

Use of Trap Trees to Detect Invasive Insect Pests and Pathogens of Black Walnut



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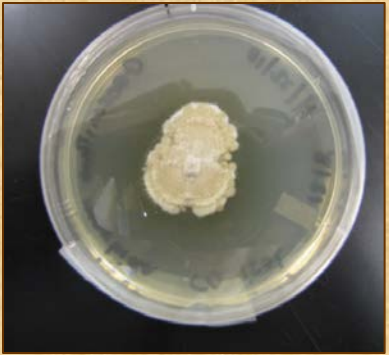
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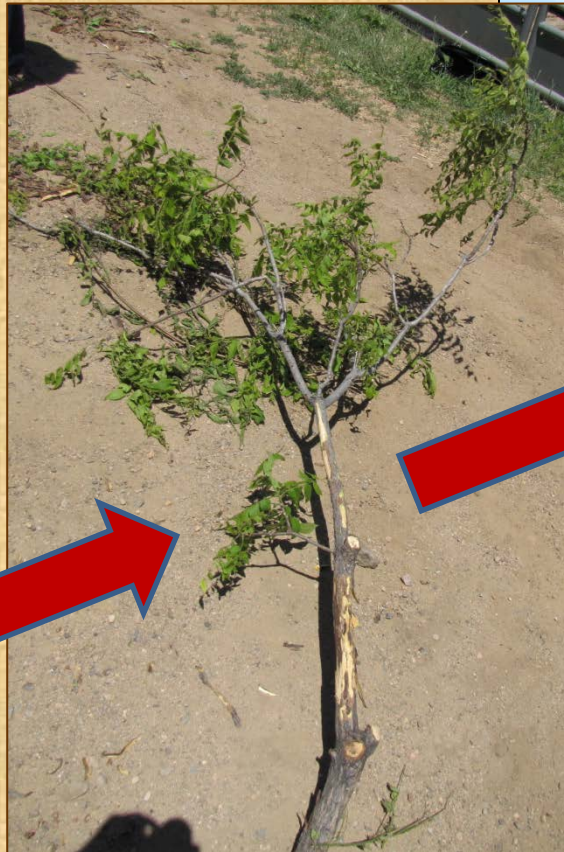
The health of black walnut in the eastern US is threatened by the insect-fungus complex known as Thousand Canker Disease (TCD).



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However, other insects and fungi colonize black walnut branches and stems, but we lack base-line information on many of these.

Insects

***Xylosandrus
crassiusculus***

(Curculionidae:Scolytinae)



Pityophthorus lautus

(Curculionidae:Scolytinae)



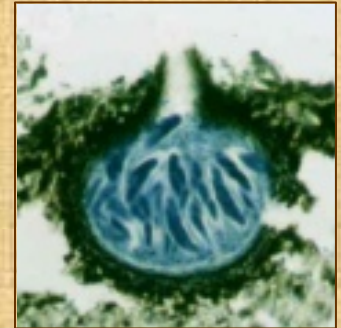
Himatium errans

(Curculionidae:Cossoninae)



Fungi

***Botryosphaeria
dothidea* – *B.
ribis* complex**



***Neonectria
galligena***



***Fusarium
lateritium*, *F.
sporotrichioides*,
and *F. solani***



Interactions or spatial overlap of these other pests/pathogens with WTB and *G. morbida* may also be important in development of TCD.

- Potential exists for other walnut insects to transmit *G. morbida*,
- Other fungi may cause cankers on TCD trees,
- Damage from different pests/pathogens may occur on the same tree and contribute to tree decline.



Weevils (Cossoninae) utilizing bark beetle galleries

Girdled black walnut branches or stems can be used as trap trees to detect WTB and other insect pests colonizing stressed walnut in the Midwest.

Girdled branch trap trees attracted WTBs in Knoxville, TN, study.

