

Biological Control of Invasive Plants FY2014

- 1. Project Title:** Biological control and management of *Ailanthus*: post-release monitoring and efficacy of *Verticillium* wilt in Ohio
- 2. Principal Investigator:** Joanne Rebbeck, USDA Forest Service, Northern Research Station, 359 Main Road, Delaware, OH 43015, 740-368-0054 (voice), 740-368-0152 (fax), jrebbeck@fs.fed.us
- 3. Cooperators and Other Participating Institutions:** Don Davis, Penn State University; Cotton Randall, Ohio Department of Natural Resources Division of Forestry; Cheryl Coon, USFS Wayne National Forest; and Shana Byrd, *The Wilds*
- 4. Amount Requested:** \$93,201 (FY14 = \$22,925; FY15 = \$36,138; FY16 = \$34,138)
Project Leveraging – This project will utilize geo-referenced aerial mapping data of *Ailanthus* populations already collected by Rebbeck and collaborators in a number of Ohio State Forests and the Wayne National Forest. Already having this data in-hand will facilitate the selection of study sites. In addition, cooperators will support the project with in-kind donations of staff time to assist in post-treatment evaluations.
- 5. 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. Develop culturing methods to scale-up production of fungal inoculum for inoculation trials.
 2. Develop post-release monitoring techniques to assess the effectiveness of the native fungus, *Verticillium nonalfalfae* as a biological control agent for *Ailanthus*.
 3. Monitor and develop techniques to assess post-treatment native plant regeneration and restoration success.
- 6. Project Justification/Urgency**

Ailanthus has been present in North American landscapes for over three hundred years (Hu 1979) and is widely distributed throughout the East and Midwest. In FIA plots in 33 of 37 states monitored east of the 100th meridian from 1998-2012 (Appendix 1), *Ailanthus* was present. 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. It is most often abundant in open sites such as roadsides, but its presence is increasing within disturbed forested sites. It possesses numerous characteristics often associated with highly invasive species. 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, *Ailanthus* 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). Vigorous sprouts can develop 50-90 feet (15-30 m) from a parent tree (Illick and Brouse 1923). *Ailanthus* can spread and persist in native plant communities, displacing native species and is most often found in mixed hardwood forests. Although the long-term effects of *Ailanthus* on native tree regeneration are not known, it likely has a negative impact because of its highly competitive traits and production of the allelopathic compound ailanthone (Hiesey 1996). 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). Given these obstacles, the use of a highly specific biological control agent such as *Verticillium nonalfalfae* shows great promise.

Penn State University researchers Donald Davis and Mark Schall identified a wilt-causing fungus as a potential biological control agent of *Ailanthus*. In 2002, they isolated *Verticillium nonalfalfae* from dead and dying *Ailanthus* trees within forested areas in Pennsylvania (Schall and Davis 2009a). After much rigorous testing and numerous trials, this soil-borne fungus was found to be very specific and deadly to *Ailanthus*. Symptoms of *Ailanthus* infected with the fungus include wilt, premature defoliation, terminal dieback, yellow vascular discoloration, and mortality. Injecting the fungus (using a hack-n-squirt type treatment) into stems of *Ailanthus* seedlings in the greenhouse and canopy trees in the forest resulted in 100% mortality within 10-16 weeks. Stem inoculations of seedlings or canopy trees of northern red oak, chestnut oak, red maple, sugar maple, white ash, and yellow-poplar did not induce wilt symptoms or mortality in these species. Within forested areas of dead and dying *Ailanthus* trees, no other tree species exhibited any wilt symptoms. Of the over 71 plant species tested to date (field and greenhouse inoculations), Kasson et al. (2012, 2013) reported that only poison ivy, staghorn sumac, red elderberry and striped maple displayed mortality ranging from 70-100% following inoculation. However, of the 64 field inoculated species, only three species appeared to acquire the fungus from natural spread (*Ailanthus*, staghorn sumac and striped maple); and the actual incidence was very low (3% for striped maple and 16% for staghorn sumac).

Since 2009, the same fungus has been found at multiple stands in Virginia (Snyder et al. 2013). In June 2012, Rebbeck and others observed dead and dying *A. altissima* trees in southern Ohio (Pike County) that exhibited symptoms similar to those on diseased *A. altissima* trees in PA and VA. Samples were collected from stems of three symptomatic *A. altissima* trees and sent to Penn State for morphological and molecular identification. All isolates were putatively identified as *V. nonalfalfae* based on the presence of verticillate conidiophores and formation of melanized hyphae. DNA was extracted and molecular analyses confirmed taxonomic placement of the OH *Ailanthus* isolates among those recovered from *Ailanthus* in PA and VA (Rebbeck et al. 2013).

