

# Current Research in Ginzel Lab

- Exploring mechanisms of ash resistance to emerald ash borer (EAB) and increasing the efficacy of its biological control agents
- Enhanced decision-making for area-wide suppression of EAB in urban settings





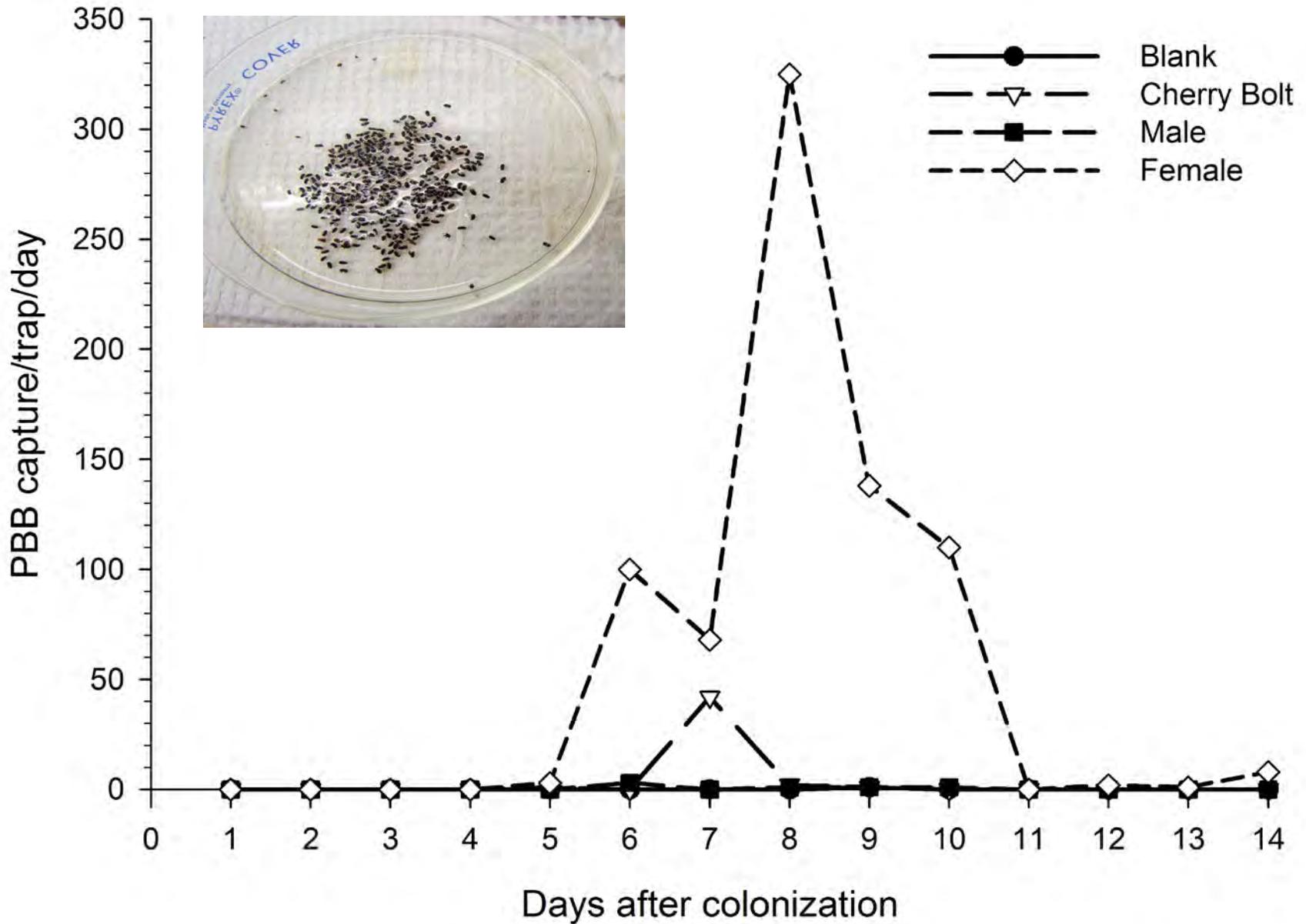


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- Enhanced decision-making for area-wide suppression of EAB in urban settings
- Understanding the chemically-mediated host colonization and mating behavior of bark and ambrosia beetles affecting native hardwoods







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- Enhanced decision-making for area-wide suppression of EAB in urban settings
- Understanding the chemically-mediated host colonization and mating behavior of bark and ambrosia beetles affecting native hardwoods
- Enhanced detection methods for the walnut twig beetle and thousand cankers disease (TCD)
- Assessment and etiology of TCD within the native range of black walnut

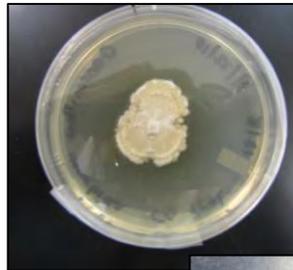
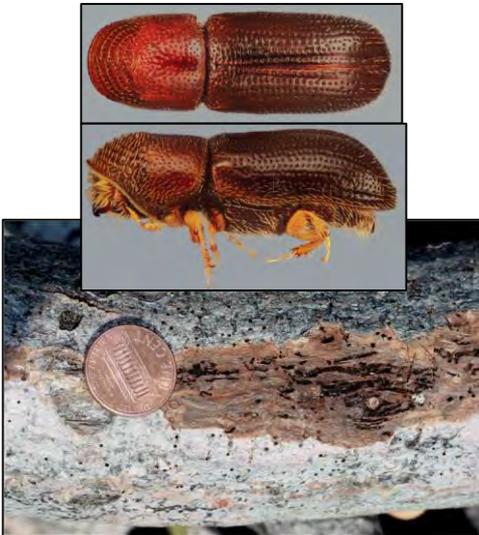


# Thousand Canker Disease (TCD)

TCD is the result of a complex interaction among

- *Pityophthorus juglandis* (walnut twig beetle),
- *Geosmithia morbida*, and
- a susceptible host, e.g. *Juglans nigra*.

