Can the invasive tree Ailanthus altissima be tamed with a native Verticillium fungus?

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Ailanthus altissima

Ailanthus or Stink Tree

Tree of heaven

The Ohio Woodland Journal Fall 2013
“It is the only introduced tree that is competing vigorously with our native tree growth . . . . unless a use is found for its wood, this tree will develop into a worthless forest weed and become a nuisance of the first magnitude.”

Joseph Illick and E.F. Brouse. 1926. PA Dept. of Forests and Waters Bulletin 38
Ailanthus wilt found in PA forests - 2002

*Verticillium nonalfalfaе*

Don Davis & Mark Schall
Matthew Kasson
Eric O’Neil

Penn State University
Forest Pathology

2009 - VA
2012 - OH
Rapid Foliar Wilting
Kills vascular tissue

Dying Ailanthus

Healthy Ailanthus
Two Verticillium wilts that attack *Ailanthus*

1. *Verticillium dahliae*
   - slow-killing $>2$ years
   - common & widely distributed
   - generalist

**2. *Verticillium nonalfalfae***
   - fast-killing $<1$ year
   - more pathogenic
   - infrequent
Windshield survey of *Verticillium nonalfafae* in Southeastern USA

• Annual monitoring of stands
• Rate of spread 300-400 ft/year
• No non-target species effects
Fungal Isolate Comparisons

• Molecular sequencing identification – All isolates are identical

• Pathogenicity on Ailanthus seedlings similar to PA and VA isolates

• Wilting began at 4 weeks
• Dead within 9 weeks

Rebbeck et al. 2013. Plant Disease 9 (7):999
http://dx.doi.org/10.1094/PDIS-01-13-0062-PDN
Verticillium nonalfalfae
"Model" Biocontrol Agent

- Native soil-borne fungus
- Highly selective for Ailanthus
- Natural transmission via root grafting, wounding
- Can persist in soil for years
2006-2009
100 canopy trees were inoculated

2011
>14,000 dead/dying trees and 10,000-15,000 sprouts killed

Kasson et al 2014
Added bonus – Ambrosia beetle may help move the fungus

*Euwallacea validus*


<1/8” long
Regulatory Approval in Ohio

- Searched for naturally occurring infected stands (2009-2012)
- Ohio Dept. of Agriculture approval for field studies in 2013
- No regulatory issues with APHIS or ODA to use Ohio isolate (in-state inoculum)
<table>
<thead>
<tr>
<th>Tree Species</th>
<th>Tree Species</th>
<th>Tree Species</th>
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</thead>
<tbody>
<tr>
<td>Boxelder</td>
<td>Autumn olive</td>
<td>White ash</td>
</tr>
<tr>
<td>Japanese maple</td>
<td>Mountain laurel</td>
<td>American sycamore</td>
</tr>
<tr>
<td><strong>Striped maple (3%)</strong></td>
<td>Great rhododendron</td>
<td>Hawthorn</td>
</tr>
<tr>
<td>Norway maple</td>
<td>Pink azalea</td>
<td>Apple ‘Rome’</td>
</tr>
<tr>
<td>Red maple</td>
<td>Lowbush blueberry</td>
<td>Sweet cherry</td>
</tr>
<tr>
<td>Sugar maple</td>
<td><strong>Silktree</strong></td>
<td>Black cherry</td>
</tr>
<tr>
<td>Red elderberry</td>
<td>Eastern redbud</td>
<td>Multiflora rose</td>
</tr>
<tr>
<td><strong>Staghorn sumac (16%)</strong></td>
<td>Honey locust</td>
<td>Amur corktree</td>
</tr>
<tr>
<td>Poison-ivy</td>
<td>Black locust</td>
<td>Korean evodia</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>American chestnut</td>
<td>White poplar</td>
</tr>
<tr>
<td><strong>Angelica tree</strong></td>
<td>American beech</td>
<td>Bigtooth aspen</td>
</tr>
<tr>
<td>European black alder</td>
<td>Northern red oak</td>
<td><strong>Royal paulownia</strong></td>
</tr>
<tr>
<td>Black birch</td>
<td>Chestnut oak</td>
<td><strong>Ailanthus</strong></td>
</tr>
<tr>
<td>American hornbeam</td>
<td>American witchhazel</td>
<td>American basswood</td>
</tr>
<tr>
<td>Hophornbeam</td>
<td>Mock-orange</td>
<td>Hackberry</td>
</tr>
<tr>
<td><strong>Japanese barberry</strong></td>
<td>Hickory</td>
<td>American elm</td>
</tr>
<tr>
<td>Northern catalpa</td>
<td>Black walnut</td>
<td>Siberian elm</td>
</tr>
<tr>
<td>Doublefile viburnum</td>
<td>Northern spicebush</td>
<td>Eastern red cedar</td>
</tr>
<tr>
<td>Blackhaw</td>
<td>Sassafras</td>
<td>Arborvitae</td>
</tr>
<tr>
<td><strong>Oriental bittersweet</strong></td>
<td>Corkwood</td>
<td>Virginia pine</td>
</tr>
<tr>
<td><strong>Burningbush</strong></td>
<td>Yellow-poplar</td>
<td><strong>Devil’s walkingstick (17%)</strong></td>
</tr>
<tr>
<td>Winter creeper</td>
<td>Cucumertree</td>
<td></td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td>Black tupelo</td>
<td></td>
</tr>
</tbody>
</table>