In 2013, Rebbeck began testing this potential biocontrol agent. Greenhouse inoculation studies are underway to verify that native tree species are not susceptible to the fungus. Preliminary greenhouse results on native Ohio seed sources of ash, beech, elm, and oak (black, chestnut, northern red oak and white) seedlings are encouraging – to date no signs of wilt have been observed. These trials will continue to be monitored through 2014. Additional species including scarlet oak and hickory (pignut, mockernut, and shagbark) will be inoculated and monitored in 2014-2015. Pilot field inoculation trials in Ohio are planned for May 2014. 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 and private forest managers have enthusiastically offered *Ailanthus*-infested stands for the proposed 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. A further benefit is that 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.

Efforts are underway to locate and confirm *V. nonalfalfae*'s presence in West Virginia, Maryland, Indiana 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.

7. **Approach**

Description of activities and methods

Goal 1: Develop culturing methods to scale-up production of fungal inoculum.

Currently fungal inoculum is generated on solid agar media as described by Schall & Davis (2009a). We propose to test and develop liquid media culturing methods as a means to increase inoculum production and efficiency. The work will commence once funding is awarded at the NRS lab in Delaware, OH.

Conidial concentrations will be determined using a hemocytometer; and viability evaluated by counting colony forming units of suspensions plated on plum extract agar. Efficacy and potency of the two culturing techniques will be compared with greenhouse inoculation trials of *Ailanthus* seedlings. These tests will be repeated over the course of the project to assess the long-term inoculum potency of the cultures.

Goal 2: Develop post-release monitoring techniques to assess the effectiveness of the native fungus, *Verticillium nonalfalfae* as a biological control agent for *Ailanthus*.

A number of potential trial areas have been identified for the field inoculations based on geo-referenced aerial maps of seed-bearing female *Ailanthus* on the Wayne National Forest, and most Ohio State Forests. When aerial survey data is not available, inoculation trial areas will be selected based on existing field-inventory data and the expert opinion of forest managers. In addition, stands within *The Wilds*, a private, non-profit wildlife conservation center located on reclaimed coal mine land in Muskingum County, Ohio will serve as replicate for the inoculation trials. Both forest and pastures within the preserve may be used. Because endangered animal species graze within the pasture areas, chemical herbicides are restricted. Therefore, finding a biological solution to *Ailanthus* invasion would provide an exceptionally valuable tool for the management of these rangelands. Building on successful treatments demonstrated at the Wilds, the application of biological control could provide a model for implementation within livestock production and pasture lands as well.

In 2014, approximately six trial sites will be selected among candidate areas (Athens District Wayne NF, Hocking State Forest, Pike State Forest, Scioto Trails State Forest, and *The Wilds*) depending on the availability of suitable areas (Appendix 2). A minimum of four stands at a given trial area will be used for a total of 24 stands. Final selection of forest stands will be based on the presence of healthy *Ailanthus* trees. Replicate stands will range in size from 1 to 2 acres and will be spaced approximately 1 to 2 miles apart. Within each stand, 10 to 20 *Ailanthus* trees with a target diameter of 2-10 in dbh will be inoculated three times at the base of each trunk using a hatchet and 1 ml of 1×10^7 conidia ml^{-1} will be injected into the cut using a calibrated spray bottle (Schall and Davis 2009ab). Small trees (<2 in dbh), will be inoculated with a syringe as previously described. 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 early May 2015.

All inoculated trees will be evaluated biweekly, beginning 4 weeks after treatment (~early June) through early October. A disease severity rating related to symptom progression (0 = healthy leaves, 1 = chlorotic leaves, 2 = necrotic leaf margins, 3 = wilting leaves, 4= dead or defoliated trees) as developed by Schall and Davis (2009a) will be utilized. Trees inoculated in 2015 will be evaluated for 2 years post-inoculation. Monitoring will be expanded beyond the initial inoculation plots to adjoining areas to assess natural spread of the fungal inoculum as well as the occurrence of non-target infections. Other non-*Ailanthus* tree species, woody shrubs and vines will be monitored for symptom development within inoculation areas as well.

Goal 3: Monitor and develop techniques to assess post-treatment native plant regeneration and restoration success.