**WTB** + **GM** + **JN** = **TCD**





## TCD symptom change (recovery) observed from 2010 to 2013 in the Knoxville, TN TCD area on black walnut tree at the TCD-founder site

- (Top photograph) This tree had a 60% live crown rating on 15 September 2010 with moderate dieback of branches.
- (Bottom photograph) New stem growth and an abundance of new healthy foliage on this tree gradually increased in 2011, 2012 and 2013 until the amount of live crown was very high (90%) on 29 August 2013. (G. J. Griffin, 2014)

*Various research questions addressed, including:*

1. Behavior: Are adult WTB attracted to volatiles emitted by black walnut and *Geosmithia morbida*?
2. Etiology: What roles do *Geosmithia morbida* and other walnut-associated insects and fungal pathogens play in affecting tree health within the native range of black walnut?



# Research Objectives

- Identify walnut and fungal volatiles and test their capacity to be used as a kairomone lure for the early detection of WTB
  - ✧ A kairomone lure could enhance the attraction of WTB to pheromone lure and increase efficacy of detection and monitoring efforts
- Determine the suite of insects colonizing bark and wood of TCD-symptomatic trees
- Identify putative fungal pathogens carried by representatives of the emerged insects



# Acknowledgements

## Collaborators

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## Students

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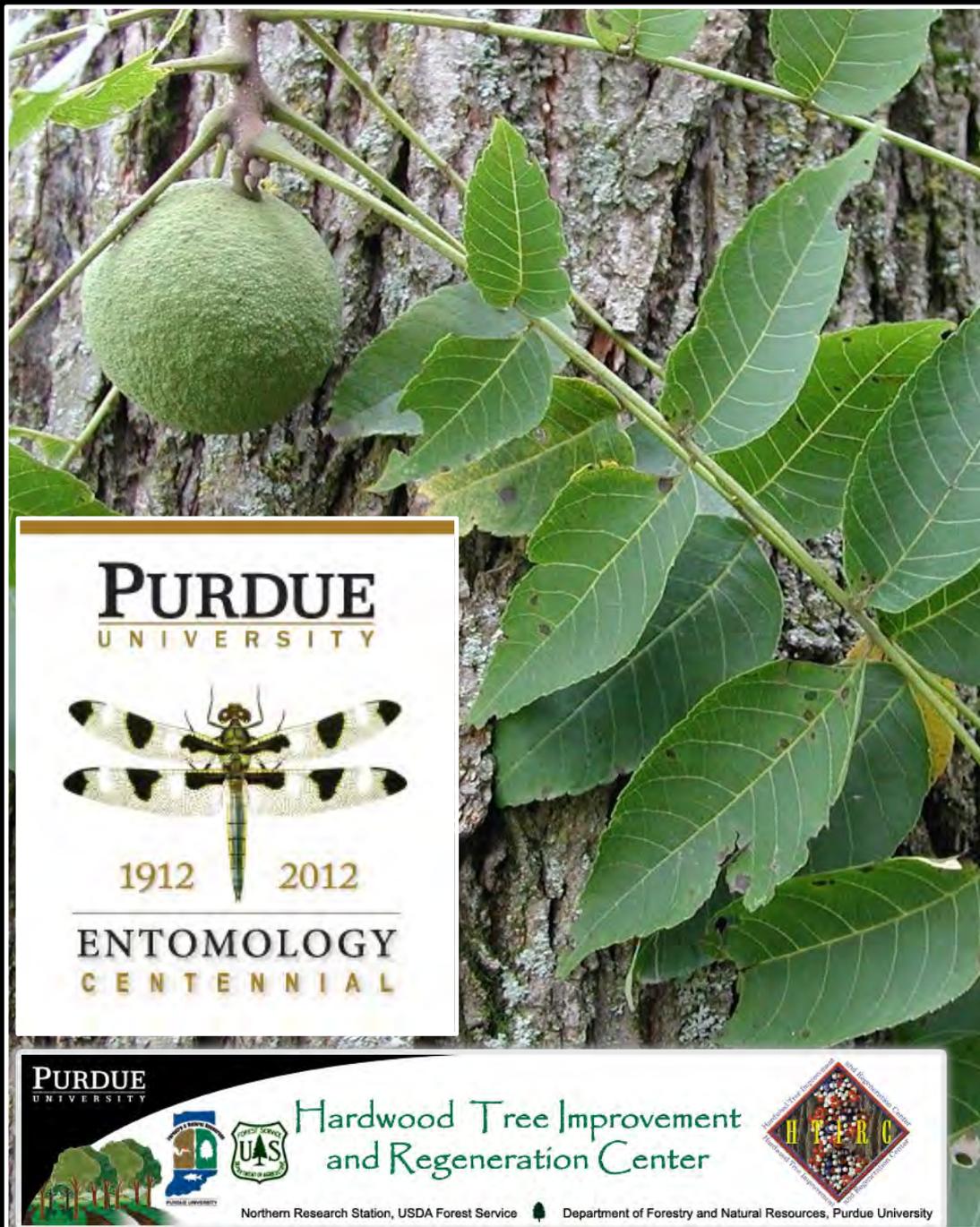
Matt Paschen

