USDA Forest Service
Forest Health Assessment and Applied Sciences Team
FHAAST-2019-03
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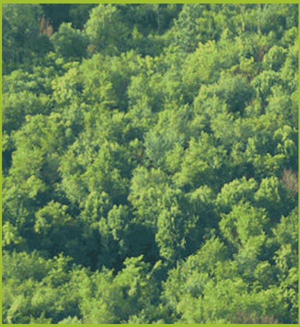
Verticillium nonalfalfae

A Promising, Naturally Occurring Biological Herbicide to Control

Tree of Heaven

(*Ailanthus altissima*)



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The Forest Health Technology Enterprise Team (FHTET) was created in 1995 by the Deputy Chief for State and Private Forestry, USDA Forest Service, to develop and deliver technologies to protect and improve the health of American forests.

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Cover images: (clockwise from top left) healthy TOH stand, diseased TOH stand, diseased TOH tree, healthy TOH tree (Photos: both stands by The Pennsylvania State University; Rachel K. Brooks, Virginia Tech; Chuck Bargeron, University of Georgia, Bugwood.org)

INTRODUCTION

Tree of heaven (TOH; *Ailanthus altissima* (Mill.) Swingle, Simaroubaceae) is native to eastern China. This deciduous hardwood tree was first introduced from England to the U.S. in 1784 to a garden in Pennsylvania, and then several more times directly from China, including to Rhode Island in 1804 and to California by Chinese immigrants in the mid-1800s. TOH now occurs across the U.S. (**Fig. 1**) and is reportedly invasive in over 44 U.S. states.

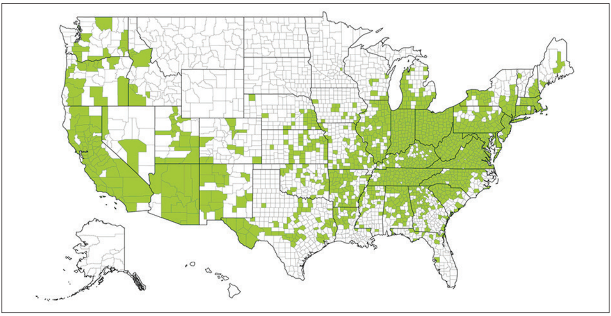


Figure 1. TOH distribution in the U.S. (Credit: EDDMapS, http://www.eddmaps.org/ accessed 11 February 2020)

- TOH invades forests, rights-of-ways, urban areas, and agricultural lands
- TOH displaces native vegetation important for wildlife habitat, timber production, recreation, and biodiversity
- TOH roots produce allelopathic compounds that may reduce the establishment of other plants
- TOH is the preferred host of other invasive pests including the spotted lanternfly, *Lycorma delicatula* (White) (**Fig. 2**), and the brown marmorated stink bug, *Halyomorpha halys* Stål
- TOH can cause allergic reactions (via pollen and direct contact) in humans
- TOH is also called Ailanthus, Chinese sumac, varnish tree, stink tree, and paradise tree

Figure 2. Spotted lanternfly colony on TOH (Photo: Rachel K. Brooks, Virginia Tech)

BIOLOGY & LIFE CYCLE

TOH is fast-growing with an extensive root system and prolific seed production. Mature trees can have a diameter up to 6 ft (1.8 m), reach up to 65 ft (20 m) tall, and can live for over 100 years. TOH tolerates a wide range of growing conditions, including air pollution and low quality soils, and is often found thriving in areas with high levels of disturbance. Once established, it is among the fastest growing trees in North America, often growing up to 6 ft (1.8 m) during its first year.

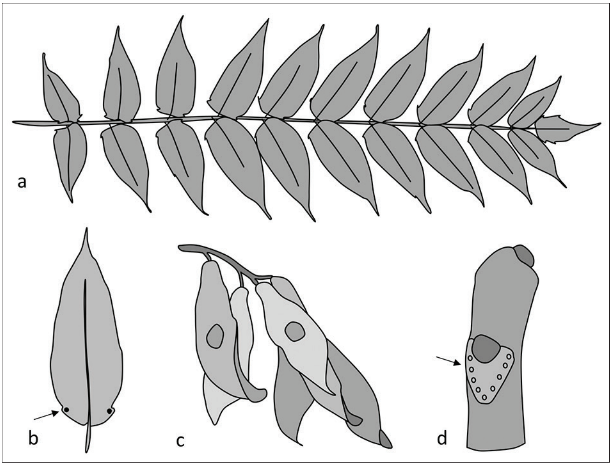
Seed production can begin within as little as 5 years (**Fig. 3a**). Flowers are first observed on TOH in late spring to early summer (**Fig. 3b**). A single tree may produce up to 1 million seeds per year and over 50 million seeds during its lifetime. Seeds are readily spread by wind and water. Unlike some other species, TOH seeds can germinate immediately upon contact with the soil and can also remain viable for over 5 years. The leaves are deciduous, falling from the tree in autumn and winter. New leaves emerge in late spring.

