkin is also exported to foreign countries (Food and Agricultural Research and Extension Institute, 2020). In November 2020, on a plantation in the super-humid zone in Cluny, pumpkin plants showing yellow lesions with pale pink fungal growth on the upper leaves were observed (Figure 1). Disease incidence was estimated to be 40% over an area of c. 15 ha. Small pieces (1 cm^2) of infected foliar tissues were excised from five plants, surface-disinfected with 1% NaOCl, air-dried and placed onto potato dextrose agar (PDA). After incubation at 23°C for seven days, the obtained isolates were uniform with white-pink fluffy colonies and an intensive red pigment visible on the underside of the agar (Figure 2). All isolates formed falcate, three- to six-septate macroconidia (33.3 × 6 μm; Figure 3) and numerous chlamydospores (12.5 μm in diameter).

DNA was extracted from mycelium of one selected isolate (designated FSI) using a modified CTAB protocol (Day & Shattock, 1997). PCR amplification and sequencing of the *tef-1α* gene and ITS region of rDNA were done using the EF1/EF2 (O'Donnell et al., 1998) and ITS5/ITS4 primer pairs (White et al., 1990), respectively. The resultant *tef-1α* and ITS sequences were deposited in GenBank (Accession Nos. OP651779 and ON738580, respectively). BLAST analyses of the *tef-1α* and ITS consensus sequences revealed >99% nucleotide

FIGURE 1 Yellow lesions observed on the upper leaf surface of pumpkin infected by blight disease.

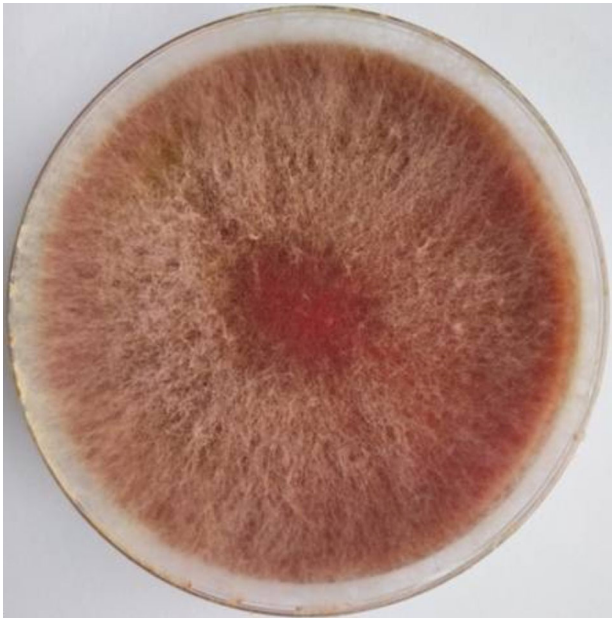

FIGURE 2 Appearance of seven-day old culture of *Fusarium acacia-mearnsii* on potato dextrose agar.

FIGURE 3 Multicellular macroconidia of *Fusarium acacia-mearnsii*.

identity with several *F. graminearum* isolates including KJ705291 and MW965280 for *tef-1α* and MT482509 and LT222056 for ITS. Similarly, a BLAST analysis using the FusariumID database revealed >99% identity with several species in the *F. graminearum* complex, including *Fusarium acacia-mearnsii* (CBS110255 for *tef-1α* and LC13787 for ITS). The morphological characteristics (Leslie & Summerell, 2006) coupled with the BLAST analyses demonstrated that isolate FSI belongs to the *F. graminearum* species complex. Species-level identification using a phylogenetic analysis of the sequences of the *tef-1α* region of representative isolates of *F. graminearum* species resulted in the construction of a neighbour joining phylogenetic tree with a well-supported branch of *F. acacia-mearnsii* including isolate FSI from Mauritius (Figure 4).

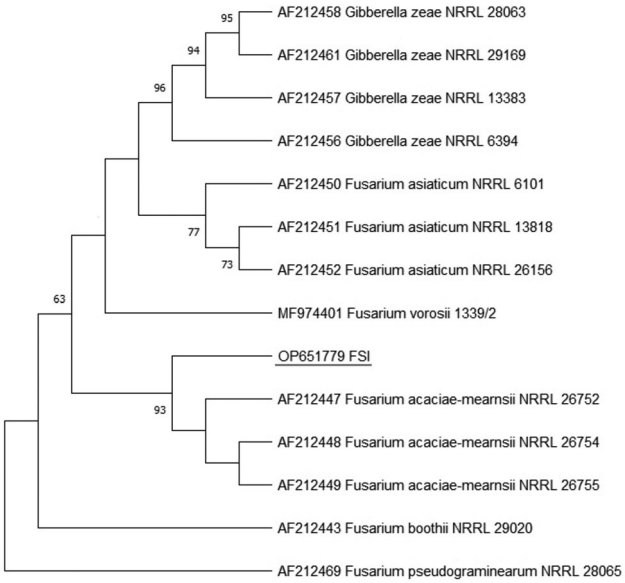

FIGURE 4 Phylogenetic tree reconstructed using the neighbour-joining method (MEGA X) based on 12 sequences of *tef 1α* gene from six representative species within the *Fusarium graminearum* complex. The values (from 1000 replicates) are indicated at the branch nodes as percentages supported by bootstrap. The phylogenetic tree is rooted to *Fusarium pseudograminearum* strain NRRL 28065 (AF212469). Isolate FSI from Mauritius is underlined.

To demonstrate pathogenicity, isolate FSI was grown on PDA at 24°C for seven days and used to inoculate leaves and roots of pumpkin cv. Olinka seedlings in the phenophase of two true leaves. Five pumpkin seedlings were inoculated by spraying with a spore suspension (10^2 spores/ml) and five seedlings were sprayed with sterile distilled water and used as control plants. Another set of five seedlings were inoculated by placing mycelial plugs on wounded stem tissue at ground level, while control seedlings were wounded and inoculated with sterile PDA plugs. After 20 days incubation at 24°C, all plants inoculated with the spore suspension had one-two necrotic spots on the leaves similar to those observed on naturally infected plants, while stem-inoculated seedlings developed lesions on stem and roots and symptoms of wilting (Figure 5). The control plants in both experiments were asymptomatic. From all symptomatic plants an isolate with a morphology resembling the original isolate FSI was recovered.

To our knowledge, this is the first study confirming *F. acacia-mearnsii* causing pumpkin blight in Mauritius. The *F. graminearum* complex comprises toxigenic species also known to cause pumpkin fruit rots (Babadoost & Zitter, 2009), a disease that can lead to considerable financial losses if not controlled effectively. Thus, this study is hoped to provide a better understanding of this disease affecting pumpkins so that proper and timely control measures can be taken.

FIGURE 5 Root and stem rot of pumpkin seedling wound-inoculated with *Fusarium acacia-mearnsii* (a) and in cross-section (b)

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