New Disease Reports

First report of *Fusarium falciforme* affecting common bean (*Phaseolus vulgaris*) in Cuba

Y. Duarte [1]*, B. Martínez [1], E. Santos do Carmo de Souza [2] and A.C. Café [2]

[1] *Plant Mycology Laboratory, Phytopathology group, National Center for Animal and Plant Health (CENSA), San José de las Lajas, Mayabeque, Cuba*; [2] *Institute of Biological Sciences, University of Brasilia, Brasil*

*E-mail: yanisia@censa.edu.cu

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Common bean (*Phaseolus vulgaris*) is affected by a range of soil pathogens that reduce the yield following root infection. *Fusarium* species are among the most significant pathogens and infection may reduce yields by up to 50% (Montiel-González *et al*., 2005)**.** During the 2015-2016 harvest, samples of wilting bean plants cv. Cuba Cueto 25-9 which had brown necrotic roots, necrosis of the stem base and leaf chlorosis, were taken to the laboratory from fields in Tapaste, Mayabeque Province, Cuba.

Necrotic stems and roots were cut into fragments (10×20mm) under aseptic conditions, surface-sterilised with sodium hypochlorite solution (1%) for one minute, ethanol solution (70%) for 45 seconds, rinsed three times with sterile distilled water, air dried and plated on potato dextrose agar (PDA, Biocen) plus chloramphenicol (0.01 g/l). Plates were incubated in the dark at 25±2°C. Pure cultures were obtained by single spore isolation (French & Hebert, 1982). Three *Fusarium* isolates were obtained, designated 010901-010903. The mycelium was white (top view) and cream (reverse view) on the PDA plates (Fig. 1). Growth rate at 25±2°C was 4.6 mm/h. Phialides were cylindrical arising from conidiophores. Macroconidia were typically falcate with 3-4-septate with a pointed apical cell and notched basal cell. The microconidia formed in false heads on monophialides and were oval to obovoid with a truncate base and 0-1-septate (Fig. 2). Chlamydospores were formed singly, in clusters, chains and terminal in pairs.

Fungal genomic DNA was extracted using the CTAB method. The translation elongation factor 1-a (*tef*) gene sequences were amplified with the EF1 (5´-ATGGGTAAGGAGGACAAGAC-3´) and EF2 (5´-GGAAGTACCAGTGATCATGTT-3´) primer pair (O'Donnell *et al.*, 1998) and sequenced. Three isolate sequences: 010901 (GenBank Accession No. MN022425), 010902 (MN022426) and 010903 (MN022427) were deposited in GenBank. BLAST analysis indicated *Fusarium falciforme* (99% identity to DQ247010, DQ247034, DQ247011) (Zhang *et al.*, 2006). Based on morphological and molecular analyses, the isolates were identified as *Fusarium falciforme.*

Pathogenicity was confirmed by a soil infestation test. Red ferralitic soil, previously sterilised on three consecutive days (121°C x 60 min), was inoculated with each *Fusarium* isolate separately. The inoculum was aseptically prepared from PDA slope cultures of each isolate grown at 28±2°C in the dark for seven days. Sterile distilled water (10 ml) was added to each tube and homogenised for one minute. Ten milliliters (10 conidia ml^{-1}) of the spore suspensions were added to the sterile soil, and three seeds of bean cv. Cuba Cueto 25-9 were sown in each pot. Only sterile distilled water was added to the control soil. The pots were placed at room temperature (c. 25°C) under semi-controlled conditions (constant white light, humidity of 60-70%), irrigating alternate days (10 ml of sterile distilled water) per pot. Symptom development was checked daily. After ten days, all the inoculated plants were observed to be infected and showing the same symptoms observed on naturally infected plants. The roots had a black colouration and brownish longitudinal grooves. The internal tissue was necrotic. No symptoms were observed on the control plants. The causal agent was isolated and identified from affected plants. Both morphological characteristics (colony and fungal structures) and sequence data of the *tef* gene were typical for *Fusarium falciforme*.

To our knowledge, this is the first report of *Fusarium falciforme* affecting *P. vulgaris* in Cuba.

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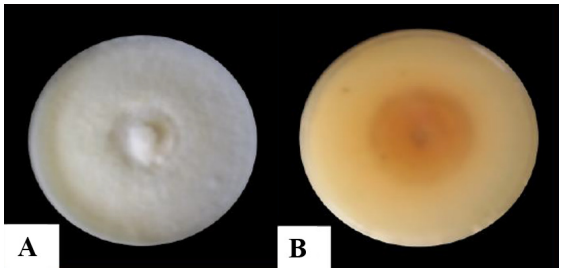

Figure 1

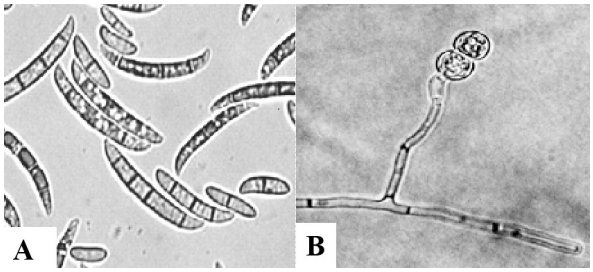

Figure 2

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