

Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Aspergillus welwitschiae* Causing Maize Ear Rot in Serbia

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In recent years, countries in Southeast Europe have been facing climate changes characterized by extreme hot weather, which contribute to the increased frequency of *Aspergillus* species. Because of these changes, *Aspergillus parasiticus* was isolated, for the first time, from maize grains in Serbia (Nikolić et al. 2018). The presence of black powdery mycelia on maize ears indicated occurrence of species of the genus *Aspergillus* section *Nigri*, which led to the need for detailed identification of these fungi. Disease incidence ranged from 10 to 15% in August 2013. Maize ears with black powdery symptoms were collected from a field in Zemun Polje, Serbia. Symptomatic kernels were surface sterilized with 1% sodium hypochlorite solution for 3 min, rinsed three times with sterilized water, and then incubated at 25°C in the dark for 7 days on potato dextrose agar (PDA). Twenty isolates were identified as the genus *Aspergillus* section *Nigri*. Monospore cultures formed black cottony colonies with a yellowish border on PDA. The average colony diameter was 50 mm. In order to reliably identify the isolates, they were transferred to malt extract agar (MEA) and Czapek yeast autolysate agar (CYA) (Samson et al. 2014). On CYA, fungal colonies consisted of a white mycelium covered by a layer of black conidiophores. On MEA, fungal colonies were dense and black, with a yellowish border. The reverse side was colorless to pale yellow, with a yellow ring in the middle. The average size of conidia was 4.3 µm. The conidia were globose to subglobose and smooth to roughened, which is in agreement with previous research (da Silva et al. 2020). Given that the fungi *Aspergillus niger* and *Aspergillus welwitschiae* are morphologically indistinguishable (Susca et al. 2016), species-level identification was completed by analysis of

a partial sequence of the internal transcribed spacer (ITS) region (ITS1/ITS4 primers) and calmodulin gene (CMD5/CMD6 primers) (Samson et al. 2014). The sequences were compared with those of *A. welwitschiae* strains registered in the GenBank database based on nucleotide similarity, and results showed 99.64 and 100% similarity with ITS (OL711714) and calmodulin (KX894585), respectively. The sequence was deposited in GenBank with accession numbers OQ456471 (ITS) and OQ426518 (calmodulin). The presence of this species was confirmed with specific primers (AWEL1/AWEL2) designed by Susca et al. (2020). The pathogenicity test was performed in Zemun Polje on the same maize hybrid from which the fungal species was isolated. A conidial suspension was injected into the silk channel 3 days after 50% of the plants reached the silking stage. Twenty ears were inoculated with each isolate in four replicates (Reid et al. 1996). The inoculum was prepared from 7-day-old colonies on PDA, and 2 ml of a conidial suspension (1×10^6 spores/ml) was used. Control plants were inoculated with sterile water. All inoculated ears showed symptoms similar to those from field infections. The control ears were symptomless. The fungus was reisolated and was morphologically identical to the original isolates, thus fulfilling Koch's postulates. Based on molecular, morphological, and pathogenic properties, the isolates were identified as *A. welwitschiae*. This is the first report of *A. welwitschiae* as the causal agent of black maize ear rot not only in Serbia but also in the other countries of the Western Balkans. Given that the fungus *A. welwitschiae* synthesizes both ochratoxin A (Battilani et al. 2006) and fumonisin (Frisvad et al. 2011), further studies should be focused on assessing its aggressiveness and toxicological profile.

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