



First Report of Ascochyta Blight on Field Pea (*Pisum sativum*) caused by *Didymella pisi* in Bosnia and Herzegovina

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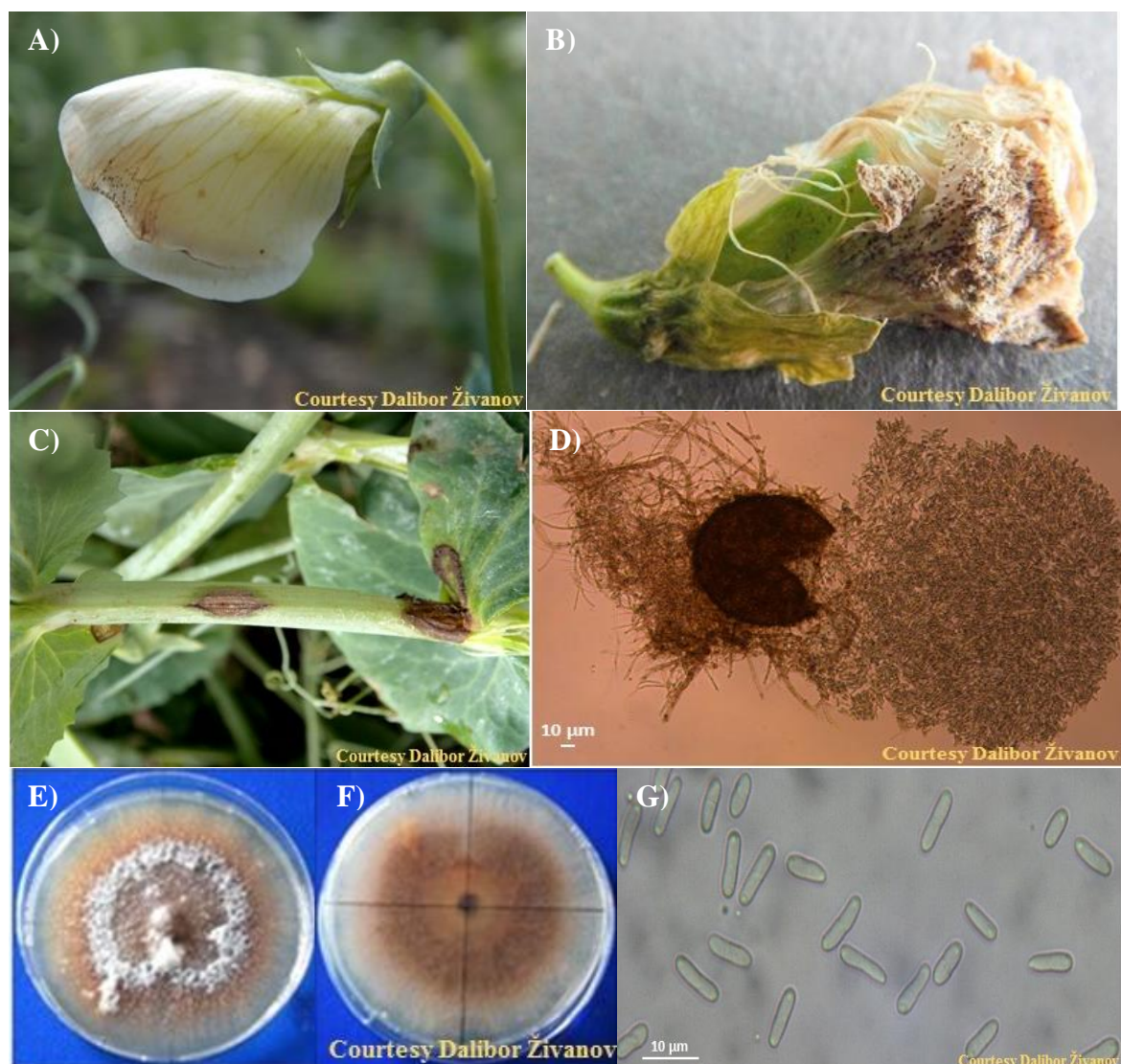
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The areas under the field pea are constantly increasing in Bosnia and Herzegovina. However, pea is susceptible to a large number of diseases, especially to Ascochyta blight, which is considered as one of the most economically important diseases of legume crops throughout the world (Le May et al. 2014; Rubiales et al. 2018). In May 2016, characteristic Ascochyta blight symptoms were observed on stems and flowers of field pea plants (*Pisum sativum* L.) grown at Velika Ilova (northern Bosnia and Herzegovina). More than 30% of field pea plants were affected in the area of 1.5 hectares. Dark brown, slightly sunken lesions with distinct dark margin were registered on infected stems. Additionally, irregular, pale brown lesions with numerous pycnidia along the nervature on petals were registered on the flowers. Symptomatic stem and flower tissues were cut in small pieces and disinfected with 1% NaOCl solution for 1 min, rinsed three times with sterile distilled water, placed on potato dextrose agar (PDA) amended with 100 mg/liter streptomycin sulfate (Sigma-Aldrich) and 50 mg/liter penicillin G sodium salt (Sigma-Aldrich), and incubated at $20 \pm 1^\circ\text{C}$ under fluorescent lights (Philips TLD 30w/08-BLB) with a 12-h photoperiod. After 7 days, the resulting colonies were transferred to water agar (WA) in order to obtain single-spore isolates as described in Leslie and Summerell (2006). Total of four single-spore isolates showed variations in colony color on PDA. Isolates produced yellowish-orange to dark orange colonies with numerous pycnidia, no chlamydospores and perithecia were observed. For each isolate one hundred pycnidia and conidia were measured. Pycnidia were pale to dark brown, round to oval, 70-240 μm in diameter. Conidia were hyaline, oblong-cylindric, with both ends rounded, mostly with one and rarely with two septa, dimensions from 8.5-13.8 x 2.9-4.4 μm . Based on morphological characteristics