**Project Title:** Biological control and management of *Ailanthus* with *Verticillium nonalfalfae*: treatment efficacy, post-release monitoring, and non-target effects

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**Cooperators and Other Participating Institutions:** Thomas Macy, Forest Health Program & Special Projects Administrator, Ohio Department of Natural Resources Division of Forestry; Gerald Scott, Zone Botanist, Supervisor's Office, USFS Wayne National Forest.

**Amount Requested:** $38,600 (FY17 = $19,800; FY18 = $18,800)

**Project Leveraging** – This project will utilize geo-referenced *Ailanthus* infestations within public forests in SE Ohio. Having this data in-hand will facilitate the selection of study sites. ODNR Division of Forestry and Wayne National Forest staff will assist with the selection of additional areas for trials and coordination of treatments. Inoculation sites established at five forest sites in 2015 will be monitored monthly at no additional cost to assess longer-term impacts of *V. nonalfalfae* on *Ailanthus* and non-target plants.

**Project Goals and Supporting Objectives**: This proposed project falls within all three priority projects to advance the technology for the biological control of *Ailanthus* (*Ailanthus altissima*) utilizing a well-studied and highly specific native fungus.

1. Streamline inoculum production by developing improved and expedient culturing methods of fungal spores.
2. Develop improved distribution of inoculum to determine optimal dose needed for effective biocontrol of *Ailanthus* and assess shelf life of formulations.
3. Monitor and develop techniques to assess the effectiveness of *V. nonalfalfae* to control high density *Ailanthus* populations and impacts on non-target plants.

**Project Justification/Urgency:** *Ailanthus* has been present in North American landscapes for over two hundred years (Hu 1979) and is widely distributed throughout the East and Midwest. *Ailanthus* was observed in 33 of 37 states monitored east of the 100th meridian from 1998-2012 (Appendix 1). The Eastern and Southern Regions of the US Forest Service rank it in Weed Category 1: an exotic species known to be invasive and persistent throughout much of both regions (USDA FS 2004). It is most often abundant in open sites such as roadsides, but its presence is increasing within disturbed forested sites. It is extremely fast-growing, reaching heights of 80-100 ft (25-30 m). It is dioecious and is a prolific seeder with up to 350,000 seeds produced per tree in a single growing season (Pannell 2002). In addition, it is capable of aggressive clonal spread, often creating dense thickets that can out-compete native trees. While considered shade-intolerant, clonal sprouts attached to a parent tree can persist in a shaded forest understory for up to 20 years (Kowarik 1995). *Ailanthus* can spread and persist in native plant communities, displacing native species and is most often found in mixed hardwood forests. Mechanical control methods are often impractical since multiple cuttings are required to deplete stored root carbohydrates. Typically mechanical methods are combined with an herbicide treatment to be effective. Chemical control is often costly and requires multiple applications (Smith & Smith 2009). Costs can quickly increase for chemical treatments of dense clonal thickets of *Ailanthus*, which are not uncommon following natural or human-induced disturbances. Given these obstacles, the use of a highly specific biological control agent such as *Verticillium nonalfalfae* which spreads naturally shows great promise.

In 2002, a native wilt-causing fungus, *Verticillium nonalfalfae* from dead and dying *Ailanthus* trees was identified within forested areas in Pennsylvania (Schall and Davis 2009a). After much rigorous testing and numerous trials, the fungus showed potential as a biological control agent of *Ailanthus*. Of the over 71 plant species tested to date (field and greenhouse inoculations), Kasson et al. (2014) only three species appeared to acquire the fungus from

natural spread (*Ailanthus*, staghorn sumac and striped maple) at very low levels. Injecting high concentrations of labcultured fungal spores into stems of *Ailanthus* seedlings in the greenhouse and canopy trees in the forest resulted in 100% mortality within 10-16 weeks. Within these forested areas of dead and dying *Ailanthus* trees, no other tree species exhibited any wilt symptoms. The genetically identical fungus has been found at multiple stands in Virginia (Snyder et al. 2013) and Ohio (Rebbeck et al. 2013).

In 2013, Rebbeck began testing the susceptibility of additional woody species to *V. nonalfalfae* in Ohio. To date, post-inoculation symptom development has been limited to devil's walkingstick (*Aralia spinosa*) and winged sumac (*Rhus copollina*). It is likely that these species serve as native hosts. Because American ginseng (*Panax quinefolius*) and devil's walkingstick are in the same botanical family Araliaceae, we began inoculation trials of ginseng in 2016. To date, no negative impacts associated with the fungus have been detected on ginseng. Throughout its range with the Appalachian region, ginseng cultivation and harvesting provides supplemental income to the public, so it is critical to assess its susceptibility to *V. nonalfalfae*. In 2014, Rebbeck established test sites in five forests in SE Ohio through past Forest Health Protection BCIP funding. Trials demonstrated that the fungus was effective in killing *Ailanthus* stems up to 16.5 in d.b.h. with no detectable non-target species effects. The fungus spread to nearby uninoculated *Ailanthus* trees. Monitoring of these trials will be ongoing to estimate rate of spread, native tree regeneration and non-target plant effects.