TOH also reproduces clonally through its suckering root system (**Fig. 3c**). Sprouts arise from the roots, root crown, and bole and typically grow much faster than seedlings (up to 10 ft [3 m] per year) because an extensive root system is already established. These develop into new plants and can quickly create large TOH monocultures.

Figure 3. TOH (a) tree, (b) flower clusters, and (c) suckers (Photos: a,c. Robert Vidéki, Doronicum Kft., Bugwood.org; b. H. Zell, Wikimedia)

IDENTIFICATION

- Leaves long, pinnately compound (**Figs. 4a, 5a**) whose leaflets have smooth margins and slightly lobed bases tipped by raised glands (**Figs. 4b, 5b**)
- Winged fruits 1 in (2.5 cm) long, turn from green/yellow to orange/red to brown (**Figs. 4c, 5c**)
- Leaf scars large, triangular, and edged by line of bundle scars (**Fig. 4d, 5d**)
- Flowers small with 5 greenish-yellow petals, produced in large clusters
- Native look-a-like trees include walnut and sumac



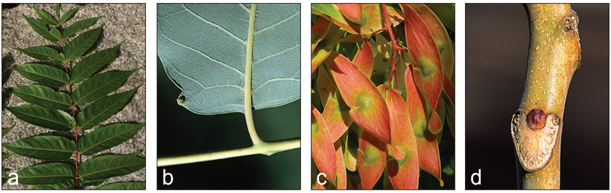


Figure 4 (top), 5 (bottom). Key identification characteristics of TOH including (a) leaf shape, (b) leaflet glands, (c) seed morphology, and (d) leaf scars (Illustrations: Rachel K. Brooks, Virginia Tech; Photos: a. Leslie J. Mehrhoff, University of Connecticut; b,d. James H. Miller Forest Service; c. Chuck Bargeron, University of Georgia; a–d Bugwood.org)

TRADITIONAL CONTROL

Small seedlings can be hand-pulled from an area. Pulling older seedlings and saplings is more difficult, and roots left in the soil will re-sprout. Cutting large trees is not recommended as the sole control method as it often results in significant basal and root sprouting (**Fig. 6**). Moreover, cutting is impractical since multiple cuttings are required to deplete root reserves.

Figure 6. TOH stand (a) cut to the ground 2 March 2007, (b) re-sprouting 14 June 2007, and (c) a monoculture 10 August 2007 (Photos: Scott Salom, Virginia Tech)

Several general-use herbicides are available that can be applied as foliar sprays, cut stump treatments, injections into the plant, or as basal sprays. These treatments are usually used on TOH at small scales but may also impact other nearby plants (non-target damage).

Chemical and mechanical control can remove TOH from a small area. However, because TOH is such an aggressive species, it will re-sprout, requiring multiple treatments and making large-scale, long-term eradication impractical and expensive.

BIOLOGICAL CONTROL

Due to the extensive negative impacts and invasive spread of TOH, as well as the shortcomings of traditional control methods for this species, there is an urgent need to develop biological control components for TOH Integrated Pest Management (IPM) programs. Bioherbicides may provide an efficient, inexpensive, and perennial control of TOH.

TOH's native range was surveyed, and multiple potential classical biological agents were identified and targeted for further research. Of these, the weevil *Eucryptorrhynchus brandti* (Harold) demonstrated the most promise. As of 2020, it is still undergoing host-range testing prior to consideration for its release in North America.

Early surveys of TOH in North America documented a lack of significant insect and pathogen agents. However, in 2002, a naturally occurring fungus was observed killing TOH trees in Pennsylvania (**Fig.** 7).

Figure 7. TOH trees exhibiting foliar symptoms of fungal infection (Photos: Rachel K. Brooks, Virginia Tech)

Verticillium nonalfalfae

HISTORY

Professor Don Davis and his students at the Pennsylvania State University first isolated this naturally occurring, deadly wilt-causing fungus from dead and dying TOH trees within forested areas in south-central Pennsylvania in 2002.

Early research at Penn State University on inoculating the fungus into TOH at several sites in south-central Pennsylvania documented its potential as an effective biological control agent.

The disease spread naturally and rapidly, creating small populations of infected TOH (**Table 1**), which prompted further interest in the fungus as a biological control agent. The fungus was originally thought to be *Verticillium albo-atrum* but has since been formally identified as *Verticillium nonalfalfae* Inderbitzin et al. in 2011.

