

**Dr. Donald D. Davis – Penn State  
Final Project Report**

**“Role of an Ambrosia Beetle (*Euwallacea validus*) in Dissemination  
of the Fungus *Verticillium albo-atrum*, Causal Agent of Verticillium Wilt  
of Tree-of-Heaven (*Ailanthus altissima*)”**

**Forest Health (FHTET) Program**

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**Project Period:** 31 August 2011 – 30 June 2015

**Recipient:** The Pennsylvania State University

**Recipient Contact Person:** Dr. Donald D. Davis, Professor

**Principal Investigator:** Dr. Donald D. Davis

**Progress Achieved in Accomplishing Project Goals & Objectives:** The overall goal of this project is to develop an effective biological control for management of the highly invasive, non-native, tree-of-heaven (*Ailanthus altissima*) using a naturally occurring, endemic, soil-borne wilt fungus (*Verticillium nonalfalfae*). Although our original project title included the words “ambrosia beetle”, we quickly abandoned this aspect of our project in favor of studies that were yielding positive results. Thus, our main project activities were: to conduct non-target (non-*Ailanthus*) risk analyses; to develop and test various inoculation protocols; to develop practical inoculation techniques for land managers; and to determine if clonal growth and intraspecific root grafts were involved and effective in short-range transmission of the biocontrol agent.

**Background:** Verticillium wilt, caused by *Verticillium nonalfalfae*, is currently killing tens of thousands of highly invasive *Ailanthus altissima* within the hardwood forests in Pennsylvania, Ohio, and Virginia and is being considered as a biological control agent of *Ailanthus*. However, more information is needed on non-target host risk analyses, inoculation protocols, and the role of intraspecific root grafts in pathogen transmission, all of which were studied during this project.

**Main Activities:** Regarding potential damage to non-target forest species, we tested 71 non-target woody species for susceptibility to the potential biocontrol agent through stem inoculation with an aqueous conidial suspension of *V. nonalfalfae*. In the field, only devil’s walkingstick and striped maple acquired minimal infections through natural spread from inoculated *A. altissima*. Staghorn sumac also exhibited wilt in close proximity to wilted *Ailanthus*, although *V. nonalfalfae* was never recovered. Stem inoculations, which are highly artificial in that they bypass root defenses and flood the xylem with millions of conidia, induced varying levels of wilt and mortality in 10 non-target species from which *V. nonalfalfae* was reisolated, although recovery and crown rebuilding occurred following initial wilt in several species including sassafras and northern catalpa. Thirty-seven of the 71 inoculated species exhibited vascular discoloration, although 23 of these species exhibited no outward symptoms (wilt, dieback) for up to 6 years after inoculation. However, *V. nonalfalfae* was reisolated from three of the 23 species,

indicating a tolerant host response. Our results suggest that *V. nonalfalfae* is generally host-adapted to *A. altissima*. Other studies that we conducted revealed that 78 of 78 *A. altissima* seed sources from 26 states and Canada were susceptible *V. nonalfalfae*. Our project offers support for adoption and dissemination of *V. nonalfalfae* as a biocontrol agent to combat the highly invasive *A. altissima*.

The above inoculations utilized a conidial suspension (which has a short shelf-life) as inoculum. Although this method generally worked well, anticipating future expanded use of *V. nonalfalfae*, we evaluated other inoculum formulations, inoculation protocols, and sensitivity of additional non-target (non-*Ailanthus*) plant species. The most effective inoculum formulation, with an extended shelf-life, was prepared by mixing water with stored, refrigerated potting-mix containing *V. nonalfalfae*. Less successful, but still positive infections were obtained by simply using infected *Ailanthus* wood as inoculum. Monthly inoculation of *Ailanthus* trees demonstrated that the optimal time for successful inoculations was April to May, but limited infections were achieved during all months, including the winter. The health of *Ailanthus* and non-target species was also evaluated in a natural field setting within a decade-old natural Verticillium wilt epicenter, where all mature *Ailanthus* trees had been naturally killed by natural *V. nonalfalfae* infections. Verticillium wilt was observed on a few newly established *Ailanthus* seedlings, whereas non-target species were asymptomatic. These realistic field findings revealed that soil-formulated and natural inocula are effective biocontrols against *Ailanthus*, and again revealed that *V. nonalfalfae* appears to pose little threat to non-target plants.

In the field, we noted that *V. nonalfalfae* caused rapid epidemics in dense stands of *Ailanthus*, and that *V. nonalfalfae* was rapidly transmitted from diseased *Ailanthus* trees to healthy *Ailanthus* trees. Root grafts could facilitate this rapid transmission, but neither root graft formation in *Ailanthus*, nor *Verticillium* transmission by root grafts in any tree species, had ever been reported. Therefore, *V. nonalfalfae* transmission between diseased and healthy *Ailanthus* trees via intraspecific root grafts and clonal growth was evaluated. Using air-spade excavation, dye translocation, and root graft inoculations, functional root grafts were illustrated between and among *Ailanthus* trees, and transmission of *V. nonalfalfae* was demonstrated across root grafts from diseased to healthy *Ailanthus* trees, as well as from diseased parent trees to connected root sprouts. With regard to the latter, in one stand, inoculation of one *Ailanthus* parent stem resulted in 187 root sprouts showing Verticillium wilt symptoms within 12 months after inoculation. Our project revealed that clonal growth and root grafts, which are normally advantageous growth habits in many trees, left *Ailanthus* stands very vulnerable to rapid and widespread *V. nonalfalfae* infection. This study also broadened our understanding of the *Ailanthus-Verticillium* pathosystem.



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