Vegetation surveys within the inoculated stands will be conducted in 2016 to estimate native plant regeneration success. Percent cover data of grasses, forbs, shrubs, vines, and tree seedlings will be estimated within four 2 x 5 m plots nested within the original inoculation stand replicates.

Length of project: 3 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. NEPA approval for inoculation trials on the Wayne National Forest are currently underway and will be completed by summer 2014.

Statistical analyses: Verticillium wilt inoculation response variables to be measured include: *Ailanthus* stem density and mortality, disease severity index, and percent cover 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 (2014): Identification and setup of test sites. As crews are installing inoculation plots within stands, they will also be surveying for natural infestations of *Ailanthus* wilt. Develop liquid fungal culture methodology. Submit progress report in September.

Year 2 (2015): Complete inoculation field trials of *Ailanthus* with *V. nonalfalfae* in Ohio forests by June. Conduct biweekly disease severity index ratings on inoculated stems. Submit progress report in September.

Year 3 (2016): Continue post-inoculation monitoring of *Ailanthus* stems. Survey percent cover of native plant regeneration within treatment areas. Submit final report in September.

Expected Products and Outcomes:

1. Development of a liquid culture method to scale-up fungal inoculum.
2. Development of a methodology to monitor post-release of *Ailanthus* wilt in forested areas.
3. Coordination with ODNR service foresters and consulting foresters to begin trial inoculations on private lands.
4. 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*.

FHP Sponsor/Contact: John Kyhl, St. Paul, MN; 651-649-5238, jkyhl@fs.fed.us

Joanne Rebbeck

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Professional Preparation

1980 B.S., Plant Science, Cook College, Rutgers University
1983 M.S., Plant Pathology, Rutgers University
1987 Ph.D., Botany, Ecology Minor, North Carolina State University

Appointments

1990- Plant Physiologist, USDA Forest Service, Northern Research Station, Delaware, OH
1988-1990 Postdoctoral Research Scientist, Northeastern Research Station, Orono, ME.
2005- Adjunct Faculty member, Department of Environmental and Plant Biology, Ohio University, Athens OH.

Selected Publications

- Rebbeck J, Malone MA, Short DPG, Kasson MT, O'Neal Eric, Davis DD. 2013. First report of *Verticillium* wilt caused by *Verticillium nonalfalfae* on Tree-of-Heaven (*Ailanthus altissima*) in Ohio. *Plant Disease* 97(7): 999 <http://dx.doi.org/10.1094/PDIS-01-13-0062-PDN>
- Rebbeck, Joanne. 2012. Fire management and woody invasive plants in oak ecosystems. *Proceedings of the 4th Fire in Eastern Oak Forests Conference*. GTR-NRS-P-102:p142-155.
- Rebbeck J, Gottschalk KW, and Scherzer AJ. 2012. Do chestnut, northern red and white oak germinant seedlings respond similarly to shading treatments? II. Gas exchange and chlorophyll responses. *Canadian Journal of Forest Research* 42:1025-1037.
- Hutchinson TF, Yaussy DA, Long RP, Rebbeck J, Kennedy Sutherland E. 2012. Long-term (13-year) effects of repeated prescribed fires on stand structure and tree regeneration in mixed-oak forests. *Forest Ecology and Management* 286:87-100.
- Rebbeck, J., Gottschalk, K., and Scherzer, A. 2011. Do chestnut, northern red, and white oak germinant seedlings respond similarly to light treatments? Growth and biomass. *Can. J. Forest Research* 41:2219-2230.
- Rebbeck J, Hutchinson T, Iverson L, Yaussy D, Boyles R, Bowden M. 2011. Studying the effects of management practices on *Ailanthus* populations in Ohio Forests. In: McManus, Katherine A.; Gottschalk, Kurt W., eds. 2010. *Proceedings. 21st U.S. Department of Agriculture interagency research forum on invasive species. 2010 January 12-15; Annapolis, MD. Gen. Tech. Rep. NRS-P-75. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: p50-51.*
- Huebner, C. D., C. McQuattie, and J. Rebbeck. 2007. Mycorrhizal associations in *Ailanthus altissima* (Simaroubaceae) from forested and non-forested sites. *Journal of the Torrey Botanical Society* 134(1):27-33.
- Rebbeck, J., Hutchinson, T.F., and Long, R.P. 2005. Invasive plants affecting the management of Ohio's forests. In 16th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species. Gottschalk, K.W., ed. *USDA Forest Service, Gen. Tech. Rep. NE-337. p.68-70.*

Proposed Budget: FY 2014-2016

Budget - should show both requested and matching (at least 25% of total project costs) funds for the following expense categories:

