

Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Brown Spot on Stored Apple Fruits Caused by *Stemphylium vesicarium* in Serbia

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Apple is one of the most economically important fruit crops worldwide, and postharvest fungal diseases can cause significant losses during storage (Petreš et al. 2020). Apple fruits (cv. Fuji) with necrosis symptoms were collected during the fall of 2022 from an ultra-low-oxygen cold storage facility in Titel, Serbia. The fruits originated from the apple orchards in Titel (45°12'47.1"N, 20°15'23.6"E). The pathogens were isolated from collected fruit samples using standard phytopathological techniques. Fruits were surface-sterilized, rinsed with sterile water, and aseptically cut in half, and small fragments collected from the border of healthy and diseased tissue were placed on potato dextrose agar (PDA) medium in Petri dishes and incubated at 25 ± 1°C in the dark for 7 days. The obtained 11 isolates were identified to the genus level as *Alternaria* (incidence, 46%), *Penicillium* (36%), *Fusarium* (9%), and *Stemphylium* (9%) based on their morphological characteristics. Pathogenicity of all the isolates was confirmed on apple fruits of cultivars Fuji and Golden Delicious. The fruits were surface-sterilized, sprayed with 5 ml of conidial suspension (1 × 10⁵ conidia/ml), and incubated at room temperature for 21 days. Symptoms developed on inoculated fruits were the same as symptoms observed on apple fruit samples collected from cold storage. Reisolation from artificially inoculated fruits resulted in colonies that morphologically corresponded with the colonies used for inoculation. The *Stemphylium* isolate was the only one included in further research. Initial symptoms and symptoms on artificially inoculated apple fruits caused by *Stemphylium* sp. occurred as circular dark brown necrosis located near the calyx, without visible sporulation on the fruit surface.

The isolate and reisolate formed aerial, white to light brown mycelia. Pigmentation of the culture medium was pale to dark brown. Conidia were singular, cylindrical, multicellular, brown to dark brown, and 22 to 35.1 µm long and 12.6 to 18.9 µm wide. Based on the morphological characteristics, the isolate and reisolate were identified as *Stemphylium vesicarium*, which is in line with the description reported by Sharifi et al. (2021) and Gilardi et al. (2022). The identification of the *S. vesicarium* isolate was confirmed by polymerase chain reaction (PCR) by amplifying and sequencing three regions using the following primer pairs: Bt2a (5'-GGTAACCAAATCGGTGCTGCTTC-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') for the β-tubulin region (Nasri et al. 2015); ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') for the internal transcribed spacer (ITS) region (White et al. 1990); and EF1 (5'-ATGGGTAAGGAGGACAAGAC-3') and EF2 (5'-GGAAGTACCAGTGATCATGTT-3') for the translation elongation factor (*TEF-1α*) region (O'Donnell et al. 1998). PCR products were separated by horizontal gel electrophoresis in 1.5% agarose gel and stained with ethidium bromide, and their visualization under UV light revealed amplified fragments of the expected size of 500 bp for the primer pair Bt2a/Bt2b, 600 bp for the primer pair ITS1/ITS4, and 700 bp for the primer pair EF1/EF2. The obtained amplicons were Sanger sequenced (Macrogen Europe BV) in both directions. BLASTn analysis showed the identity of amplified fragments of the isolates with sequences of *S. vesicarium* present in GenBank was 100% (MT881940.1 and JQ671944.1) for the β-tubulin region, 99.40% (MT520589.1 and OR256793.1) for the ITS region, and 99.49% (DQ471090.2 and MT394642.1) for the *TEF-1α* region. The sequences were deposited to NCBI GenBank (accession nos. OQ653540 for the β-tubulin region, OQ678016 for the ITS region, and OR232710 for the *TEF-1α* region). To our knowledge, this is the first finding of *S. vesicarium* on apple fruits in the Republic of Serbia and the finding of a new causal agent of postharvest apple fruit rot.

References:

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