Since many forested areas within Ohio have varying densities of *Ailanthus*, developing and testing *V. nonalfalfae* as a biocontrol agent of *Ailanthus* is highly desirable. Many public forest managers and private landowners have enthusiastically offered *Ailanthus*-infested stands for inclusion in biocontrol trials. This potential biocontrol agent provides an added benefit - the fungus is native to North America so we are not introducing a new exotic organism. Once the fungus is introduced into a stand, it can spread from tree to tree through root grafting and naturally build up in the forest (O'Neil and Davis 2014). Efforts are underway to locate and confirm the presence of *V. nonalfalfae* in Indiana, West Virginia, Maryland, and Kentucky. Once the fungus is found within a given state, initiation of inoculation trials in that given state is only subject to approval by State officials. Because there is no interstate movement of the fungus, USDA APHIS has no jurisdiction.

**Approach**

**Description of activities and methods:**

**Goal 1:** Testing efficacy of alternative production methods of fungal inoculum Currently fungal inoculum is generated on solid agar media as described by Schall & Davis (2009a). Culturing on solid agar media takes 3 to 4 weeks to allow adequate hyphal growth and spore production. Recently, we developed a liquid media culturing methods as a means to increase inoculum production and efficiency (Appendix 2). Adequate concentrations (3 x 104 spores/ml) of viable conidia were produced in approximately 5 days. We propose to test the efficacy and potency of the conidia produced in liquid-cultures first with techniques with greenhouse inoculation trials utilizing *Ailanthus* seedlings prior to field testing. If successful, this technique could shorten the time and labor needed by 3 weeks. The proposed liquid method of conidial production has the potential to quickly produce much larger quantities of inoculum.

**Goal 2**: Develop improved distribution of inoculum to determine optimal dose needed for effective biocontrol of *Ailanthus* and assess shelf life of formulations. We will also implement trials to determine the lowest concentration of inoculum needed to effectively kill *Ailanthus* stems. To date, only conidial concentration of 107 ml-1 have been tested as an effective dose. While testing soil-based formulations, O'Neil and Davis (2015) found that 10 to 20 g *V. nonalfalfae*-colonized soils were equally effective as a biocontrol treatment. Preliminary dose-response trials will be conducted with seedlings in the greenhouse to efficiently identify three target concentrations (plus controls) for field trials. Seedlings will be evaluated biweekly for disease symptoms using a 0–8 scale, where 0 = healthy foliage, 1 = chlorosis and/or necrotic margins on leaves, 2 = slight wilt (<15% wilting foliage) with no or slight defoliation (<15%), 3 = moderate wilt (15% to <50% wilting foliage) with no or slight defoliation (<15%), 4 = severe wilt (50–100% wilting foliage) with no or slight defoliation (<15%), 5 = moderate defoliation (15% to

<50%), 6 = severe defoliation (50% to <90%), 7 = very severe defoliation (90–100%) with epicormic sprouting and 8 = dead (O'Neil and Davis 2015). The shelf life of inoculum produced from liquid and solid media cultures will also be similarly tested.

**Goal 3:** Monitor and develop techniques to assess the effectiveness of *V. nonalfalfae* to control high density *Ailanthus* populations and impacts on non-target plants. Since *Ailanthus* is an aggressive sprouter, dense areas of rapidly-growing saplings can quickly establish follow burning, harvesting and ineffective herbicide treatments. Chemically treating these high density areas is extremely labor intensive, increasing operational costs. We propose to utilize these areas to test the rate of spread of the fungus and determine the minimum number of stems treated to kill high density patches of *Ailanthus* sapling/poles in lieu of utilizing limited resources towards chemical control. Many known areas have already been identified by groundtruthing geo-reference aerial mapping and timber management projects within public forests. In early 2017, approximately four to five trial high-density "patch" areas will be selected among candidate sites (Athens District Wayne NF, Blue Rock, Hocking, Pike, Tar Hollow, Perry and Scioto Trail State Forests) with the assistance of ODNR Division of Forestry and WNF staff. A minimum of five high density sapling areas at a given trial site will be used for a minimum of total of 20 plots. Replicate plots will vary in size depending on size of a given sapling-pole patch. Inoculation areas within a given site will be physically separated by at least 100 ft. Within each patch, the largest 2 to 3 *Ailanthus* saplings (>5 in d.b.h.) will be inoculated three times at the base of each trunk using a hatchet and injected with 1 ml of 1 x 107 conidia ml-1 (Schall and Davis 2009ab). Each stand within a site will have a control stand where trees will be wounded with a sterile hatchet at three points at the stem base and treated with sterile distilled water. All trees will be inoculated in late May to early June 2017. Disease severity of inoculated seedlings and trees will be evaluated monthly from June through October using a scale of 0–8, previously described. Other non-*Ailanthus* tree species, woody shrubs and vines will be monitored for symptom development within inoculation areas as well.

