

First Report of Verticillium Wilt and Mortality of *Ailanthus altissima* Caused by *Verticillium dahliae* and *V. albo-atrum sensu lato* in SpainC. Moragrega,^{1,†} J. Carol,¹ E. Bisbe,² E. Fabregas,² and I. Llorente¹¹ Laboratory of Plant Pathology, Institute of Food and Agricultural Technology-CIDSAV-XaRTA, University of Girona, 17003, Girona, Spain² Galanthus, Associació per a l'estudi i divulgació del medi ambient, 17460, Celrà, Spain**Funding:** This work was funded by the Diputació de Girona (Spain) (2017/8719, 2019/3091, 2020/7565, and 2021/1468). Plant Dis. 00:00, 2021; published online as <https://doi.org/10.1094/PDIS-03-21-0463-PDN>. Accepted for publication 30 April 2021.

Ailanthus altissima (Mill.) swingle is a highly invasive tree that has become established worldwide, especially in the Mediterranean Basin because of its good drought resistance. *A. altissima* is included in the list of invasive alien species of the European Union, so measures for eradication and management are required. Assessment for potential biological control agents is of great interest to manage this invasive tree in natural ecosystems. *Verticillium dahliae* Kleb. and *Verticillium nonalfalfae* Inderb. et al. (formerly *V. albo-atrum* Reinke & Berthold) have been reported as the causal agents of Verticillium wilt and mortality of ailanthus (Brooks et al. 2020; Rebbeck et al. 2013; Schall and Davis 2009; Snyder et al. 2013). Ailanthus trees with Verticillium wilt symptoms (wilt, premature defoliation, terminal dieback, yellow vascular discoloration, and mortality) were detected for the first time in 2007 in Celrà (42.040466N, 2.864436E) (Catalonia, northeastern Spain) and then spread to neighboring ailanthus populations. In 2018, ailanthus trees in a 570-km² area in Catalonia were surveyed for disease symptoms. The incidence of wilt disease in ailanthus trees in forest ecosystems ranged from 50 to 90%, and the severity ranged from 60 to 92%. One hundred fifty branch samples showing wilt symptoms were collected, disinfected by immersion in 1% sodium hypochlorite for 2 min, and then cut into 5-mm pieces. These were placed onto potato dextrose agar (PDA) plates and incubated at 22.5°C and 12-h light photoperiod for 7 to 10 days. Eighty-four tentative *Verticillium* sp. isolates were recovered and subcul-

tured on modified water agar (WA-p) and PDA for identification (Inderbitzin et al. 2011, 2013). The majority of isolates (77%) were identified as *V. dahliae* based on morphology: production of brown-pigmented microsclerotia and conidia features and dimensions (5.7 ± 0.9 µm long). Sequencing of mycelial DNA using primer pair ITS1-F and ITS4 resulted in consensus sequences of 503 bp. BLASTn analysis of the ITS sequence of native isolate VdGi688 gave 100% identity to the ITS sequences of *V. dahliae* type strain PD322 (92% coverage) and Vd16_9 (100% coverage). In addition, 23% of isolates morphologically corresponded to *V. albo-atrum* or *V. nonalfalfae*: melanized resting mycelia and round to oval-shaped conidia (5.2 ± 0.9 × 2.2 ± 0.5 µm). The ITS consensus sequence (544 bp) of native isolate VaaGi02 gave 99% identity (90 to 100% coverage) to *V. albo-atrum* isolates CBS 127169, PSU 140, and Vaa_TN1 and to *V. nonalfalfae* type PD592, CBS5451.88, and Vert 18. Sequences from isolates VdGi688 and VaaGi02 were deposited in GenBank as MW624723 and MW624724, respectively. Koch's postulates for seven *V. dahliae* isolates and eight *V. albo-atrum* isolates were fulfilled by injection of 1 ml of 1 × 10⁷ conidia/ml suspension into the stem of *A. altissima* seedlings under greenhouse conditions. Six plants were inoculated per isolate in two independent experiments. Control plants were inoculated with sterile distilled water. All isolates caused leaf chlorosis, defoliation, and apical stem death, as well as internal necrosis and vascular discoloration. Control plants remained asymptomatic. The pathogens were reisolated from internal symptomatic tissues of inoculated plants. To our knowledge, this is the first report of *V. dahliae* and *V. albo-atrum sensu lato* causing Verticillium wilt on *A. altissima* in Spain. The study suggests the potential of native isolates of *Verticillium* spp. in the biological control of ailanthus in the Mediterranean Basin.

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