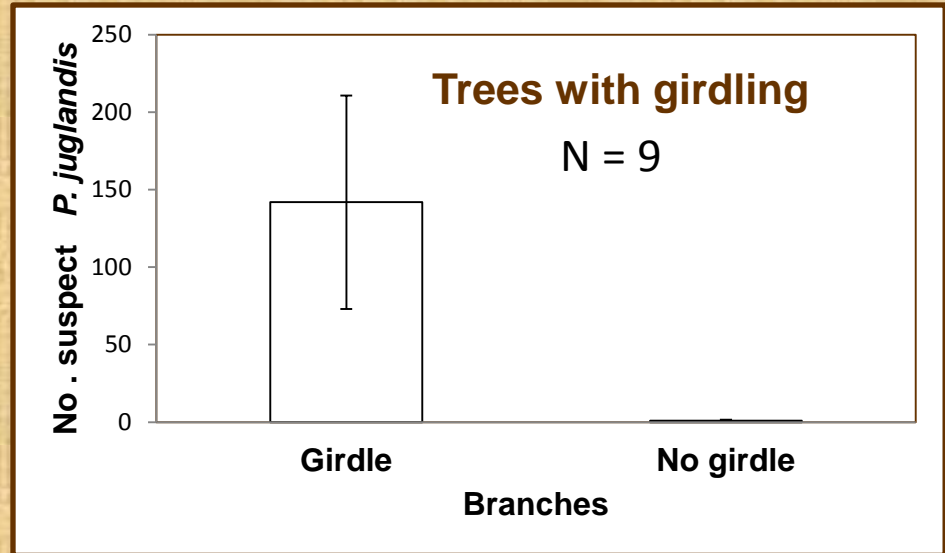


Photo & Data Source: Alicia Bray, TSU

Furthermore, fungi carried by the insects emerged from trap tree samples can be determined.

Frequencies of *Geosmithia morbida* isolation from WTBs emerged from girdled branches in Tennessee, 2011.

Source of WTBs	No. Beetles Emerged	No. Beetles Assayed	Percentage with <i>G. morbida</i>
Girdled tree 3	22	15	67
Girdled tree 5	29	22	64
Girdled tree 6	9	7	29
Girdled tree 7	18	13	8
Girdled tree 4 control	4	0	N/A

* Based on serial dilution plating of macerated beetle suspension on ¼ ++ PDA

Data Source: J. Juzwik

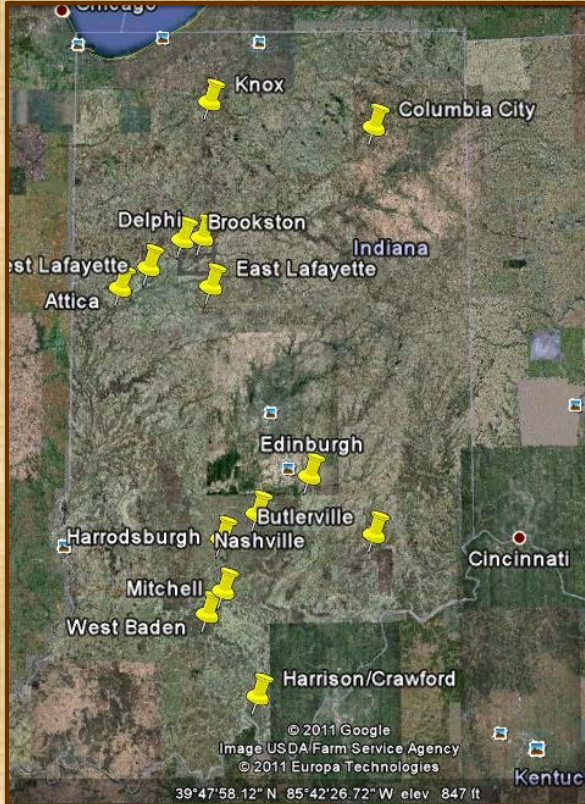
Today's presentation

Field study conducted in 2011

- To determine the range of insects colonizing bark and wood of girdled black walnut in Indiana and Missouri, and
- Identify putative fungal pathogens carried by representatives of the emerged insects.