## Funding

USDA-NIFA

US Forest Service

Purdue University College of Agriculture  
Indiana DNR



# Thousand Cankers Disease:

Attraction of *Pityophthorus juglandis* to volatiles of black walnut and *Geosmithia morbida*

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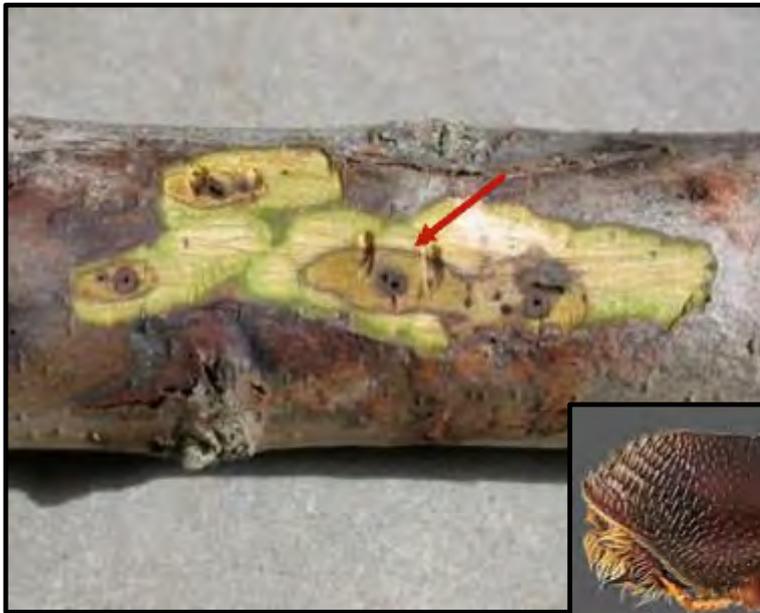
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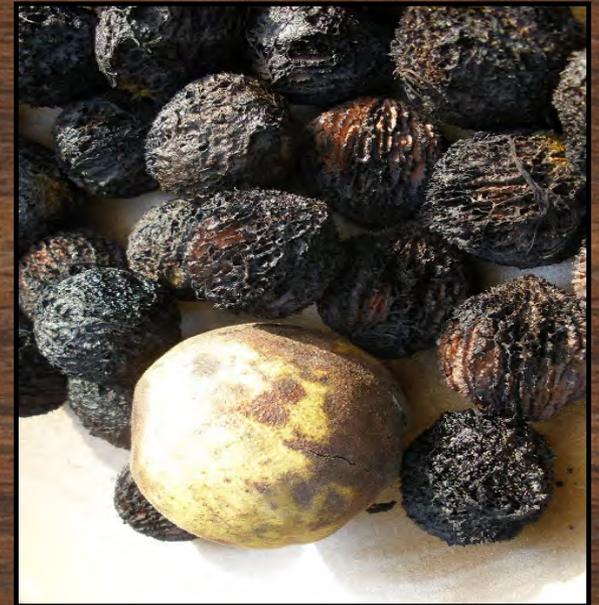
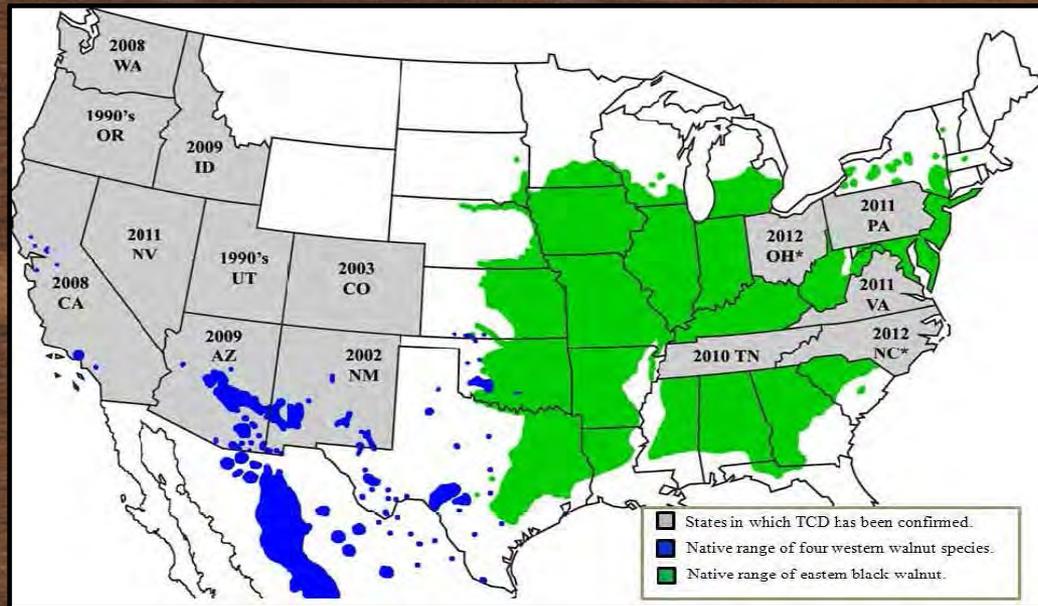
# Thousand Cankers Disease

