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DISEASE NOTES



First Report of *Alternaria tenuissima* and *Alternaria infectoria* on Organic Spelt Wheat in Serbia

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Spelt wheat (*Triticum aestivum* ssp. *spelta* L. Thell) is one of the oldest cultivated hulled grains with high nutritional properties and great adaptability in different ecosystems. Spelt is an ideal grain crop for sustainable and organic farming due to hulls that serve as barriers against pathogens ([Vučković et al. 2013](#)). A marked increase in black point disease incidence on spelt wheat was detected in Vojvodina, North Serbia in 2012. The potential risk of black point disease on cereal production in Serbia is not so much yield decrease but postharvest losses and reduction in baking quality due to decreased nutritive value, discoloration, and insipidness. Five different spelt wheat genotypes from organic production were collected. From each sample, 100 kernels were surface-disinfected in 0.4% NaOCl for 2 min, rinsed with sterilized distilled water, then placed on filter paper in petri plates in four repetitions. Incubation was performed at 25°C for 7 days in darkness. *Alternaria* single spores were isolated from diseased spelt kernels and cultivated on potato carrot agar (PCA) at 24°C for 7 days under 8/16-h periods of light/dark ([Simmons et al. 2007](#)). Selection of five *Alternaria* isolates was conducted randomly from each spelt cultivar. A total of 22 isolates formed compact cottony mycelia with two to three concentric zones varying from greenish black to olive gray and 1 to 2 mm white margin. Chains of six to 14 conidia occasionally branched in

a sympodial manner with three to five conidia in each lateral chain. Conidia were ovoid to obclavate with a narrowly tapered upper half, septated with three to seven transepta, and ranged in size from 20.5 to 45 × 9 to 16.5 µm. Based on morphological and growth features, isolates were identified as *Alternaria tenuissima*. Three other isolates produced woolly whitish colonies, with dark orange under surface. Concentric rings varied from white center to olive green zones with light orange pigmentation. Conidial chains consisted of three to eight conidia with numerous branching patterns. Very small one- to two-celled conidia were narrow ovoid or ellipsoid and 15 to 25 × 4.5 to 7 µm. Such morphological features indicated *A. infectoria*. Pathogenicity of both species was tested by soaking kernels in conidial suspensions (10^6 conidia/ml) harvested from 7-day-old cultures on potato dextrose agar (PDA). Control kernels were dipped in sterile distilled water. After 7 days, kernels that developed black point symptoms were similar to those naturally occurring on the original kernels. No symptoms were observed on any of the control kernels. Morphological identification was confirmed by amplification and sequencing of the internal transcribed spacer (ITS) region of rDNA (White et al. 1990). Total DNA was extracted directly from fungal mycelium with a DNA Isolation Kit (Agilent Technologies, Santa Clara, CA) and PCR amplification performed with primers ITS1F/ITS4. Amplified fragments of isolates were in the range of 569 to 579 bp with clear separation of three isolates yielded in length of 613 to 618 bp. ITS sequence analysis revealed that 22 isolates (GenBank Accession Nos. KM516062 to KM516083) shared 100% identity with reference isolate *A. tenuissima* CBS 918.96 (EF031053) while three isolates (KM516084 to KM516086) 100% matched to *A. infectoria* CBS 210.86 (FM958526). Black point disease on spelt kernels caused by *A. alternata* was previously detected in Poland (Kurowski and Wysocka 2009). To our knowledge, this is the first report of *A. tenuissima* and *A. infectoria* detected on spelt wheat in Serbia.



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