

# Disease Note

## Diseases Caused by Fungi and Fungus-Like Organisms

### First Report of *Fusarium verticillioides* Causing Fusariosis on Triticale Grain in Serbia

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Triticale (*Triticosecale* Wittmack) is a cereal crop species developed to combine positive traits of wheat and rye into a single plant (Arendt and Zannini 2013). Due to its high protein content, favorable amino acid composition, and nutritional values higher than maize, triticale is an excellent component for preparing feed for domestic animals (Đekić et al. 2019). In May 2017, discolored spikes of triticale were observed in a field in Zemun Polje, Serbia, with a disease incidence between 15 and 20%. In total, 400 kernels obtained from discolored spikes were surface sterilized with 1% sodium hypochlorite solution for 3 min, then rinsed three times with sterilized water, plated on potato dextrose agar (PDA) at 25°C, and incubated in the dark for 7 days. In total, 15 isolates of genus *Fusarium* on PDA formed cottony white to greyish-purple colonies with dark yellow to purple-gray reverse and average colonies. Obtained isolates were transferred to carnation leaf agar (CLA) and synthetic nutrition agar (SNA) for a 7-day incubation in the dark at 25°C. Ten isolates formed “rabbit ear” monophialides, which is the main characteristic of *Fusarium verticillioides* (Sacc.) Nirenberg. Microconidia are formed from monophialids in long chains. Their shape is oval with a flattened base, without septa (4.0 to 18.0 by 1.5 to 4.5 µm). The shape of macroconidia is slightly falcate to almost straight (3.5 to 4.5 by 31 to 58 µm). Chlamydospores are not produced (Leslie and Summerell 2006). The genomic DNA from one *F. verticillioides* isolate was extracted using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Molecular identification was confirmed by sequencing the internal transcribed spacer (ITS), translation elongation factor 1 $\alpha$  (EF-1 $\alpha$ ), and RPB2 (O’Donnell et al. 2000).

The sequence was deposited in GenBank as MZ664391, MZ666958, and MZ666957. This sequence was compared with the sequences of *F. verticillioides* strains registered in the GenBank database based on nucleotide similarity. It showed 100% identity to the sequences MT180471.1 (ITS), MN861767.1 (EF-1 $\alpha$ ), and MT264836.1 (RPB2). Pathogenicity of all isolates was tested on 20 randomly selected triticale spikes in four replicates (Mesterházy et al. 1999). Inoculation was performed when half of the plants reached the flowering stage by spraying with 20 ml of spore suspension (1 × 10<sup>6</sup> spores/ml from 7-day-old cultures on PDA). *F. verticillioides* RBG 1603 Q27 was used as a positive control. Inoculated spikes were covered with PVC bags for 48 h. Discolored spikes were observed after 3 weeks of incubation in a greenhouse at 20°C with a photoperiod of 16 and 8 h (light and dark). All inoculated spikes showed symptoms, similar to those from field infections. Control spikes were symptomless. The fungus was reisolated and was morphologically identical to the original isolates, thus completing Koch’s postulates. Based on morphological, molecular, and pathogenic features, the isolated fungus was identified as *F. verticillioides*. *Fusarium* spp. infect crop plants worldwide, resulting in yield loss and reduced grain quality due to mycotoxin contamination (Asam et al. 2017). In Serbia, *F. verticillioides* was isolated from wheat and barley grains (Stanković et al. 2012) and, to the best of our knowledge, this is the first report of *F. verticillioides* on triticale grain in Serbia.

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#### e-Xtra

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