- Pest complex that has caused the widespread death of walnut species throughout the Western United States
- The walnut twig beetle (*Pityophthorus juglandis*, WTB) vectors the fungal pathogen *Geosmithia morbida*



# Black Walnut in the Eastern U.S.

- 3.4 billion ft<sup>3</sup> of black walnut growing in the Eastern US has an estimated value of over a half trillion dollars
- Black walnut is valued for timber, veneer, nuts, nursery stock production, and ecosystem services



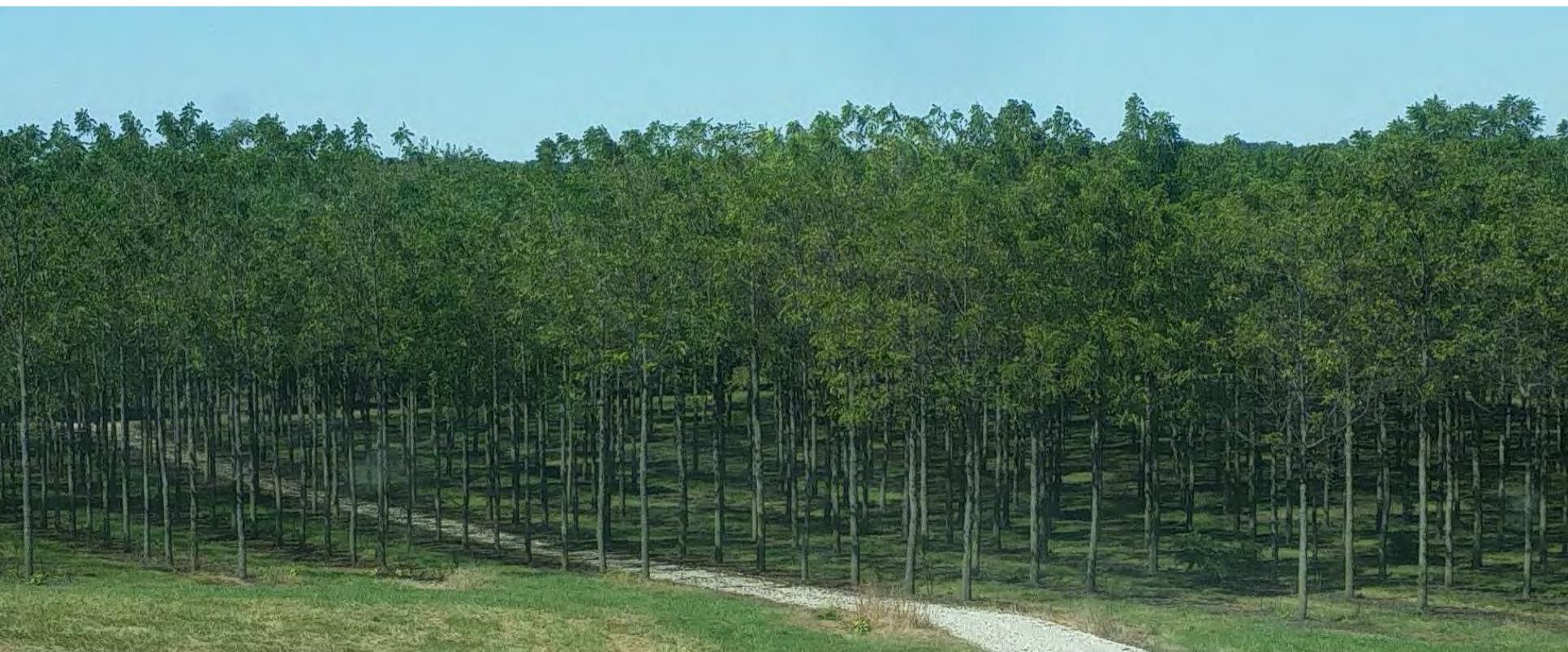
# Host Colonization

- Male WTB locate suitable hosts by orienting to host odors and releasing volatile aggregation pheromones to coordinate mass attack and mating
- Pheromone lure bioactivity is limited
- Plant- and fungal-derived kairomones that may mediate host location by WTB remain poorly understood



# Objective

To test the hypothesis that WTB are attracted to volatile organic compounds emitted by black walnut and *Geosmithia morbida*



# Volatile Collection

- Collected head-space volatiles of intact and girdled branches from two known genotypes of black walnut growing at Martell Forest (Tippecanoe Co., IN)
- Branches were girdled on May 24, 2012
- Volatiles were collected every two weeks from two ramets of each genotype from June 13-Sept. 6, 2012