Continue to monitor inoculation trials initiated in a previously funded Forest Health BCIP project at five sites (Appendix 3).

**Length of project:** 2 years

**Required Documentation** (Technical Advisory Group, APHIS, NEPA): In 2013, Rebbeck secured approval to begin inoculation trials with the Ohio isolate *V. nonalfalfae* by Ohio Department of Agriculture. In 2014, it was determined *V. nonalfalfae* inoculations on the Wayne National Forest were categorically excluded from NEPA approval by Forest Supervisor Tony Scardina.

**Statistical analyses:** *Verticillium* wilt inoculation response variables to be measured include: disease severity index, *Ailanthus* stem density and mortality estimates. Data will be analyzed via ANOVA, Repeated Measures Analyses and other appropriate statistical methods such as regression analyses in a factorial design, testing for effectiveness of *V. nonalfalfae* inoculations.

**Year 1 (2017):** Initiate liquid culture methods testing in spring 2017. Complete greenhouse inoculation trials comparing efficacy of conidial inoculum produced from liquid and solid media culture. Initiate in greenhouse preliminary trials to identify conidial concentrations to test in field trials. Identify and setup, test sites for sapling inoculations. Monitor non-target trials of ginseng, devil's walkingstick and sumac initiated in 2015 and 2016. Continue monthly disease ratings of inoculation trials initiated at five sites in 2015. Submit progress report in September.

**Year 2 (2018)**: Continue monthly disease severity index ratings on inoculated stems at all sites.

Submit progress report in September.

**Expected Products and Outcomes:**

1. Development of a liquid culture method to scale-up fungal inoculum production.
2. Development of a methodology to monitor post-release of *Ailanthus* wilt in forested areas.
3. Presentations at various professional meetings sponsored by the following: ODNR Forest Health, Ohio Chapter of American Society of Foresters, Ohio Invasive Plant Council Ohio, Midwest Invasive Plant Network, SE Ohio Invasive Plants Interest Group, and OSU Extension Service. These oral presentations have the potential to inform a large number of land managers and private landowners regarding the efficacy of *V. nonalfalfae* as a biological control of *Ailanthus* and possible non-target effects.
4. Initiate coordination with ODNR service foresters and consulting foresters to begin future trial inoculations on private lands, contingent on limited off-target species effects.

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**Proposed budget: FY 2017 - 2018**

| FY 2017 | Item | Requested Funding | Indirect Costs Other Source Funding | Source |
|---|---|---|---|---|
| Administration | Salary* | 16,800 | 44,000 | USDA FS RESEARCH & NFS REGION 9 (in-kind salary) |
| | Travel | 1,000 | 500 | USDA FS |
| Procurements | Contracting | 0 | 0 | |
| | Equipment | 0 | 0 | |
| | Supplies | 2,000 | 0 | |
| Year Totals | | 19,800 | 44,500 | |

*Field technician - 1 seasonal staff at $15/hr (includes fringe) for 28 weeks

| FY 2018 | Item | Requested Funding | Other Source Funding | Source |
|---|---|---|---|---|
| Administration | Salary | 16,800 | 41,500 | USDA FS RESEARCH & NFS REGION 9 (in-kind salary) |
| | Travel | 1,000 | 500 | USDA FS |
| Procurements | Contracting | 0 | 0 | |
| | Equipment | 0 | 0 | |
| | Supplies | 1,000 | 0 | |
| Year Totals | | 18,800 | 42,000 | |