Four trees were girdled on multiple sites in two states and samples collected 3 months later.



Study sites in Indiana



Girdling treatment on a Missouri site



Cutting branch sample

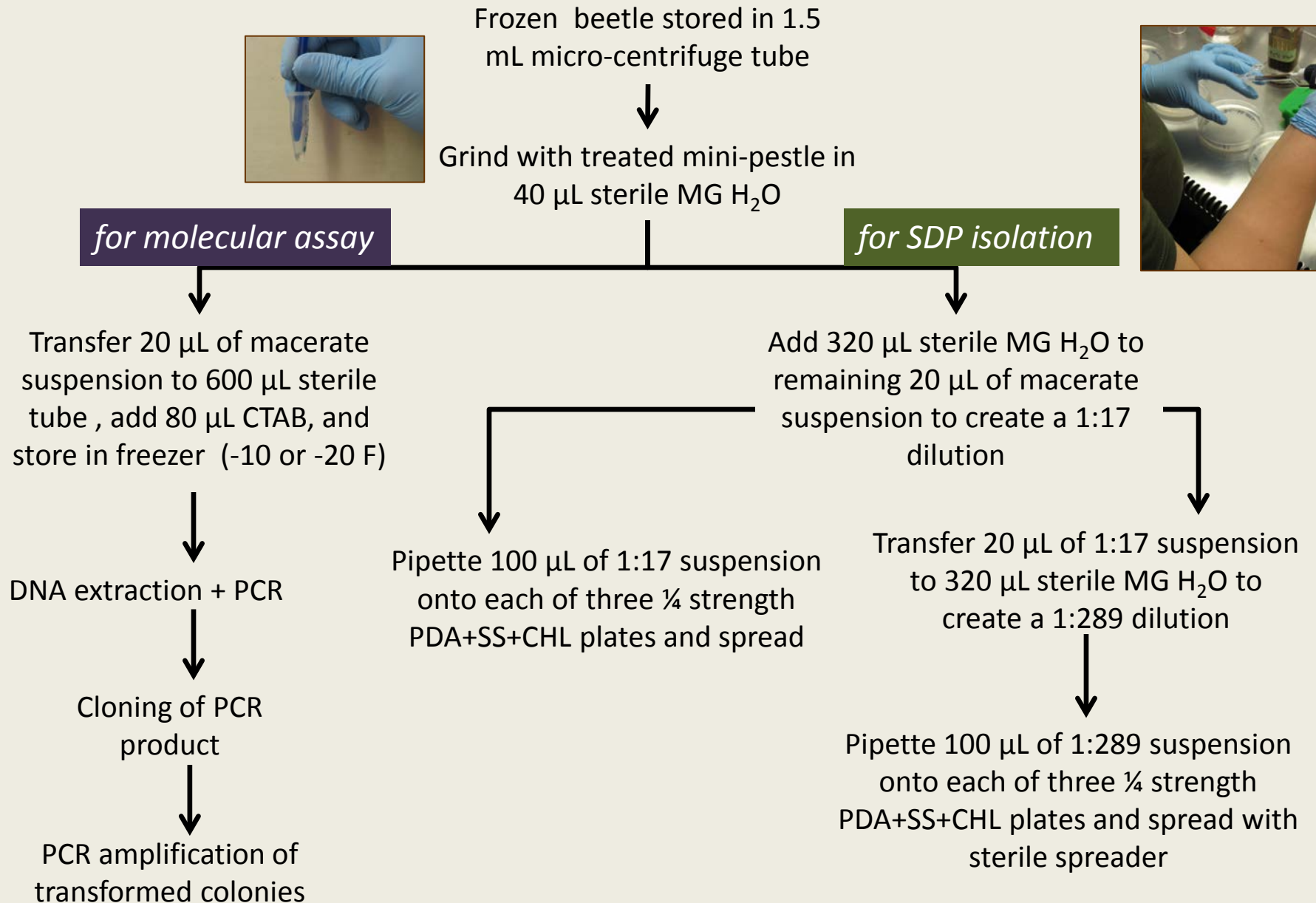
Insects were obtained from tree samples, tentatively identified and stored for further processing.