Verticillium nonalfalfae was subsequently found on TOH in Virginia in 2008 by scientists at Virginia Tech and in Ohio in 2012 by U.S. Forest Service scientists. This promising, host-restricted biological control currently occurs over thousands of acres in these states.

By 2011, natural spread of the fungus had resulted in the mortality of >30,000 TOH trees, effectively removing most TOH from the landscape at these inoculated sites.

Table 1. The spread of *V. nonalfalfae* from inoculated TOH to adjacent TOH at 4 sites in Pennsylvania, USA (Source: Don Davis, unpub. data 2011)

Site	2006 # TOH inoculated	2010 # TOH dead or dying
1	20	2,663
2	20	503
3	10	941
4	10	701

LIFE CYCLE AND IMPACT

As a soil-borne plant pathogen, *Verticillium nonalfalfae* attacks only plants, not humans or other organisms. This fungus produces resting structures (dormant melanized mycelia) that can survive in the soil or within plant debris. Stimulated by root exudates, these resting structures germinate, form mycelia that penetrate the tree's root, and invade the xylem. The mycelia then spread quickly throughout the tree's vessels, ultimately clogging the xylem and eliciting typical wilt symptoms. This disease develops fairly quickly and kills mature trees within just a few months. All TOH growth stages are susceptible, from seedling to mature tree, as well as the roots and subsequent root suckers. It is thus a strong candidate for use as a biological control agent.

SYMPTOMS

- **Foliar (leaves) symptoms:** rapid onset of wilting, chlorosis (yellowing), necrosis (browning, **Fig. 8**), premature leaf drop, emergence of new leaves late in the season (epicormic sprouting and flushing, **Fig. 9**)
- **Wood symptoms:** branch drop and orange coloration of wood visible when bark is removed (**Fig. 10**)
- **Stand symptoms:** numerous diseased or dead TOH in one area (**Fig. 11**)

SAFETY

All native trees and understory plants that currently coexist with TOH are unaffected by this wilt, with the exception of striped maple, *Acer pensylvanicum*. Natural *V. nonalfalfae* infections have killed only 1% of associated striped maple, and striped maple populations were even observed increasing after TOH control. A large host-range testing has been conducted on *V. nonalfalfae* isolate VnAa140. Of the 71 plant species tested in a greenhouse and in the field, significant disease impact occurred only on TOH. Work is underway to screen VnAa140 against a much larger list of nontarget plant species representative of agriculturally and ecologically important plants.

Figure 8. Foliar symptoms on a *V. nonalfalfae*-inoculated TOH (Photo: Donald D. Davis, The Pennsylvania State University)

Figure 9. Epicormic sprouting on a *V. nonalfalfae*-inoculated TOH (Photo: Rachel K. Brooks, Virginia Tech)

Figure 10. Bark removed exposing color differences between healthy (left) and infected TOH (right) (Photo: Kristen Wickert, West Virginia University)

Figure 11. Aerial view of a TOH stand with several trees exhibiting foliar symptoms following *V. nonalfalfae* inoculations (Photo: The Pennsylvania State University)

SPREAD

Verticillium nonalfalfae can spread naturally from diseased to nearby healthy TOH through root contacts, soil movement, and transmission by insects. Due to this tree's aggressive growth and clonal tendencies, TOH stands include many functional root grafts (roots that have grown together, **Fig. 12**). Consequently, the fungus readily spreads within a stand and is highly effective at controlling TOH over large areas.

Figure 12. Excavated TOH roots showing a functional root graft (Photo: Kristen Wickert, West Virginia University)

Between or among stands, the fungus can be spread passively by ambrosia beetles, which feed on dead and dying trees. *Euwallacea validus* (Eichhoff) is the most common ambrosia beetle associated with symptomatic TOH. In laboratory studies, the weevil *E. brandti* demonstrated the ability to carry and transfer *V. nonalfalfae* to TOH. These insects would be able to spread the fungus to TOH stands otherwise unreachable for short-range pathogen dissemination.

It is relatively easy to infect TOH by injecting a suspension of spores into the stem or by the "hack and squirt" method. Disease develops quickly, killing 100% of the trees within three months following infection. Because the fungus can spread naturally from diseased to nearby healthy TOH through root contact, this ability for natural spread would greatly increase the biological control efficacy of the pathogen and decrease application coverage needs.

