Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Verticillium Wilt Caused by *Verticillium dahliae* on Russian Olive (*Elaeagnus angustifolia*) in Iran

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Russian olive, also known as Persian olive or oleaster (*Elaeagnus angustifolia* L.), is a species in the Elaeagnaceae family native to western and central Asia. In some orchards in Iran, intercropping Russian olive or *Prunus* trees with vegetables is a common practice. In 2018, about 130 ha of *E. angustifolia* orchards in Shahrood, Semnan Province, Iran, showed branch wilting and dieback. Symptoms on affected trees started with yellowing of the lower leaves, followed by wilting and finally death of affected branches. Sections of stems indicated brown or black streaks in the vascular tissues under the bark. Isolations were made from discolored vascular tissues by surface disinfecting small pieces of symptomatic tissue with 0.5% sodium hypochlorite for 2 min, plating them onto potato dextrose agar amended with 25 mg/liter of streptomycin sulfate, and incubating them in the dark for 14 days at 25°C. Fungi consistently isolated from symptomatic tissues. Fungal isolates were identified as *Verticillium dahliae* Kleb. based on characteristics of verticillate conidiophores, hyaline, elliptical, single celled conidia measuring 4.7 to 6.0 × 2.3 to 3.4 µm ($n = 100$), and irregular, dark microsclerotia measuring 27 to 34 × 22 to 26 µm ($n = 50$) that developed after 14 days of growth at 25°C in the dark. The identification of two isolates was further confirmed by performing a real-time PCR assay using a pair of specific primer for the internal transcribed

spacer region of *V. dahliae*, as previously described (Hiemstra et al. 2013). In addition, the molecular subdivision of isolates was further determined to discriminate defoliating (D) and non-defoliating (ND) molecular types (Keykhasaber et al. 2017). According to molecular assays, isolates were identified as *V. dahliae* and grouped with ND types. The pathogenicity of isolates was evaluated by root dipping 1-year-old *E. angustifolia* seedlings (10 seedlings) into conidial suspensions of 1×10^7 CFU/ml. Inoculated plants were transplanted into pots containing autoclaved soil and maintained in a greenhouse at 25°C until symptom appearance. Two seedlings were treated with sterile distilled water as controls. All inoculated seedlings started to show wilting symptoms similar to those present in naturally affected trees within 30 days after inoculation and died thereafter. Furthermore, *V. dahliae* was consistently isolated from symptomatic tissues. No symptoms were observed on the control plants. The pathogenicity test was repeated twice with similar results. To the best of our knowledge, this is the first report of Verticillium wilt on Russian olive trees in Iran. In Iran, Verticillium wilt is the cause of serious losses in many woody and herbaceous plants with economic importance, including many trees belonging to the genus *Prunus* that are highly susceptible to the disease. In Shahrood (Semnan Province), most agricultural fields have a potato- or tomato-growing history. Verticillium wilt might become an important economic problem in many Russian olive and *Prunus* orchards in the future because their cultivation is expanding rapidly in many agricultural areas previously dedicated to tomato and potato crops, the majority of which are infested with *V. dahliae*.

References:

- Hiemstra, J. A., et al. 2013. Page 62 in: 11th International Verticillium Symposium, Georg-August-Universität, 5-8 May 2013. DPG Spectrum Phytomedizin, Göttingen, Germany.
- Keykhasaber, M. 2017. Unravelling aspects of spatial and temporal distribution of *Verticillium dahliae* in olive, maple and ash trees and improvement of detection methods. PhD thesis, Wageningen University & Research. DOI:10.18174/396604.

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