Buckets used to emerge beetles from branch and stem samples.

- Groups of insects forwarded for species identification:
 - Bark beetles
 - Ambrosia beetles
 - Weevils
- Other insects found included buprestids and cerambycids

Two assay methods are used to detect fungi present on the same beetles.



Fungi detected by either method are identified based on DNA sequencing.

Molecular Assay

DNA sequencing of PCR product of clones



DNA sequences aligned in Sequencher®



BLAST search (NCBI GenBank) to identify matches

SDP Assay

Pure cultures obtained



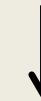
DNA extraction



PCR amplification



DNA sequencing



BLAST search (NCBI GenBank) to identify matches

Although no WTBs were detected 14 ambrosia beetle species, 5 bark beetle species, and 4 weevil species were obtained .

Taxonomic Group	No. Species - Indiana -	No. Species - Missouri -	No. Collected - Indiana -	No. Collected - Missouri -
Ambrosia beetles (Scolytinae)	14	7	9,024	7,185
Bark beetles (Scolytinae)	4	2	104	5
Weevils (Cossoninae)	4	6	455	114

Exotic beetle species obtained

- Seven exotic beetle taxa accounted for 94% of the 16,318 adults collected.
- Black walnut is a host for at least 8 of the 19 beetle species collected; other species found attack closely related genera.

Beetle Group	Species	Black walnut host?
Ambrosia	<i>Ambrosiodmus rubricollis</i>	Y
	<i>Euwallacea validus</i>	Y
	<i>Xyleborinus saxeseni</i>	Y
	<i>Xyleborus californicus</i>	?
	<i>Xylosandrus crassiusculus</i>	Y
	<i>Xylosandrus germanus</i>	Y
Bark beetle	<i>Dryoxylon onoharensium</i>	N

Number of each ambrosia beetle species obtained

Species	Indiana	Missouri
<i>Ambrosiodmus rubricollis</i>	199	2
<i>Ambrosiophilus atratus</i>	47	0
<i>Anisandrus sayi</i>	1	0
<i>Euwallacea validus</i>	63	0
<i>Monarthrum fasciatum</i>	2	0
<i>Monarthrum mali</i>	142	40
<i>Euplatypus compositus</i>	15	0
<i>Xyleborus ferrugineus</i>	22	15
<i>Xyleborinus saxeseni</i>	3,282	6,055
<i>Xyleborus affinis</i>	277	431
<i>Xyleborus californicus</i>	1	0
<i>Xyleborus celsus</i>	14	0
<i>Xylosandrus crassiusculus</i>	4,168	240
<i>Xylosandrus germanus</i>	791	402

Four species accounted for 94% (IN) and 99% (MO) of all ambrosia beetle species obtained.

- *Xyleborinus saxeseni* (58%)
 - Wide host range
 - Economic damage to fruit trees



- *Xylosandrus germanus* (7%)
 - Key pest of deciduous nursery stock
 - Toothpick frass spikes



- *Xylosandrus crassiusculus* (27%)
 - Serious pest of woody ornamentals, fruit and nut trees
 - Toothpick frass spikes



- *Xyleborus affinis* (4%)
 - Native species
 - Minor pest



Bark beetle species obtained

State	Species	Frequency (no.)
Indiana (n = 104)	<i>Dryoxylon onoharensium</i>	61
	<i>Pityophthorus lautus</i>	19
	<i>Hylocurus rudis</i>	10
	<i>Hypothenemus eruditus</i>	14
Missouri (n = 5)	<i>Hypothenemus eruditus</i>	2
	<i>Hypothenemus interstitialis</i>	3



