First Report of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 associated with Panama Disease of banana outside Southeast Asia

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Fusarium wilt or Panama disease of banana, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), is among the most destructive plant diseases (3). Race 1 ravaged ‘Gros Michel’-based export trades until the cultivar was replaced by resistant Cavendish cultivars. However, a new variant of Foc, tropical race 4 (TR4), was identified in Southeast Asia in 1992 and has spread throughout the region (3). Cavendish clones, which are most important in subsistence and export production, are among the wide range of cultivars that are affected, and there is a huge concern that TR4 will move into Africa and Latin America, thereby threatening other vital banana-growing regions. In Jordan, Cavendish bananas are produced on 1,000-1,500 ha. in the Jordan Valley (ca. 32°N, 35.5°E). In 2006, symptoms of Fusarium wilt were observed and sampled for the isolation of Foc. On half-strength PDA amended with 100-ppm streptomycin sulfate, pale salmon-colored colonies with floccose mycelia developed consistently from surface-disinfested xylem. Single microconidia from these colonies were transferred to half-strength PDA, and conidia and mycelia from these monospore colonies were stored at -80°C in 15% glycerol. On banana leaf agar (Co60-irradiated leaf tissue on water agar), isolates resembled *F. oxysporum* phenotypically by producing infrequent three- to five-celled macroconidia, copious, usually aseptate microconidia on monophialides, and terminal and intercalary chlamydospores after two weeks (2). With nitrate-nonutilizing (nit) mutants and testers for different vegetative compatibility groups (VCGs), each of seven examined monospore isolates were placed in VCG 01213, which contains only strains of TR4 (3). Total DNA was extracted from six isolates and PCR analyses, which confirmed their identity as TR4 (1). Subsequently, one of the isolates (JV11) was analyzed for pathogenicity. Inoculum production and inoculation were according to (1) by dipping (30 min.) root-wounded 10 week-old plants of the Cavendish cv. Grand Naine in 2 L of spore suspension (1.0 x 10⁶ spores/ml). Inoculated plants were then placed in sand in 3L pots under 28°C, 70% relative humidity and a 16/8h light/darkness photoperiod. Sets of three plants were each treated with either JV11 or two TR4 controls (isolate II-5 and a strain isolated from an affected Cavendish plant in Mindanao, Philippines, both of which were diagnosed as TR4 by PCR and pathogenicity analyses). Control sets were either treated with race 1 (originating from Cruz das Almas, Bahia, Brazil see (1)), or water. After 2 weeks, plants inoculated with JV11 and TR4...
controls produced typical symptoms of Fusarium wilt. After 4 weeks, tissue was collected from all plants and plated on Komada’s medium. TR4 was directly confirmed by PCR (1), either directly from symptomatic plants (JV11 and TR4 controls), or from isolates that were recovered from these plants. Nothing was reisolated from race 1 inoculated plants and water controls, which remained asymptomatic. This is the first report of TR4 affecting Cavendish outside Southeast Asia, is its northernmost outbreak, and represents a dangerous expansion of this destructive race. Currently, 80% of the Jordan Valley production area is affected by Fusarium wilt, and 20-80% of the plants are affected in different farms.


e-Xtra Links to Approved Websites

http://panamadisease.org/news/13
Biological confirmation of pathogenicity of *Fusarium oxysporum* f. sp. *cubense* collected from diseased *cv.* Grand Naine Cavendish banana plants in Jordan. **A,** inoculation of *cv.* Grand Naine, superior, after inoculation; inferior, four weeks after inoculation with negative and positive controls as follow; **A1:** water, **A2:** race 1, **A3:** pure culture of JV11, **A4:** TR4 from Philippines **A5:** TR4 strain II-5. **B,** a close-up of Foc symptoms on *cv.* Grand Naine after inoculation with JV11. **C,** longitudinal-section of *cv.* Grand Naine corm four weeks after inoculation with JV11. **D,** a close-up of the infected veins in the pseudostem. **E,** Growth of Foc on Komada medium after re-isolation from *cv.* Grand Naine plants infected with Foc isolate JV11.
Identification of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4) by multiplex PCR and Vegetative Compatibility Group (VCG) analysis. **A**, amplicons of multiplex PCR on DNA from infected *cv. Grand Naine* plants tissues, four weeks after inoculation, using the elongation factor-1α (EF-1/EF-2) primer set as a internal control for amplifications, TR4 specific primers TR4 (FocTR4-F/FocTR4-R and the banana actin primers (BanAct-F/BanAct-r) as an internal control for banana DNA. Three panels each (Race 1, JV11 and TR4 from the Philippines) containing lanes 1-3 for infected roots, corm and pseudostem tissue from *cv. Grand Naine* plants, respectively. Panels are separated by positive controls of DNA from a pure culture of TR4 reference isolate II-5. Specific bands for TR4 (463 bp), elongation factor-1α (648 bp) and the banana actin gene (217 bp) are indicated on the left. M, molecular marker 1-kb DNA ladder plus. **B**, identification of VCG01213 of *Foc* isolate JV11, isolated from infected Jordanian Cavendish banana plants.