Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium proliferatum* Causing Garlic Clove Rot in Russian Federation

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Garlic (*Allium sativum* L.) is a widely consumed bulbous crop both worldwide and in Russia. About 200,000 tons of garlic is produced in Russia annually (<https://rosstat.gov.ru/>). Significant pre- and postharvest losses of garlic regularly occur due to *Fusarium* sp. (Taylor et al. 2013). Since September 2018, rotting has been observed in Russia during garlic bulb storage (data of the Federal Scientific Vegetable Center, FSVC, Moscow Region). The outer bulb surface looked healthy, but underneath the integumentary scales, the cloves had light brown and brown spots. When grown, diseased plants were characterized by root and bulb disruption and leaf drying; for some cultivars, up to 100% of plants died. In January 2020, cultivars Strelets and Dubkovsky bulbs, collected in July 2019, with rot symptoms, were taken from the FSVC storage. Necrotic clove tissue fragments (0.2 to 0.5 cm) were cut, sanitized with 70% ethanol for 3 min, rinsed with sterile water, and incubated on potato dextrose agar (PDA) with 1 mg/ml of ampicillin at 22°C in the dark. Four single-spore cultures were obtained from four diseased bulbs. After 6 days of incubation, the isolates produced abundant aerial white mycelia and acquired a purple pigmentation. The hyphae were hyaline with septation. All isolates (Dubkovsky, Dubkovsky 2, Strelets, and Strelets 2) produced numerous oval unicellular microconidia without septa, 4.1 to 11.6 × 1.3 to 3.4 µm ($n = 50$) and very few macroconidia with three to four septa (21 to 26 × 3 to 4 µm [$n = 30$]), narrowed at both ends. The cultural and conidial characteristics of the isolates corresponded to *Fusarium* species (Leslie and Summerell 2006). To determine the species, DNA was extracted from four isolates, and the internal transcribed spacer (ITS), and genes of translation elongation factor 1α (*EF1α*) and subunits 1 and 2 of

DNA-directed RNA polymerase II (*RPB1* and *RPB2*) were amplified and sequenced with primers ITS1/ITS4 (White et al. 1990), EF1/EF2 (O'Donnell et al. 1998a), RPB1-F5/RPB1-R8 (O'Donnell et al. 2010), and fRPB2-5F/fRPB2-7cR (Liu et al. 1999). The obtained sequences were identical for all four isolates. The isolate Strelets sequences were deposited in NCBI GenBank (MW149129 [ITS], MW161161 [*EF1α*], MW413302 [*RPB1*], and MW413303 [*RPB2*]); their analysis in MLST (<https://fusarium.mycobank.org/>) showed 98.8 to 99.8% similarity to *Fusarium proliferatum* (NRRL 13582, 13598, and others), which is part of the *F. fujikuroi* complex (O'Donnell et al. 1998b). The test of pathogenicity was performed two times according to the method of Leyronas et al. (2018). For this, three replicates of 10 cloves (cv. Strelets) were soaked in a conidial suspension (~10⁶ conidia/ml; Strelets isolate) for 24 h. Ten control cloves were soaked in sterile water. The cloves were incubated on Petri dishes (five cloves on a dish; on filter paper wetted with sterile water) in the dark at 23°C. After 5 days, brown lesions and white mycelium developed on the surface of the treated cloves. The taxonomic status of the fungus isolated from necrotic tissue was determined as *F. proliferatum* according to the ITS, *EF1α*, *RPB1*, and *RPB2*. Garlic basal and bulb rot is known to be caused by *F. oxysporum* f. sp. *cepae* and *F. proliferatum* (Snowdon 1990). This study is the first report of *F. proliferatum* causing rot of garlic bulbs during storage in Russia. *F. proliferatum* produces a variety of mycotoxins during bulb infestation, and our findings are important for diagnosing a *Fusarium* disease and because of the use of garlic crop in food and medicine.

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