- Salaries (principal investigator, post-doctoral fellows, students, and technicians)
- Benefits (tuition costs are not allowable)
- Supplies
- Equipment (all items; non-expendable equipment costs above \$5,000 are strongly discouraged)
- Travel

Budgets should include all year costs, as agreements will be funded in their entirety. A minimum 25 percent of the total project cost is required (i.e. to determine the amount of match for a 75/25 cost share, multiply the federal amount of funds by .3333). Indirect charges can be used to meet the cost sharing requirement but requires some in-kind funding other than indirect charges – this can be Principal Investigator salary, travel, supplies, etc. The leveraging of funds and in-kind contributions among project participants is an important criterion.

FY 2014	Item	Requested Funding	Indirect Costs Other-source Funding	Source
Administration	Salary*	21,425	46,425	ODNR, The Wilds, USFS
	Travel	1,000	1,000	USFS
Procurements				
	Contracting			
	Equipment			
	Supplies	500	500	USFS
Year Totals		22,925	47,925	

*Field technician (\$20/hr) for 4 months

FY 2015	Item	Requested Funding	Other-source Funding	Source
Administration	Salary	32,138	56,425	ODNR, The Wilds, USFS
	Travel	2,000	2,000	USFS
Procurements				
	Equipment*	1,000		
	Supplies	1,000	500	USFS
Year Totals		36,1385	58,925	

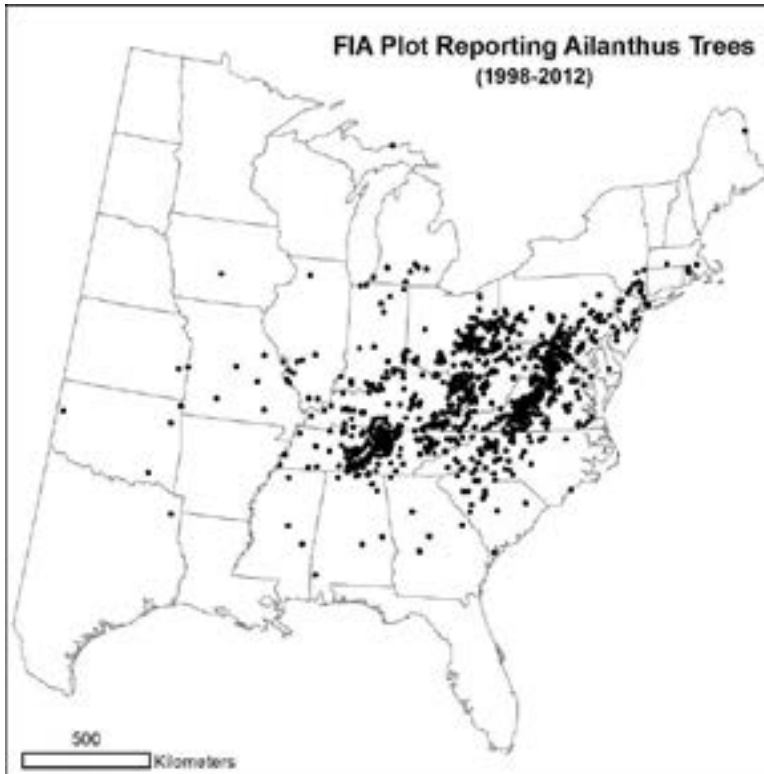
*2 Hypo-hatchet for field inoculations

FY 2016	Item	Requested Funding	Other-source Funding	Source
Administration	Salary	32,138	56,425	ODNR, The Wilds, USFS
	Travel	2,000	2,000	USFS
Procurements				
	Equipment			
	Supplies		500	
Year Totals		34,1385	58,925	