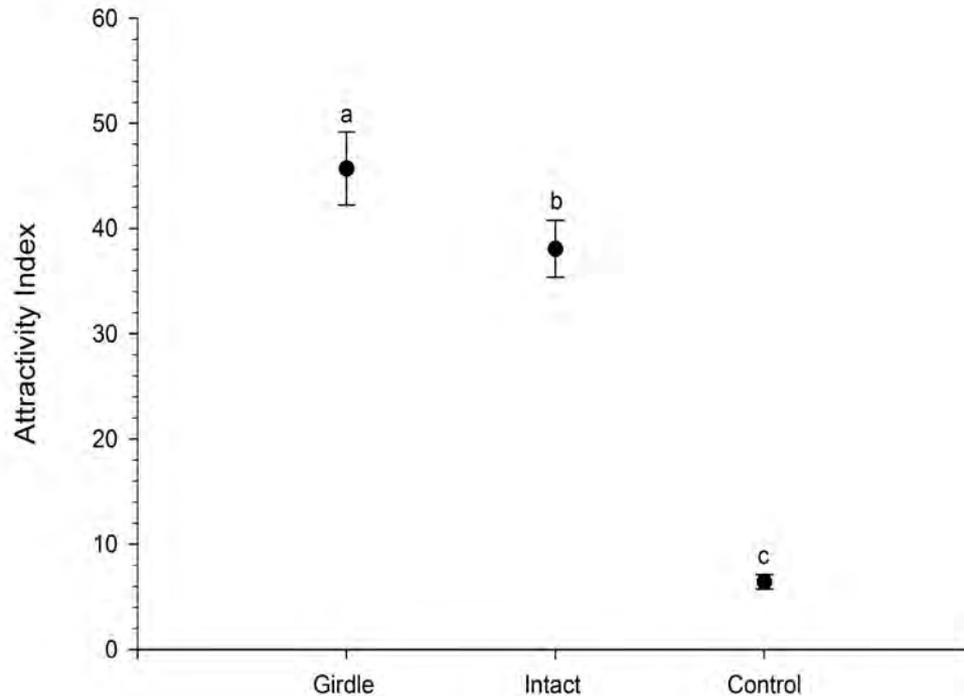


# Olfactometry Bioassay

- Measured walking response of adult WTB (5/trial) in a straight-tube glass olfactometer divided into five equal sectors to odor sources
- Location of each beetle within the olfactometer is recorded after 30 minutes
- This index has been used to assess the colonization behavior of *Phloeotribus scarabaeoides* (Peña et al. 1992)



- Branches were girdled on May 24, 2012
- Volatiles were collected every two weeks from two ramets for each of the three genotypes from June 13 to September 6, 2012



Attraction of adult WTB to volatiles of girdled and intact branches of black walnut and a blank control (ANOVA  $F_{2,111} = 75.75$ ,  $P < 0.001$ ). Bars marked with the same letter are not significantly different (Duncan's test,  $P < 0.05$ ).



# Attraction of WTB to synthetic walnut volatiles

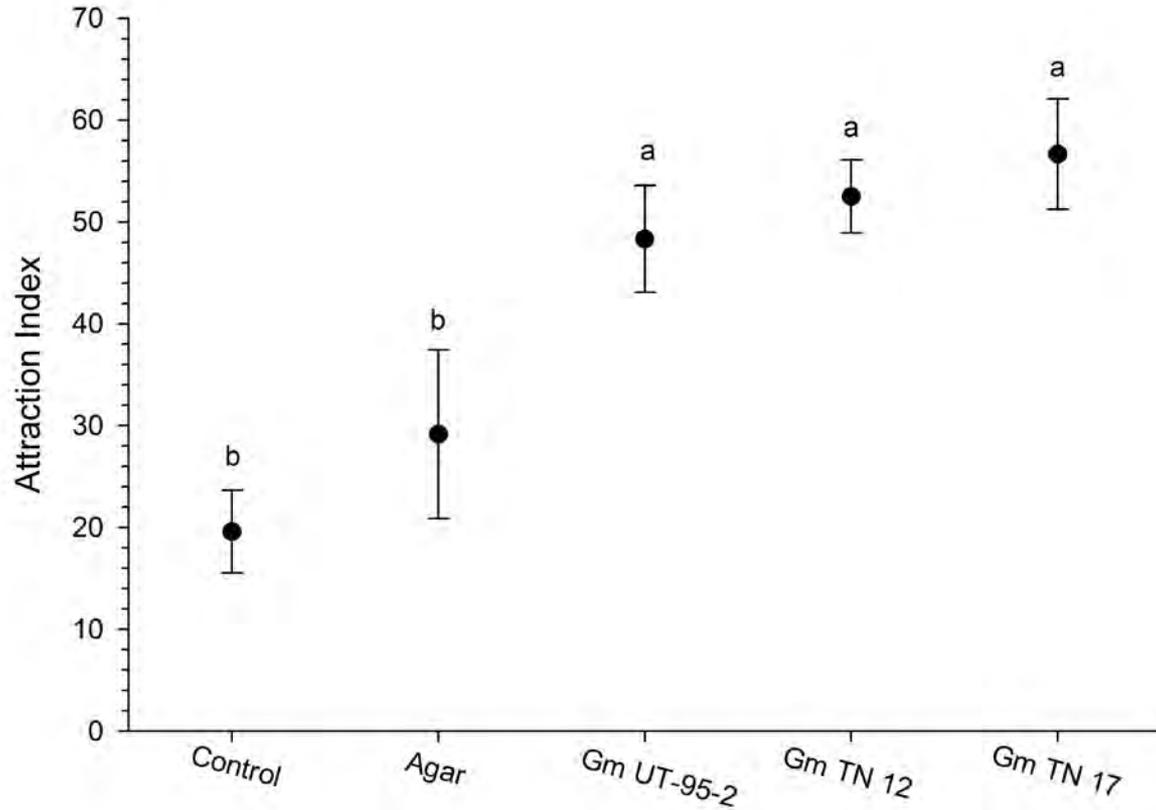


# Attraction of WTB to volatiles

- ☑ Walnut trees
- ☑ Girdled branches
- ☑ synthetic walnut volatiles
- ? Fungal symbiont

