Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium verticillioides* Causing Banana Fruit Rot in Jordan

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Banana (*Musa* spp.) is widely cultivated in the Jordan Valley. Fruit rot caused by *Fusarium verticillioides* (Sacc.) is a damaging postharvest disease (Van Hove et al. 2011). In June and July of 2018 and 2019, dark brown spots were observed on the epidermis of banana fruit in the Jordan Valley. Occasionally, spots coalesced. Spots were brown to dark brown with lacunae at the center and typically surrounded by light brown halos. Larger spots had cracks, which exposed the pulp. Whitish fungal colonies were frequently observed on the spots. Initially, virtually no pulp damage was observed. Cut surfaces of crowns and pedicels sometimes were also blackened and often covered with whitish mycelia. As the disease progressed, spots were covered by whitish colonies over the whole fruit, and the pulp showed extensive decay. The disease affected ~80% of fruits during the harvest and first 3 weeks postharvest. Pieces of skin (0.5 × 0.5 cm) from symptomatic bananas were disinfected by dipping into 5% NaOCl for 1 min followed by rinsing with sterile distilled water three times. Pieces were placed on PDA and incubated at 25°C for 5 days. The hyphal agar plugs were subcultured on new PDA plates. Isolates were cultured on synthetic low-nutrient agar with a ~1 × 2-cm strip of sterile filter paper on the surface (Nirenberg 1976). Cultures were incubated for 10 to 14 days at 20°C in darkness. When sporulation was observed, agar blocks were mounted on a microscopic slide with a drop of lactophenol cotton blue and examined at 400×. Colonies grew rapidly with abundant pink to violet aerial hyphae. Sporodochia formed on the agar, and the aerial conidiophores branched sparsely, often alternately or oppositely, terminating with up to three verticillate phialides. Macroconidia were abundant, falcate to straight, three- to five-septate, with a distinct foot cell, 25 to 71 × 3.3 to 5.4 µm. Microconidia produced on polyphialides and aggregating in heads, were unicellular, ovoidal, ellipsoidal, or allantoid, 4.1 to 18 × 1.7 to 4.3 µm. Chlamydospores were not observed. Based on morphological characteristics, it was tentatively identified as *F. verticillioides* (Al-Hatmi et al. 2016;

Guarro 2013). The ITS region, *TEF1*, and *RPB2* for five isolates were amplified by PCR using the primers and protocols of White et al. (1990), O'Donnell et al. (1998), and Liu et al. (1999), respectively. PCR products of the ITS and *TEF1* were purified and ligated into pGEM T-Easy Vector (Promega), and two clones for each PCR product were bidirectionally sequenced (Macrogen). PCR products of *RPB2* were isolated directly from the gel using a Zymoclean Gel DNA Recovery Kit (Zymo Research) and sent for direct sequencing from both directions (QuintaraBio). All sequences were deposited in GenBank (MN335231 to MN335240). BLASTn analyses of the nucleotide sequences were 100% identical to *F. verticillioides* sequences. A phylogenetic tree was constructed using the maximum likelihood method (MEGA X) with the combined *TEF1* and *RPB2* sequences. The isolates clustered with *F. verticillioides* strains. Koch's postulates were fulfilled to confirm *F. verticillioides*. Inoculation tests were carried out in duplicate on healthy green bananas (cv. Grand Naine) that were lightly wounded by piercing the skin with a bundle of 20 sterile sewing needles and placing hyphal agar plugs (5 × 5 mm) from a 5-day-old culture of isolate JO-B499 on wounded (W) and nonwounded (NW) areas. Uninoculated PDA plugs on W and NW fruit were a negative control. Inoculated fruit was incubated at 25°C for 2 weeks in plastic boxes. JO-B499 induced light to dark brown spots on W and NW parts. Spots on W parts were larger and developed faster than on NW parts. Some larger brown spots, especially on W parts, had whitish mycelial colonies surrounded by yellow halos. These spots extended after 2 weeks, yielding symptoms similar to those in the field. This is the first report of *F. verticillioides* causing fruit rot of banana in Jordan. It is a major pathogen of maize and a producer of fumonisin mycotoxins (Blacutt et al. 2018). Our results open an avenue to future studies on the spread and impact of this fungus, which may pose a threat to banana and other plants in Jordan.

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