Ohio Greenhouse Studies

Greenhouse inoculations at Delaware Lab 2013-2015

• *Ailanthus*
• Oaks: black, chestnut, pin, red, scarlet, & white
• Hickories: bitternut, butternut, mockernut, pignut & shagbark
• Elm
• Ash
• Beech
• Tomato, alfalfa & soybeans

To date – no off-target effects
10 weeks post-inoculation
Ohio Field Research

• Demonstration study installed at Vinton Furnace and Tar Hollow State Forests in June 2014
Hack-n-Squirt Stem “Injection” of Ailanthus

1. Fungus is cultured on agar in lab

2. High concentration of spores in aqueous solution prepared

3. Ailanthus trees cut with hatchet & injected with fungus
2 weeks after inoculation
8 weeks after inoculation
15 weeks after inoculation
US FS Forest Health Program Biocontrol Grant

- Inoculated and monitoring Ailanthus in 5 SE Ohio forests
- Monitoring for off-target effects
- Studying “aftermath” regeneration within inoculated stands

Partners: Ohio Division of Forestry, Wayne National Forest, The Wilds, & Penn State
5 study sites:
• 4 plots inoculated + 1 control plot per site
• 10 Ailanthus trees per plot (>2.4” dbh)

Plots are 20 X 50 m (66 x 164 ft)
Summary of percent cover vegetation at each of the five study areas within 2 x 5 meter subplots prior to inoculation of Ailanthus trees with Verticillium wilt

<table>
<thead>
<tr>
<th>Vegetation type</th>
<th>Mean</th>
<th>±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Native plants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasses</td>
<td>11.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Native vines/lianas</td>
<td>12.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Herbs &amp; forbs</td>
<td>18.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Shrubs - Spicebush</td>
<td>76.6</td>
<td>8.5</td>
</tr>
<tr>
<td>Tree seedlings</td>
<td>8.6</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>B. Non-native plants excluding Ailanthus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese stiltgrass</td>
<td>15.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Japanese honeysuckle</td>
<td>50.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Garlic mustard</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Multiflora rose</td>
<td>72.6</td>
<td>16.8</td>
</tr>
<tr>
<td>Bush honeysuckle</td>
<td>24.0</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Other non-natives observed include privet, periwinkle, wineberry, and autumn olive.
## Biweekly Disease Severity Index Ratings