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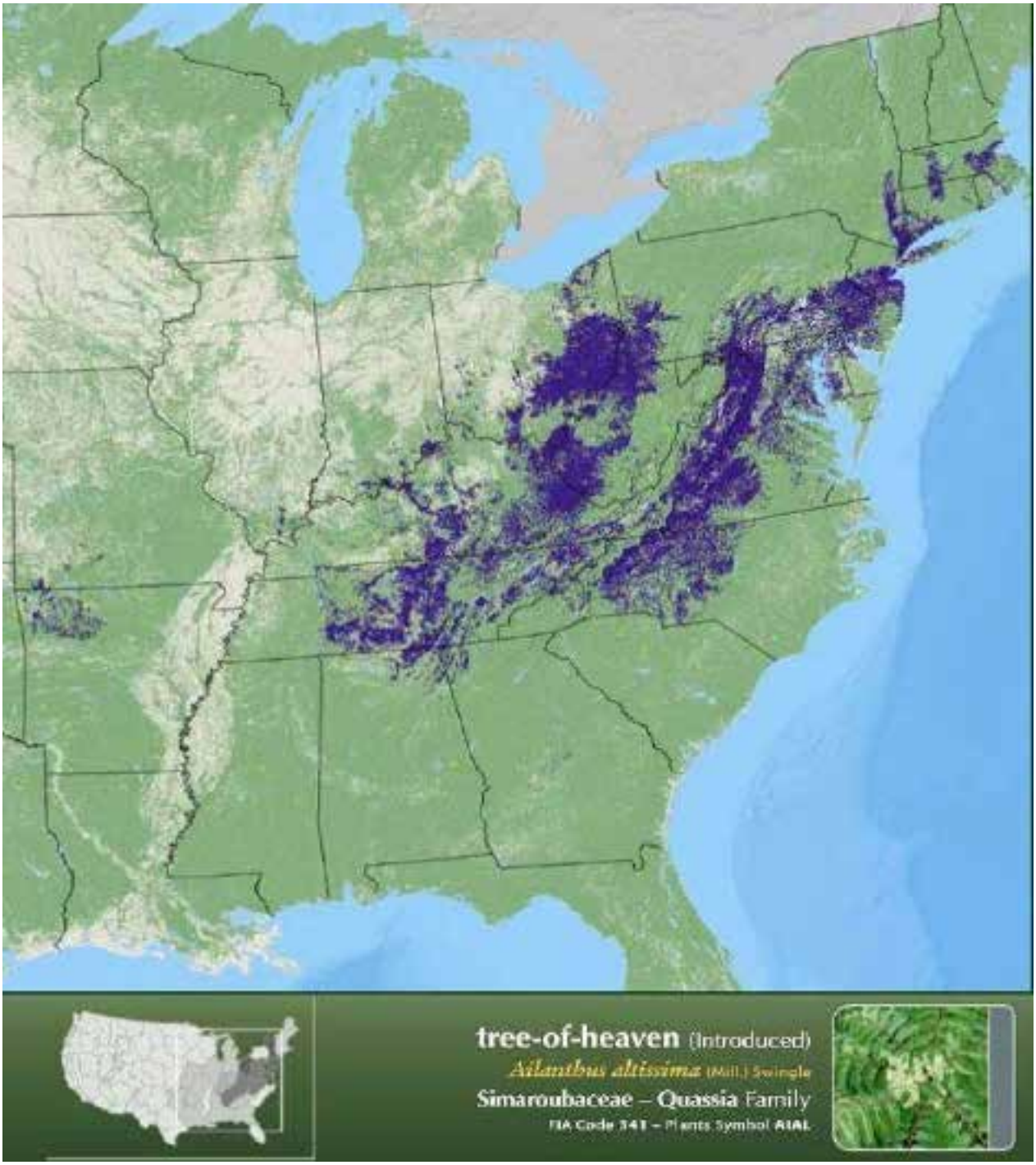
**Appendix 1**. Distribution of Ailanthus using FIA data with a resolution of 240 m. Source: from National Individual Tree Species Atlas. 2015, FHTET-15-01. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team. 1998 -2012.



**APPENDIX 2.** Inoculum Production and Distribution: Testing New Streamlined Method

**A. Standard practice to generate inoculum**

**Solid Media Spore Production and Inoculum Preparation**

Prepare solid agar & plate with spore culture
3-4 weeks

Mycelia and conidia loosened with sterile spatula into sterile water

Mixed and filtered through sterile cheese cloth or milk filter

Centrifuge spin to remove hyphae
Verify spore counts & dilute to 107 conidia ml-1 with sterile water

Conidial viability evaluated by counting colony forming units of suspensions plated on plum extract agar
2-3 day shelf life

Keep solution cool, take to field. Apply 1ml inoculum per incision into Ailanthus stem (3 cuts/tree)

**B. Goal: Develop & test as streamline method to generate inoculum**

**Liquid Media Spore Production and Inoculum Preparation**

Prepare sterile liquid media & inoculate with spore culture.
~5 days

Agitate to release spore. Verify spore counts & dilute to 107 conidia ml-1 with sterile water

Conidial viability evaluated by counting colony forming units of suspensions plated on plum extract agar

Keep solution cool, take to field. Apply 1ml directly into incision into Ailanthus stem 3 cuts/tree

**Appendix 3.** Map of sites for field inoculation trials to be monitored. Four stands within each site were inoculated with V. nonalfalfae in May 2015. An additional site at each site was used as a control with sterile water inoculations.