Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Wilt Caused by *Fusarium proliferatum* and *F. brachygibbosum* on Date Palm (*Phoenix dactylifera*) in Tunisia

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Since 2017, a new leaf wilt syndrome was observed in plantations of date palm in Tunisia. Its incidence increases sharply from year to year, especially in 'Deglet Nour' trees, aged between 5 and 15 years. In severe cases, the large number of dried leaves per tree can lead to complete cessation of date production. Symptoms appear on one or more leaves in the center of the crown. Whitening and drying start at the top of the leaflets and proceed to their base, whereas the midrib remains green. Then the whole leaf dies. Small white-creamy leaflet fragments and roots were collected from five different regions in the Djerid Oases. They were disinfected with diluted bleach (0.8% NaOCl) and ethanol (80%) (each 2 min), rinsed with sterile distilled water, dried, and finally plated in Petri dishes containing potato dextrose agar (PDA) amended with 50 mg/liter of neomycin. After incubation for 7 days at 25 ± 2°C, emerging fungal colonies were single-spored by serial dilution. They were transferred to PDA, carnation leaf agar (CLA), and Spezieller Nährstoffarmer agar (SNA) for morphological identification. Based on the colony color on PDA, conidial morphology, and phialide structures on CLA and/or SNA, of the 85 *Fusarium* isolates, around 90% were identified as *F. proliferatum* and around 10% as *F. brachygibbosum* (Leslie and Summerell, 2006). *F. proliferatum* colonies rapidly developed white aerial mycelium that became

purple in old cultures. Microconidia were abundant in the aerial mycelium and formed chains of variable length, on monophialides and polyphialides, a characteristic that distinguishes *F. proliferatum* from *F. verticillioides*. Less often, they were observed in false heads. Chlamydospores were absent. On CLA, microconidia were mostly 2 × 15 µm in size; a large number of sickle shaped macroconidia (2 × 25 µm) had one septum, and some were larger (2 × 50 µm) with three septa and tips at both ends. Molecular identification was carried out based on elongation factor (*EF-1α*) gene sequencing. The region between the EF1 and EF2 primers (O'Donnell et al. 1998) was amplified, and the sequences were compared with *Fusarium* reference sequences (GenBank). The sequences of the isolates Fus 1953 (539 bp), Fus 1962 (618 bp), and Fus 1965 (605 bp) shared respectively 100, 99.51, and 99.51% homology with that of *F. proliferatum* JF740713.1 and were deposited in GenBank with the following accession numbers: MT630418, MT630419, and MT630420, respectively. The sequences of isolates 7F, 28F, Fus 1955, and Fus 1956 shared 100% homology with that of *F. brachygibbosum* (GQ505418.1), whereas those of Fus 1955 and Fus 1956 showed 99.02 and 98.91% identity, respectively, with *F. brachygibbosum* JX118981.1. The sequences of 7F (535 bp), 28F (535 bp), Fus 1955 (608 bp), and Fus 1956 (647 bp) were deposited in GenBank with the following accession numbers: MT630409, MT630410, MT630411, and MT630412, respectively. Two milliliters of suspension of 10⁶ conidia/ml of each isolate was sprayed separately or in combinations on in vitro cloned Deglet Nour plants, placed in a greenhouse at 28 ± 2°C and 70% relative humidity. Isolates of *F. proliferatum* led to dryness and wilted leaflets after 3 weeks. *F. brachygibbosum* only induced mild leaf yellowing, whereas in combination they were more virulent. Fungal isolates of both species were reisolated and their identity confirmed to be the same of those isolated from leaflets infected in the open field, confirming Koch's postulates. Control plants lacked symptoms. *F. proliferatum* is known as date palm pathogen in many countries (Saleh et al. 2017); however, to our knowledge, this is the first report of *F. proliferatum* and also *F. brachygibbosum* causing leaf wilt symptoms on *P. dactylifera* in Tunisia.

References:

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