Advantages of *V. nonalfalfae*

- Native to the USA; not an introduced pathogen
- Found killing TOH in Pennsylvania, Ohio, and Virginia
- Host-restricted; needs further confirmation
- Kills TOH of all growth stages
- Can be mass produced and formulated
- Easy to inoculate TOH with existing tools
- 100% TOH kill—consistent and fairly quick
- Disease spreads from infected to untreated TOH—a highly desirable feature
- Could be integrated with other biological control agents (e.g., TOH weevil *Eucryptorrhynchus brandti* and spotted lanternfly pathogens and parasites)

BIOHERBICIDE POTENTIAL

An email survey was designed and conducted by the Florida Survey Research Center, University of Florida to determine the preference of using a registered bioherbicide as opposed to a chemical herbicide to control TOH. A total of 500 potential users (e.g., highway departments, state parks and foresters, extension scientists, and pest control companies) were contacted. Of the 100 responders ⅓ said they were "very likely" and another ⅓ responded they were "likely" to use the bioherbicide instead of the chemical herbicide.

Because *V. nonalfalfae* isolate VnAa140 from Pennsylvania has shown such excellent potential as a biocontrol agent for TOH, efforts are currently underway to register this isolate as a bioherbicide with the U.S. EPA. Registration would allow for the widespread general use of this biopesticide, in addition to its movement across state lines.

The registration process can take several years, although bio-based pesticides (e.g., bioherbicides) are usually granted "fast track" status and receive an expedited process. When registered by the U.S. EPA, this bioherbicide is expected to become a major component in IPM programs to control TOH.

REGISTRATION PROCESS

A preliminary meeting (15 February 2018) between the U.S. EPA Biopesticides and Pollution Prevention Division, the private company BioProdex, and Dr. Michael Braverman (Manager Biopesticide, Organic and International Capacity Building Programs, IR-4 Project, Rutgers University, NJ) outlined the requirements and work plan for registration of *V. nonalfalfae*.

- Beginning in 2019, BioProdex (contact Dr. Raghavan Charudattan), in collaboration with the USDA ARS U.S. Horticultural Research Laboratory, agreed to conduct laboratory and greenhouse trials on approximately 250 plant species to determine the pathogenicity of *V. nonalfalfae* isolate VnAa140, the host range of the isolate, and the potential non-host species at risk, if any.
- Dr. Braverman agreed to serve as Bioprodex's EPA liaison throughout the registration effort and to advise and assist the company regarding agency rules and regulations, data requirements, submissions, reviews, and other matters. His services are provided free of cost, as part of the IR-4's assistance to promote biopesticide registrations.
- Sylvan Bio, Kittanning, PA, a renowned world leader in fungal biomass production, agreed to industrially develop isolate VnAa140 biomass for the proposed effort and, after EPA registration, serve as the manufacturer of the fungal bioherbicide product for BioProdex.
- A certified commercial toxicology lab must still be subcontracted to gather toxicity data on the fungal isolate according to EPA Tier 1 testing guidelines.
- Gathering empirical data and submitting a full registration data package to the EPA is expected to take 3 years. An additional year might be needed to develop bioherbicide product specifications, application tools and methods, a pesticide label and use instructions, and packaging.
- During the EPA registration review, the bioherbicide is expected to be available for field testing under an Experimental Use Permit.

WHAT YOU CAN DO

Because industrially developing and registering the fungus as a bioherbicide would provide a powerful tool to hasten the control of TOH, there is an urgent need to identify funding sources to support its registration.

It is estimated that a total of $185,000 is needed to complete the host range trials, and a total of $215,000 is needed to complete the toxicology tests. These funds would cover studies conducted over 2 years and provide the initial critical data to determine whether or not to continue the registration process (e.g., field trials, formulation development). Research proposals have been submitted to potential funding sources (e.g., Farm Bill, Spotted Lanternfly Program, USDA Special Projects) to develop registration data, but to date funding has not materialized.

Funding is imperative as this native fungus has been demonstrated as a highly effective bioherbicide that kills TOH and spreads naturally. It is the only long-term, environmentally compatible control for TOH, either as a standalone treatment or as a component for an IPM approach. Contact your state department of agriculture, department of forestry, or department of highways to inquire about the registration status of this fungus.

UNTIL *V. NONALFALFAE* IS REGISTERED

STOP

- Movement of infected plant material or associated soil is highly discouraged as this practice can lead to the spread of other pests or diseases (such as spotted lanternfly or fusarium canker)
- Movement of *V. nonalfalfae* across state borders requires a government permit. Movement without a permit is punishable by law
- Use, production, and sale of *V. nonalfalfae* as a bioherbicide requires U.S. EPA registration

Booklet back cover image: A TOH stand whose center-most trees were inoculated with *V. nonalfalfae* two months prior to this photo (Photo: Rachel K. Brooks, Virginia Tech)

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