the pathogen was initially identified as *Didymella pisi* Lib. (Tadja, 2012). To confirm this assumption based on morphological identification of a new pathogen, the molecular identification of four isolates (K501, K502, K503, K504) was determined by sequencing the internal transcribed spacer (ITS) region and translation elongation factor 1-alpha gene (TEF1- α). After the BLAST search of the NCBI nucleotide database, the ITS sequences (GenBank no. KY703786.1., KY703790.1., KY703787.1., KY703778.1., respectively) of isolates K501 and K502 had 100% identity with the isolate *D. pisi* CBS 126.54 (MH857263.1), while isolates K503 and K504 had 100% identity with *D. pisi* CBS 108.26 (EU167557.1). The TEF1- α sequences (GenBank accession no. MK225296, MK225297, MK225298 and GenBank no. MF974609.1. respectively) had 92-100% identity with *D. pisi* isolate AP2 (DQ386494.1). Specific primers, LR12R and MpPmpRev1, used for differentiation of *D. pinodes* from *D. pinodella* (Le May et al., 2018) were used to amplify extracted DNA. Amplification profiles of tested isolates corresponded to neither *D. pinodes* nor *D. pinodella*. To confirm pathogenicity of tested isolates, fifteen 3-week-old (BBCH 13) pea plants were sprayed with single-spore

suspensions (3.5×10^5 conidia/ml) of each *D. pisi* isolate, and incubated in humid chamber at constant temperature of 20°C, 12h day/ night, and 100% relative humidity for 72h. Control plants were sprayed with sterile distilled water. The first Ascochyta blight symptoms occurred 8 days after inoculation with all tested isolates as dark brown lesions on both leaves and stems of pea. The fungus was reisolated as previously described. No symptoms were observed on the control plants. To the best of our knowledge, this is the first report of Ascochyta blight of pea caused by *Didymella pisi* in field condition in Bosnia and Herzegovina.

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Supplementary figure 1. Ascochyta blight symptoms on field pea plant and morphological characteristics of *Didymella pisi*. Flower (A-B) and stem (C) symptoms observed in the field; Pycnidia releasing hyaline conidia (D); Colony growth on PDA (E-F), Conidia (G)