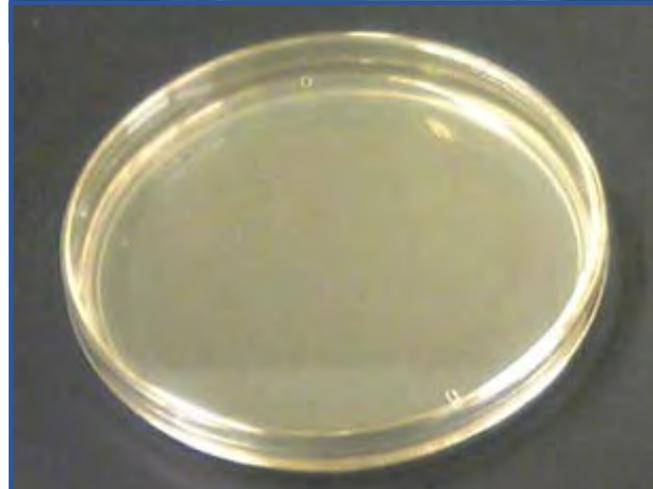


# Olfactometry bioassay *G. morbida* volatiles



ANOVA  $F_{4, 31} = 10.41, p < 0.001$

Bars marked with different letters are significantly different (Duncan's test,  $p < 0.05$ )



# Conclusions

Adult WTB are attracted to synthetic walnut and *Geosmithia morbida* volatiles

# Future Directions

Test the bioactivity of these volatiles in the field and determine the extent to which they enhance the efficacy of a commercially available pheromone lure



# Thousand Cankers Disease:

Scolytine beetles and fungal pathogens associated with symptomatic eastern black walnut (*Juglans nigra*)

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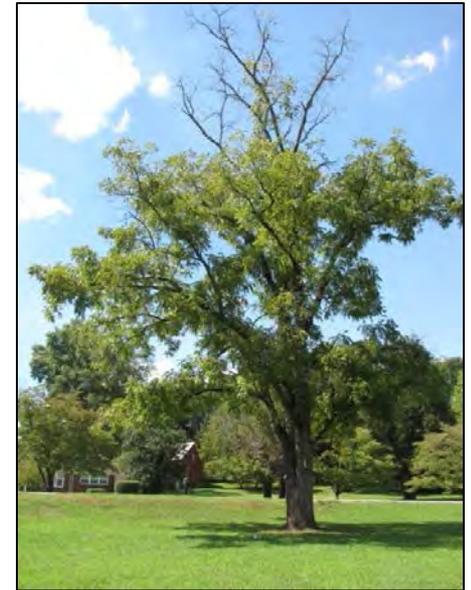
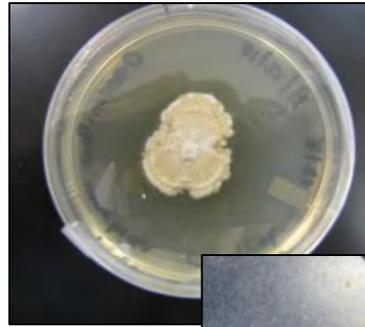
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# Thousand Cankers Disease (TCD)

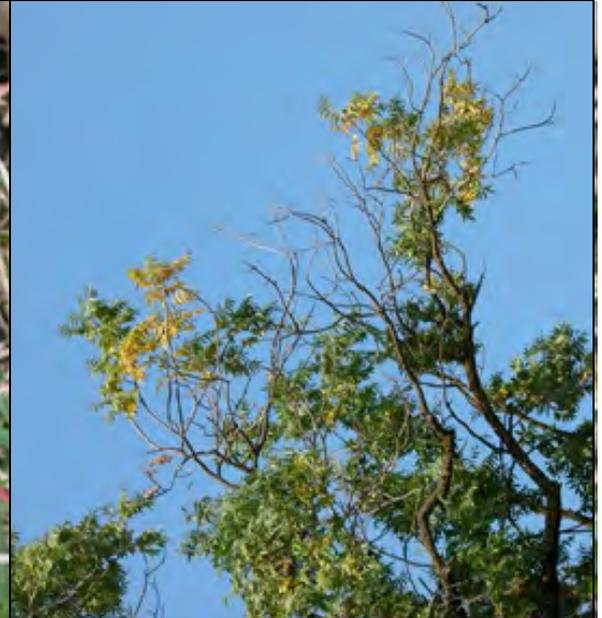


**WTB + Gm + Jn = TCD**

**TCD is the result of a complex interaction among:**

- A beetle, *Pityophthorus juglandis* (walnut twig beetle)
- A pathogen, *Geosmithia morbida*
- A susceptible host, e.g. *Juglans nigra* (black walnut)

# Thousand Cankers Disease (TCD)



# Thousand Cankers Disease (TCD) of Walnut Disease Distributions and Quarantines as of April 15, 2015



 Exterior Quarantine

 County Quarantine

 Black Walnut Native Range

 Thousand Cankers Disease  
(includes presence of diseased trees,  
walnut twig beetle and/or fungal pathogen)