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>healthy foliage</td>
</tr>
<tr>
<td>1</td>
<td>chlorosis and/or necrotic margins on leaves</td>
</tr>
<tr>
<td>2</td>
<td>slight wilt (&lt;15% wilting foliage) with no or slight defoliation (&lt;15%)</td>
</tr>
<tr>
<td>3</td>
<td>moderate wilt (15 to &lt;50% wilting foliage with no or slight defoliation (&lt;15%)</td>
</tr>
<tr>
<td>4</td>
<td>severe wilt (50 to 100% wilting foliage with no or slight defoliation (&lt;15%)</td>
</tr>
<tr>
<td>5</td>
<td>moderate defoliation (15 to &lt;50%)</td>
</tr>
<tr>
<td>6</td>
<td>severe defoliation (50 to 90%)</td>
</tr>
<tr>
<td>7</td>
<td>very severe defoliation (90 to 100%) with epicormic sprouting</td>
</tr>
<tr>
<td>8</td>
<td>dead</td>
</tr>
<tr>
<td>Forest Site</td>
<td>Mean dbh (in)</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Blue Rock SF</td>
<td>5.5</td>
</tr>
<tr>
<td>Marietta WNF</td>
<td>6.3</td>
</tr>
<tr>
<td>Perry SF</td>
<td>5.5</td>
</tr>
<tr>
<td>Tar Hollow SF</td>
<td>5.9</td>
</tr>
<tr>
<td>The Wilds</td>
<td>5.9</td>
</tr>
<tr>
<td>Mean</td>
<td>5.8</td>
</tr>
<tr>
<td>Controls (sterile water)</td>
<td>5.9</td>
</tr>
</tbody>
</table>

*DAI = Days after inoculated with Verticillium fungal spores
What are the off-target effects?

Natural infections of *V. nonalfalfa* fungus in PA

Striped maple (3%)  Staghorn sumac (16%)

http://faculty.etsu.edu
Devil’s Walkingstick, *Aralia spinosa*

- PA - 17% were naturally infected – 2 sites
- Isolated fungus from leaves
- 2015 – began trial inoculations of aralia & sumac in OH
Devil’s walkingstick
• 5 weeks post-treatment
• all defoliated and spreading

Glossy sumac
• 3 weeks: no symptoms

Possible native hosts for fungus?

Muskingum Watershed Conservancy District, Leesville, OH
Family Araliaceae - Mostly tropical shrubs and trees
USDA PLANTS Database - 18 Genera and 47 species

- Devils walkingstick
- English ivy
- Schlefflera (Umbrella plant)
- Ginseng - 4 species

American ginseng, *Panax quinquefolius* L.

Credit: Gary Kauffman, USFS
• Ginseng is difficult to grow artificially

• Monitoring ginseng in current inoculation research plots

• 2016 - Inoculate Ailanthus trees within ginseng areas
The work continues...

- Assess off-target effects
- Alternate ways to culture fungus (scaling up)
- Stability of fungus (has short shelf life)
- Restoration plantings in inoculated stands
- Infected wood chips to “inoculate” Ailanthus
Collect 10-20 large wood chips
Store in ziplock freezer bag
Keep samples cool – store in cooler or fridge

*Wood chips: both healthy and discolored
THANKS

• Matt Malone, Jeremy Scherf, & Brad Wireman
• OSAF & Ohio forestry community
• ODNR Division of Forestry, Wayne NF, & The Wilds
• Penn State & WVU - Don Davis, Matt Kasson, & Eric O’Neal
• US Forest Service State & Private Forest Health Biological Control Program and NRS for financial support
SPECIAL THANKS FOR FIELD AND LAB ASSISTANCE

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QUESTIONS?

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740-368-0054
Alternative inoculation formulations

- Infected soil
- Infected Ailanthus wood & leaves

O’Neal & Davis. 2015. Biocontrol Science & Technology

Optimal inoculation time in PA – April to May