Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Sea Buckthorn Stem Wilt Caused by *Fusarium proliferatum* in Liaoning, China

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Sea buckthorn (*Hippophae rhamnoides* L.) is a flowering shrub native to cold-temperate regions of Eurasia that is valuable for its berries and leaves containing various vitamins and flavonoids (Pundir et al. 2021). In late June 2020, high mortality (more than 70%) was observed in sea buckthorn in a 1.6-ha seedling nursery in Chaoyang City, Liaoning province, China, where 16 Chinese and Russian cultivars had been planted since 2014 ('Shenqihong', 'Eshi01' through 'Eshi15'). The mortality of two introduced sea buckthorn cultivars ('Eshi02', 'Eshi04') was 100% (125 trees died in total). The symptoms include massive drooping leaves and dried-up stems on 6-year-old infected trees. Pieces of tree roots and stems with brown discoloration in the xylem vessels were selected. Small tissue fragments (0.2 to 0.5 cm) were surface disinfested (3 min in 75% ethanol, rinsed with sterile distilled water), air dried, and placed on potato dextrose agar (PDA) medium for 5 days at 25°C in the dark. A fungus was consistently isolated from both diseased roots and stems tissues, and a representative isolate (LC-1) was harvested. Genomic DNA was extracted for amplification and sequencing of the partial translation elongation factor-1 α (EF1 and EF2 primers, accession no. MZ669853) (O'Donnell et al.

1998) and RNA polymerase II second largest subunit (RPB2) (7cf/11aR primers, accession no. MZ669854) (O'Donnell et al. 2007). The sequences were further analyzed at the *Fusarium* MLST (<https://fusarium.mycobank.org/>) for identity confirmation, and showed 99.8% (over 95.2% query coverage) and 96.4% (over 88.4% query coverage) similarity to *Fusarium proliferatum* (NRRL 13584, 13591). Isolates on Spezieller Nährstoffarmer agar (SNA) produced abundant aerial white mycelia and yellow pigmentation. The 30 macroconidia measured were 28.5 to 62.5 $\times$ 3.2 to 5.4 μ m, thin, slender, and had three to five septa. The aseptate microconidia ranged from 4.7 to 13.6 $\times$ 2.2 to 4.3 μ m ($n = 30$). Pathogenicity tests were performed on healthy, potted 1-year-old sea buckthorn seedlings ('Eshi05') using two isolates in a greenhouse at 25°C, 80% relative humidity, and 12-h light/dark photoperiod. Ten potted seedlings were inoculated on the stems by placing a 5-mm-diameter mycelial plug (5-day-old PDA cultures for each isolate) into the surface of a wound created with a needle, and the inoculation sites were covered with Parafilm to maintain moisture. Ten seedlings were inoculated with PDA plugs as controls. Six to 10 days after inoculation, the color of the leaves in the middle of the stems was variegated, and then dark necrotic lesions on leaf margins were observed. Three weeks after inoculation, 80% of inoculated stems were wilted, and control plants remained asymptomatic. The pathogen was consistently reisolated, and the recovered isolates were identified as *F. proliferatum* by amplifying the EF-1 α gene. The typical symptoms on inoculated plants were dark to brown necrotic lesions on chlorotic leaves initially, and black withered stems in the terminal stage, similar to those observed on sea buckthorn trees infected with *Fusarium sporotrichioides* in Gansu and Heilongjiang provinces (Song et al. 2010; Xia et al. 2021). To our knowledge, this is the first report of sea buckthorn stem wilt caused by *F. proliferatum* in Liaoning province, China, which will be beneficial for expanding knowledge of *Fusarium* disease in sea buckthorn and provide more information for sustainable disease management in sea buckthorn.

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