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Disease Notes

First Report of Foliar and Stem Blight on Sunflower Caused by *Alternaria helianthiinficiens* in Croatia

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Abstract

Sunflower (*Helianthus annus* L.) is the most important oilseed crop in Croatia. In August 2009, in six localities of eastern Croatia, severe foliar and stem blight symptoms were observed on several genotypes with disease incidence ranging from 10 to 50%. At the initial stage of the infection, irregular to oval, brown spots different in size, surrounded by a chlorotic halo, appeared on the leaves that gradually became enlarged and coalesced, and whole leaves turned yellow and necrotic, followed by defoliation. Lesions on the stems were light to dark brown, randomly distributed, rounded and tapered on the ends; later becoming large and elongated causing stem breakage. Tissue within the lesion was reddish on the cross section. To

determine the causal agent, small pieces of symptomatic leaves and stem tissue of sunflower were surface disinfested and placed on potato dextrose agar. A total of 17 isolates from leaves as well as six from stems were obtained and all formed cottony, dark olivaceous to black colonies under 12 h of fluorescent light per day. All isolates formed uniform solitary, pale brown to brown, long ovoid conidia with five to eight transverse and one to two longitudinal septa. The conidia of all isolates were slightly constricted at the transverse septa, measuring 55 to 90 × 14 to 20 µm. Based on morphological characteristics, the pathogen was identified as *Alternaria helianthiinficiens* E.G. Simmons, Walcz & R.G. Roberts (4). The pathogenicity was tested with one representative isolate (Alt5) by injection of a conidial suspension (10⁶ conidia/ml) into stems of 20 healthy sunflower seedlings and by spraying 20 non-wounded detached leaves with a suspension of spores. Small necrotic spots on all inoculated seedlings and leaves formed 5 and 9 days after inoculation, respectively. The control sunflower seedlings and detached leaves, inoculated with sterile water, showed no reactions. The identity of isolate Alt5 was further confirmed by amplification and sequencing of the internal transcribed spacer (ITS) region of rDNA. Because there are no available corresponding ITS sequences of *A. helianthiinficiens* in the GenBank, reference type strain CBS 208.86 (publicly purchased, CBS, Utrecht, Netherlands) was also sequenced in this study. Total DNA was extracted directly from fungal mycelium and PCR amplification and sequencing were performed with primers ITS1F/ITS4. Sequence analysis of ITS region revealed 100% nucleotide identity between isolate Alt5 (GenBank Accession No. JX101648) and isolate CBS 208.86 (GenBank Accession No. JX101649). The nucleotide identity of both isolates compared with *A. helianthi* (HM449991), another sunflower pathogenic fungus, was only 80%. *A. helianthiinficiens* has previously been reported on sunflower in Hungary and the USA (3), Serbia (1), and Korea (2). However, to our knowledge, this is the first report of *A. helianthiinficiens* occurrence in Croatia as a new and harmful parasite of sunflower, illustrating an expansion of its geographical range and underscoring the need for phytosanitary control because it is a seedborne fungus.

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