

First Report of *Verticillium* Wilt Caused by *Verticillium nonalfalfae* on Tree-of-Heaven (*Ailanthus altissima*) in Ohio

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Verticillium wilt of the highly invasive tree-of-heaven [*Ailanthus altissima* (Mill.) Swingle], caused by *Verticillium nonalfalfae* Interbitzin et al. (1), formerly classified as *V. albo-atrum* Reinke and Berthold, has been reported in the United States from two states: Pennsylvania (2) and Virginia (3). Infected *A. altissima* in both states exhibited similar symptoms of wilt, premature defoliation, terminal dieback, yellow vascular discoloration, and mortality. In June 2012, the second author observed dead and dying *A. altissima* trees in southern Ohio (Pike County) that exhibited symptoms similar to those on diseased *A. altissima* trees in Pennsylvania and Virginia. Samples were collected from stems of three symptomatic *A. altissima* trees and sent to Penn State for morphological and molecular identification. Immediately upon arrival, samples were surface-disinfected and plated onto plum extract agar (PEA), a semi-selective medium for *Verticillium* spp., amended with neomycin and streptomycin (2). The samples yielded six isolates, two from each of the three symptomatic trees, all of which were putatively identified as *V. nonalfalfae* based on the presence of verticillate conidiophores and formation of melanized hyphae. DNA was extracted from three isolates and molecular analyses performed using known primers (1) coding for elongation factor 1- α (EF), glyceraldehyde-3-phosphate dehydrogenase (GPD), and tryptophan synthase (TS). A BLAST search generated sequences that revealed 100% similarity to *V. nonalfalfae* for all three protein coding genes among the three Ohio isolates and reference sequences from *Ailanthus*, including isolates VnAaPA140 (GenBank Accession Nos. KC307764, KC307766, and KC307768) and VnAaVA2 (KC307758, KC307759, and KC307760), as well as isolate PD592 from potato (JN188227, JN188163, and JN188035), thereby confirming taxonomic placement of the Ohio *Ailanthus* isolates among those recovered from *Ailanthus* in Pennsylvania and Virginia. Aligned sequences from one representative isolate, VnAaOH1, were deposited into GenBank as accessions KC307761 (EF), KC307762 (GPD), and KC307763 (TS). In August 2012, the pathogenicity of all six isolates was confirmed by root-dipping 10 healthy 3-week-old *A. altissima* seedlings (seeds collected in University Park, PA) into conidial suspensions of 1×10^7 cfu/ml, wherein all inoculated seedlings wilted and died within 4 and 9 weeks, respectively. *V. nonalfalfae* was reisolated from all inoculated seedlings; control seedlings inoculated with distilled water remained asymptomatic. Ohio is the third state from which *V. nonalfalfae* has been reported to be pathogenic on *A. altissima*. If *V. nonalfalfae* proves to be widespread, it may represent a natural biocontrol for the invasive *A. altissima*. Also, since USDA APHIS evaluates and regulates new potential biocontrol agents on a state-by-state basis, it is important to document each state in which *V. nonalfalfae* is killing *A. altissima*, so that in-state inoculum can be used for biocontrol efforts, simplifying the regulatory process.

References: (1) [P. Inderbitzin et al. 2011 PLoS ONE, 6, e28341, 2011.](#) (2) [M. J. Schall and D. D. Davis. Plant Dis. 93:747, 2009.](#) (3) [A. L. Snyder et al. Plant Dis. 96:837, 2013.](#)

Source

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