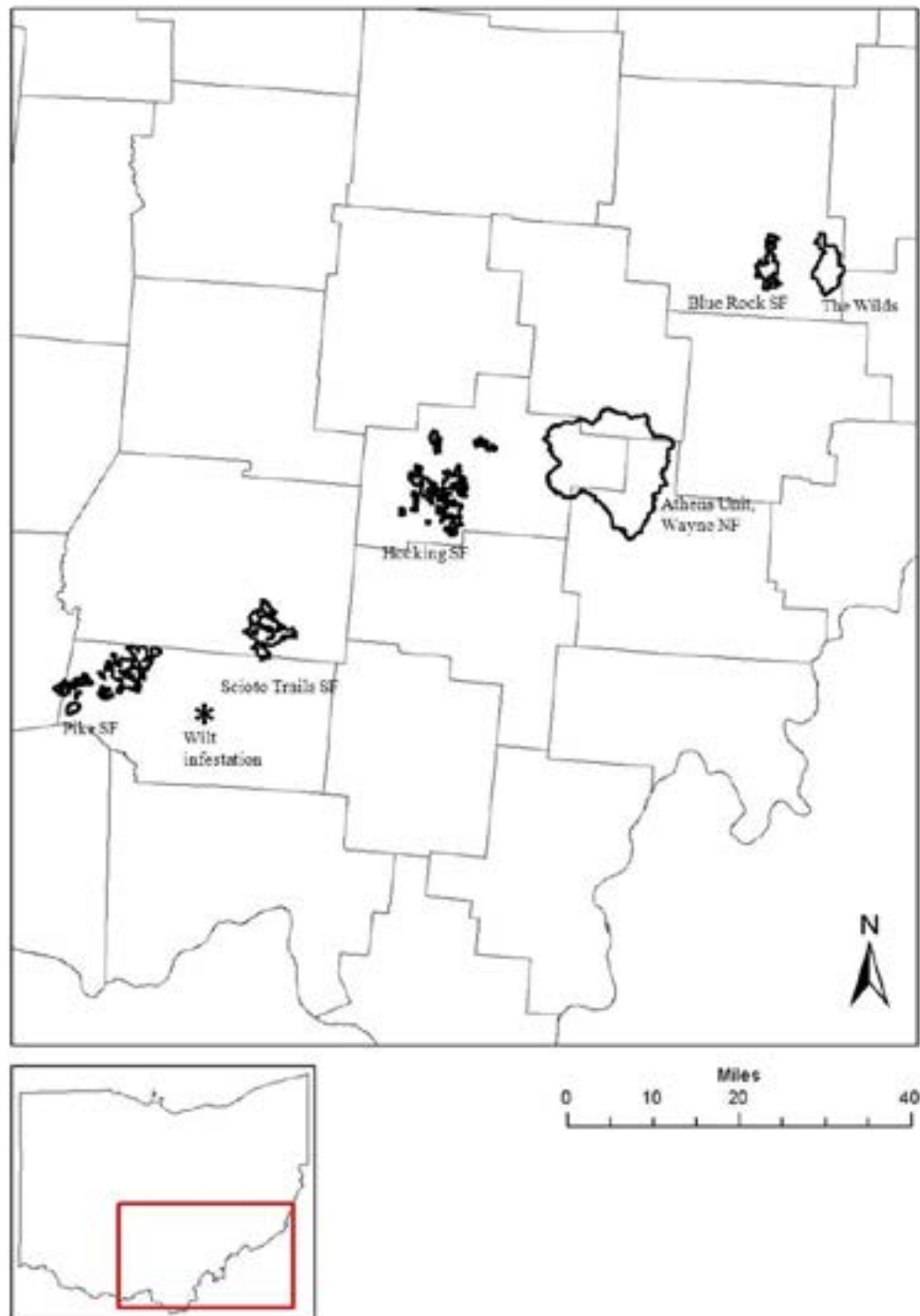
Literature cited

- Hiesey RM. 1996. Identification of an allelopathic compound from *Ailanthus altissima* (Simaroubaceae) and characterization of its herbicidal activity. *American Journal of Botany* 83:192-200.
- Hu SY. 1979. *Ailanthus*. *Arnoldia* 39:29-50.
- Illick JS and Brouse EF. 1926. The *Ailanthus* tree in Pennsylvania. *Pennsylvania Department of Forestry and Water Bulletin* 38:1-29.
- Kasson MT. 2012. *Verticillium nonalfalfae*: A potential biological control of the invasive *Ailanthus altissima* in Pennsylvania. A dissertation in Plant Pathology, The Pennsylvania State University.
- Kasson MT, Short DPG, O'Neal ES, Subbaro KV, Davis D. 2013. Comparative pathogenicity, biological efficacy, and multilocus sequence typing of *Verticillium nonalfalfae* from the invasive *Ailanthus altissima* and other hosts. *Phytopathology* <http://dx.doi.org/10.1094/PHYTO-06-13-0148-R>
- Kowarik I. 1995. Clonal growth in *Ailanthus altissima* on a natural site in West Virginia. *Journal of Vegetation Science* 6:853-856.
- Pannell PD. 2002. Tree-of-heaven control. Maryland Department of Natural Resources Forest Service Stewardship Bulletin, Hagerstown, MD.
- Rebbeck J, Hutchinson T, Yaussy D, Iverson I, 2008. Prescribing fire in managed oak forest landscapes: interactions with the invasive tree *Ailanthus altissima*. Joint Fire Science Program Project number: 08-1-2-07 http://nrs.fs.fed.us/units/sustainingforests/local-resources/docs/REBBECK_JFSP_study%20plan.pdf
- Schall MJ and DD Davis. 2009a. *Ailanthus altissima* Wilt and Mortality: Etiology. *Plant Disease*. 93:747-751.
- Schall MJ and DD Davis. 2009b. *Verticillium* Wilt of *Ailanthus altissima*: Susceptibility of associated tree species. *Plant Disease* 93:1158-1162.
- Smith, K and Smith A. 2009 Controlling non-native invasive plants in Ohio forests: *Ailanthus*. OSU Fact Sheet F-65-09.
- Snyder et al. 2013. First Report of *Verticillium* Wilt of *Ailanthus altissima* in Virginia caused by *Verticillium nonalfalfae*. *Plant Disease* 97 (6):837