## Black Walnut

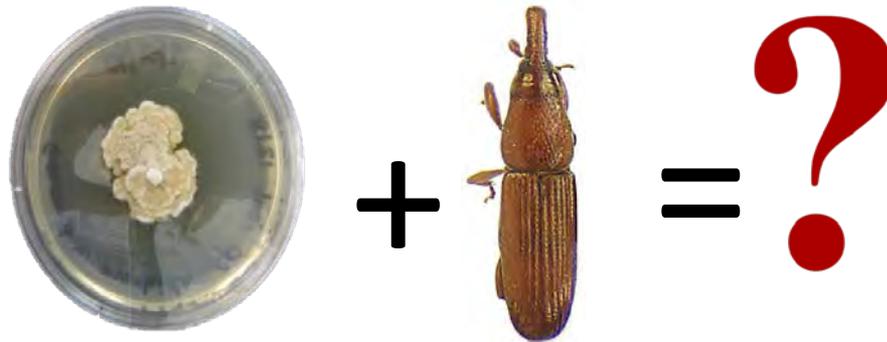
- Approximately 3.4 billion cubic feet
- Estimated value of over \$569 billion

# 2011: *Geosmithia morbida* recovered from a weevil, *Stenomimus pallidus*

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- *Stenomimus pallidus* recovered from walnut trees on 12 sites in Indiana
- *G. morbida* was detected on 3 (n=21) individuals reared from 2 trees growing at Yellowwood State Forest, Brown Co., Indiana

What are the implications of the discovery of *G. morbida* on *S. pallidus*?



# Is the current TCD model complete?

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- What beetles other than walnut twig beetle are capable of transmitting *G. morbida*?
  - Interaction & spatial overlap
- What fungi in addition to *G. morbida* play a role in TCD?



Weevil (Cossoninae) utilizing bark beetle galleries

# Other insects and fungi associated with black walnut

## INSECTS

*Xylosandrus crassiusculus*  
(Curculionidae: Scolytinae)



*Xyleborinus saxeseni*  
(Curculionidae: Scolytinae)



*Himatium errans*  
(Curculionidae: Cossoninae)



## FUNGAL PATHOGENS

*Botryosphaeria dothidea*  
(Botryosphaeriales:  
Botryosphaeriaceae)



*Diplodia seriata*  
(Botryosphaeriales:  
Botryosphaeriaceae)



*Fusarium solani*  
(Hypocreales: Nectriaceae)



# Project Objectives

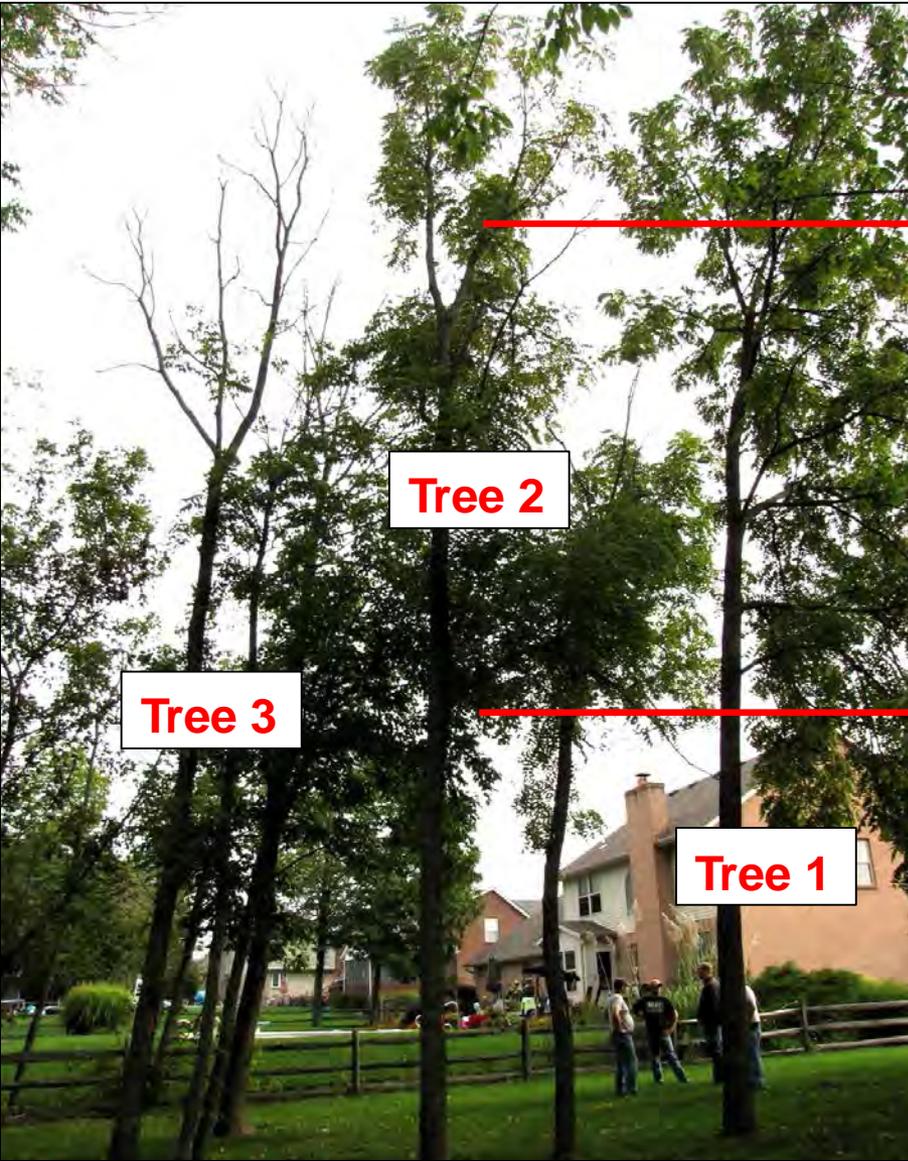
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- Identify insect species associated with TCD symptomatic black walnuts that may be capable of carrying *G. morbida*
- Identify phytopathogenic fungi other than *G. morbida* that could exacerbate branch dieback and tree death in TCD-affected trees