Dryoxylon onoharensium



Pityophthorus lautus

Weevil species obtained

Species	No. from IN	No. from MO
<i>Himatium errans</i>	10	91
<i>Caulophilus dubius</i>	0	5
<i>Tychius picirostris</i>	0	1
<i>Plocamus hispidulus</i>	1	2
<i>Dryophthorus americanus</i>	0	6
<i>Stenomimus pallidus</i>	435	9
<i>Sitophilus seamais</i>	9	0



Himatium errans

(Cossoninae: Rhyncolini)



Stenomimus pallidus

(Cossoninae: Dryotribini))

Source of *S. pallidus*:
www.bugguide.net

Fungi detected on three SDP-assayed insect species*

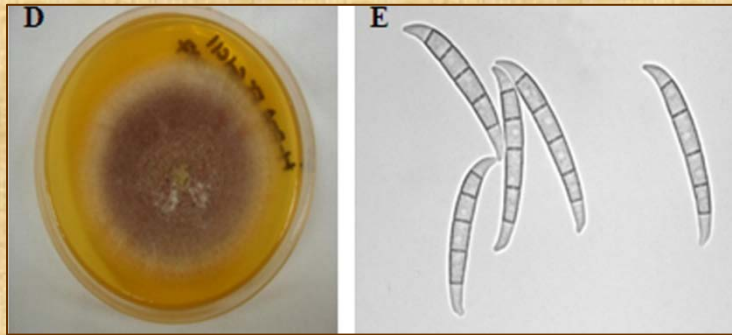
Fungal taxa	<i>Xyleborinus saxeseni</i> (%) n = 111	<i>Xylosandrus crassiusculus</i> (%) n = 74	<i>Stenomimus pallidus</i> (%) n = 51
<i>Aspergillus niger</i>	12	5	0
<i>Fusarium solani</i>	41	42	50
<i>Graphium spp.</i>	22	9	0
<i>Graphium penicilloides</i>	13	16	3
<i>Penicillium sumatrnse / meleagrinum</i>	40	32	0
<i>Penicillium kloeckeri</i>	10	12	13
<i>Scytalidium cuboideum</i>	2	1	16
yeasts	34	46	0

*Fungal taxon listed only if occurrence was > 10% on one or more assayed insect species.

Putative pathogenic and wood-staining fungi obtained

Stem canker pathogen

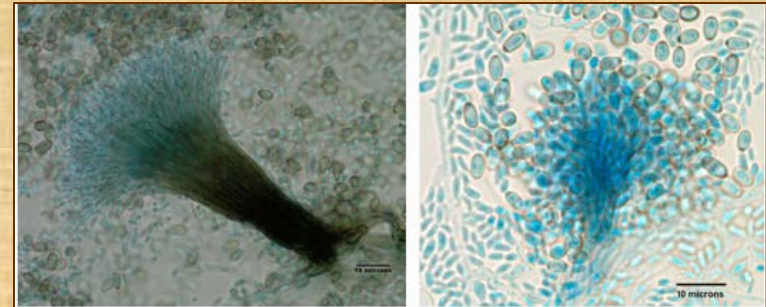
- *Fusarium solani*



Elongate
canker

Wood-staining fungi

- *Scytalidium cuboideum*
- *Graphium penicilloides*



Conclusions to date

Insect pests detected

- *Pityophthorus juglandis* – NOT detected
- Ambrosia beetle pest species were detected
 - *X. crassiusculus* – major
 - *X. germanus* – major
 - *X. saxeseni* – minor
 - *X. affinis* – minor
 - *E. compositus* - minor

Putative pathogens carried by insects

- *Geosmithia morbida* – NOT detected on assayed insects,
- Documented canker pathogen (*F. solani*) is common, and
- Two wood stain-causing fungi detected.

Potential interaction with TCD organisms

- Potential exists for any of the insect species colonizing bark or boring through bark to casually acquire *G. morbida* spores if attacking a TCD black walnut,
- and*
- Fusarium cankers would be expected on TCD-symptomatic black walnut colonized by any of the insects assayed in this study.

Acknowledgements



Healthy black walnut

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