Appendix 1: Distribution of Ailanthus trees and seedlings reported present in FIA plots from 1998-2012.



Appendix 2. Map of potential sites for field inoculation trials. Asterisk shows first verified location of natural infection of *Ailanthus* by *V. nonalfalfae* in June 2012.





Ohio Department of Natural Resources

JOHN E. KASICH, GOVERNOR

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October 30, 2013

Richard Reardon
National Program Manager, Biological Control
USDA Forest Service

Dear Mr. Reardon:

I am writing in support of the grant application "Biological control and management of *Ailanthus*: post-release monitoring and efficacy of *Verticillium* wilt in Ohio" being submitted by Joanne Rebbeck, USDA Forest Service. *Ailanthus altissima* is widespread in Ohio, and it represents a major management challenge in many Ohio State Forests. The development of an effective and safe biological control for *Ailanthus* would greatly enhance our ability to manage this forest health issue both on public and private forest lands in Ohio by adding to the suite of possible control methods. The Ohio Division of Forestry looks forward to partnering with the USDA Forest Service and other project partners on this important project and is offering three of our state forests as potential trial sites.

Thank you for your consideration of this important project.

Sincerely,

A handwritten signature in blue ink that reads "Robert L. Boyles".

Robert L. Boyles
State Forester and Chief
Ohio Division of Forestry

Cc: Joanne Rebbeck, USDA Forest Service



October 29, 2013



USDA Forest Service
Forest Health Protection
Forest Health Technology Enterprise Team

RE: Support for Proposed Project: "Biological control and management of *Allanthurus*: post-release monitoring and efficacy of *Verticillium* wilt in Ohio"

To Whom It May Concern:

On behalf of *The Wilds* conservation center, I am writing to express our support for the proposed project entitled, "*Biological control and management of Allanthurus: post-release monitoring and efficacy of Verticillium wilt in Ohio*".

As outlined in the request for proposals issued by **Technology Development for the Biological Control of Invasive Native and Non-Native Plants**, priority invasive species threaten ecosystem integrity on a substantial scale, and therefore addressing the issue requires collaborations and innovation. *The Wilds* is eager to serve as a partner and help facilitate deployment of biological solutions to invasive species through technology and applied ecosystem management.

The Wilds is a unique biological field station located on nearly 10,000 acres of reclaimed surface mined land in southeastern Ohio. Following reclamation, this landscape has evolved into a diverse refuge for native wildlife as well as endangered species from Africa, Asia and North America. Within this setting, researchers are working in fields of Restoration Ecology, Conservation Science and Animal Health and Education and providing opportunities for training the next generation of conservation professionals. Similar to other Ohio landscapes that have experienced disturbance, the tenacious *Allanthurus altissima* is a priority invasive species of concern, given the plant's ability to degrade ecosystem health. As such, we are working to identify and control *Allanthurus* in the most efficient and ecologically sensitive manner possible.

Implementing a biological control treatment within our wildlife pastures would provide an exceptionally valuable management option that would not pose the potential risks to animal health associated with traditional chemical herbicide treatment options. Additionally, *The Wilds* will contribute in-kind support of professional staff oversight for project implementation as well as basic training of research technicians to assist with monitoring efforts.

Given the considerable benefits that could be provided by a biological control for *Allanthurus*, we are excited about the potential this project and offer our sincere support.

Best regards,

Shana Byrd
Director of Restoration Ecology
The Wilds
sbyrd@thewilds.org