**Methods:** Four TCD-symptomatic *J. nigra* in Hamilton, OH, were felled in early September 2014 for insect and fungal analysis

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Four 30 cm segments from each of four branches per tree

Sixteen 30 cm sections from main stem of each tree

## Methods: Fungal isolations from *J. nigra* phloem tissue

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Isolated fungi from branches and main stems of TCD-symptomatic trees in Hamilton, Ohio (2014)



Main stem section exhibiting typical *G. morbida* canker coalescence and *P. juglandis* galleries



Branch section exhibiting typical *G. morbida* canker coalescence and *P. juglandis* galleries

**Methods:** Insects were obtained from tree samples, tentatively identified, and stored for further processing

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**Identified all emerged:**

- Bark beetles
- Ambrosia beetles
- Weevils

Other insects such as  
buprestids and cerambycids  
were not assayed for

*Geosmithia*

**Results:** Three reported pathogens of *J. nigra* also were commonly isolated from cankers on OH branches and stems

Frequency of fungal species isolated							
Sample Source	Canker Description	Total Number of Cankers	<i>G.m.</i>	<i>F.s.</i>	<i>B.d.</i>	<i>G.m.</i> + <i>F.s./B.d.</i>	<i>F.s.</i> + <i>B.d.</i>
Branches	Typical <i>Geosmithia</i>	96	38	8	9	28	2
Stems	Typical <i>Geosmithia</i>	102	14	34	8	13	6



# Results: Eight insect species (n = 152) were reared from Ohio TCD symptomatic tree stem samples in 2014

Taxonomic group	Species	No. specimens
Ambrosia Beetles	<i>Xyleborus affinis</i>	1
	<i>Xylosandrus crassiusculus</i>	26
	<i>Xyleborinus saxeseni</i>	73
	<i>Monarthrum mali</i>	1
Ironclad Bark Beetles	<i>Synchyta fuliginosa</i>	1
Bark Beetles	<i>Pityophthorus juglandis</i>	1
Weevils	<i>Himatium errans</i>	2
	<i>Stenomimus pallidus</i>	47

**Three species (*X. crassiusculus*, *X. saxeseni*, and *S. pallidus*) accounted for 95% of total.**

**Results:** *G. morbida* isolated from specimens of three different beetle species emerged from TCD symptomatic *J. nigra*

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*Xylosandrus crassiusculus*

17 specimens of *X. crassiusculus* (n=26)



*Xyleborinus saxeseni*

15 specimens of *X. saxeseni* (n=68)



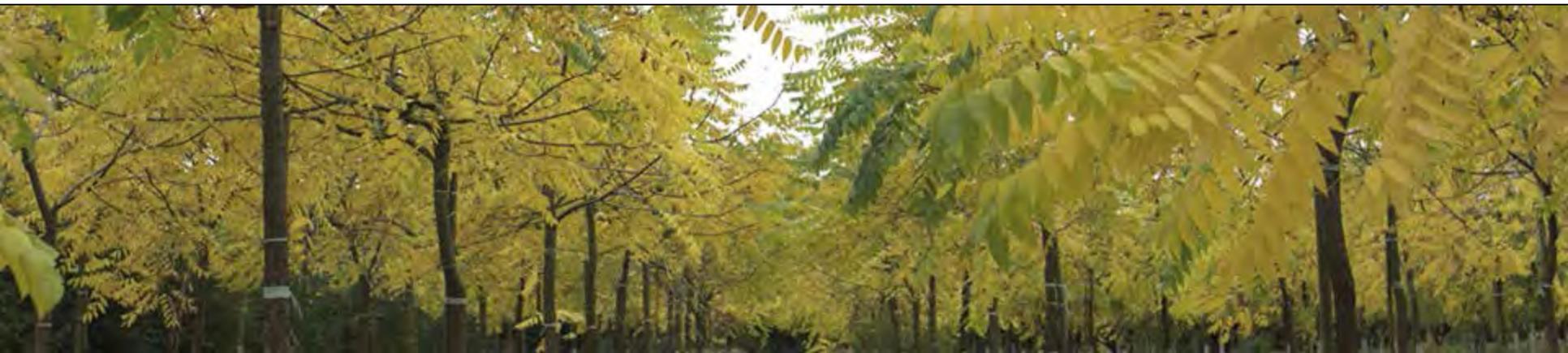
*Stenomimus pallidus*

13 specimens of *S. pallidus* (n=47)

# Summary

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- ✧ There appears to be a suite of insect species colonizing *J. nigra* as TCD develops
  - Discovered *G. morbida* on two species of ambrosia beetles, both are exotic species in the U.S. and aggressive colonizers
  - *G. morbida*-laden ambrosia beetles may exacerbate TCD symptom progression in areas with the disease
- ✧ Other phytopathogenic fungi may contribute to canker development and branch death in TCD affected trees



# Future Directions

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- Determine to what extent other phytopathogenic fungi contribute to canker development and branch death in TCD affected trees
- Establish an invasion sequence of insect colonization of TCD trees
- Determine dispersal and phoresy rates from TCD sites in other beetles established as *G. morbida* vectors

