

## Diseases Caused by Fungi and Fungus-Like Organisms

### First Report of *Fusarium oxysporum* f. sp. *ciceris* on Chickpea (*Cicer arietinum*) in Serbia

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Chickpea (*Cicer arietinum* L.) is one of the most commonly consumed legume crops worldwide, cultivated in more than 55 countries (FAO 2017). However, in Serbia it is a novel crop grown on approximately 120 acres, but the area under this crop slightly increases each year. Fusarium wilt caused by *F. oxysporum* f. sp. *ciceris* is one of the most economically important diseases in most chickpea-growing areas (Jiménez-Díaz et al. 2015), but there has been no formal report of the Fusarium wilt of chickpea in Serbia. In June 2018, the first symptoms of Fusarium wilt were registered at Rimski Šančevi (Vojvodina Province), Serbia (N 45°19.311', E 19°49.933'), as wilted chickpea plants grouped in patches on approximately 5% of plants in a 3-acre area. Symptoms of yellowing and necrosis of foliage appeared as late wilt in the podding stage. Roots of affected plants showed no external discoloration, but a cross-section showed dark-brown discoloration of xylem tissue. To isolate the causal agent, symptomatic plants were collected and 10 cuttings of root symptomatic tissue were surface disinfected with 2% sodium hypochlorite solution for 5 min, rinsed three times in sterile distilled water, air dried on sterilized filter paper, and plated on potato dextrose agar (PDA) and water agar (WA) amended with streptomycin sulfate. After 7 days incubation at 25°C in the dark, isolates were preliminarily identified according to their morphological characters described by Leslie and Summerell (2006). For each isolate, 100 microconidia, macroconidia, and chlamydospores were measured. Conidia were hyaline; macroconidia sickle shaped, with blunt ends, two to four septa (10.1 to 17.7 × 3.1 to 5.8 μm); microconidia ellipsoidal, zero to two septa (4.9 to 8.6 × 2.7 to 3.5 μm). Chlamydospores were globose (4.3 to 8.8 μm).

Representative isolates (K343, K375, and K378) were purified by a single-spore technique for further analyses (Leslie and Summerell 2006). To confirm isolate identification, molecular identification of three representative isolates (K343, K375, and K378) was done by sequencing the rRNA internal transcribed spacer (ITS) region and translation elongation factor 1 $\alpha$  (TEF1) gene. For all isolates, the ITS and TEF1 genes were amplified and sequenced with primers ITS1/4 (White et al. 1990) and EF1-728 and EF1-986 (Rehner and Buckley 2005), respectively. Based on a BLAST search of the NCBI nucleotide database, the ITS sequences (GenBank MK920204.1, MK928423.1, and MK928424.1) had 99.8% identity with *F. oxysporum* f. sp. *ciceris* isolate (MK074845.1). The TEF1 (GenBank MN788462.1, MN788463.1, and MN788464.1) had 96.3 to 100% identity with *F. oxysporum* f. sp. *ciceris* isolate (FJ538245.1). A pathogenicity test was conducted on 7-day-old plants using the drench method described by Maitlo et al. (2016). The concentration of inoculation suspensions was adjusted to 1 × 10<sup>6</sup> conidia/ml, and 10 plants per isolate were tested and inoculated with 10 ml of suspension. Control plants were drenched with 10 ml of sterilized distilled water. Nine days after inoculation, the first symptoms of leaves wilting and white mycelia present around the stem base occurred on plants inoculated with isolate K378. On day 11 after inoculation, the first symptoms occurred on plants inoculated with isolates K343 and K375. By day 13 after inoculation all plants were wilted, and the pathogen was successfully reisolated and confirmed as *F. oxysporum* f. sp. *ciceris*. To the best of our knowledge, this is the first report of *F. oxysporum* f. sp. *ciceris* causing Fusarium wilt on chickpea in Serbia.

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