



Invasive Ambrosia Beetle Conference
The Situation in California
August 12 - 14, 2012

Meeting sponsored by:

The Hofshi Foundation

University of California, Riverside

UC Center for Invasive Pest Research

The Huntington Botanical Gardens

The Los Angeles Arboretum



Invasive Ambrosia Beetle Conference
The Situation in California
August 12 - 14, 2012

Session 4
Biology of the Fungal Symbiont

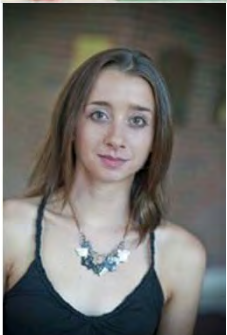
Yet another ambrosia beetle with a *Fusarium* symbiont: *Euwallacea validus* associated with *Verticillium* wilt of *Ailanthus*.



Matt Kasson,
now postdoc at VA Tech



Dylan Short,
Now postdoc at
UC Davis



Kristi Fenstermacher,
currently @PSU

Matthew T. Kasson

Dylan P.G. Short

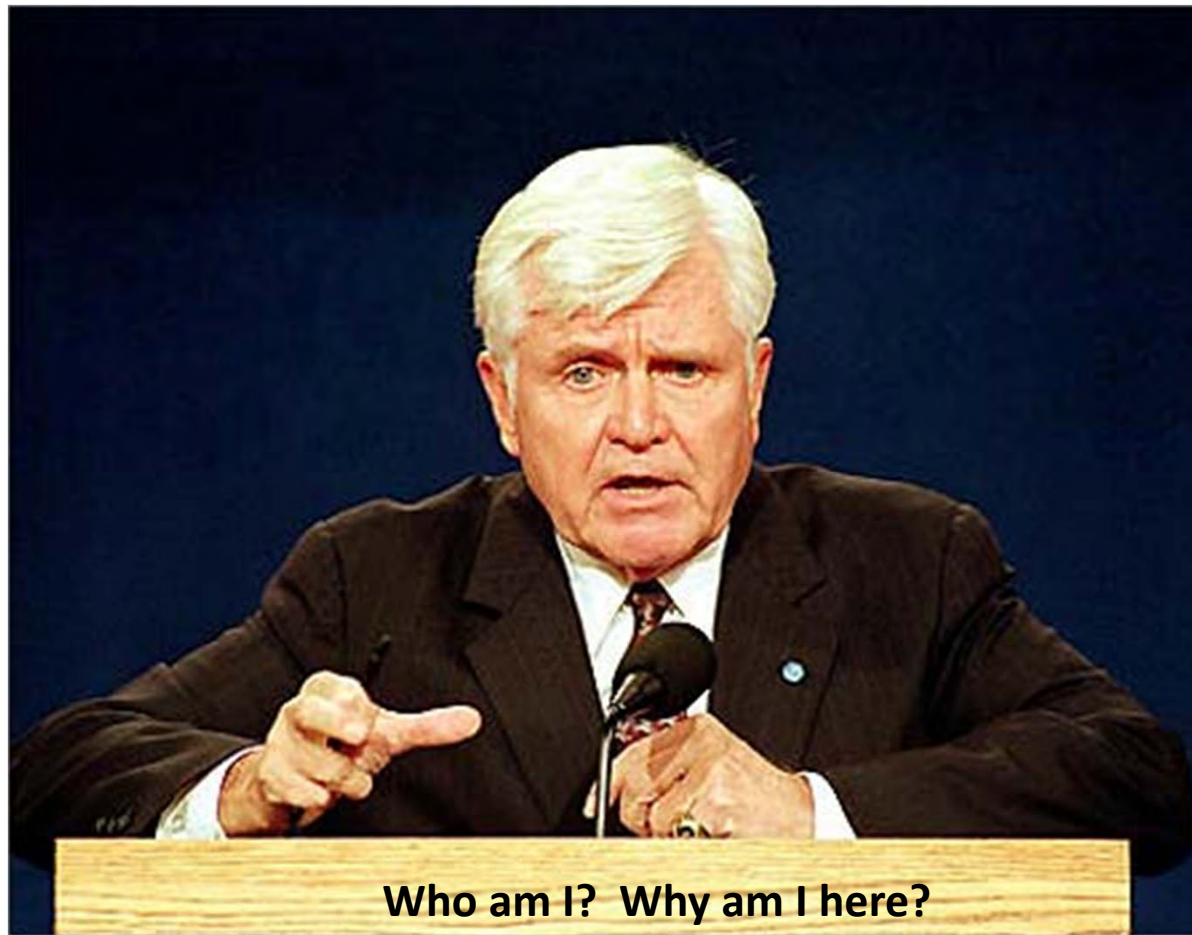
Kristi A. Fenstermacher

Donald D. Davis

Kerry O'Donnell (USDA-ARS, Peoria)

Stanley Freeman, Zvi Mendel (Volcani Inst.)

David M. Geiser



I am here to help:

***Fusarium* systematics**

Cultures

Basic *Fusarium* biology



2013 SYSTEMATIC INTERNSHIP PROGRAM



[Home](#)

[About the Program](#)

[Who May Apply](#)

[Stipend Information](#)

[Application Requirements](#)

[Internship Selection Process](#)

[Notification of Applicants](#)

[More Information](#)

[Training Topics and Mentors](#)

Are you a graduate student or early career professional interested in Systematics?

Then the 2013 Systematic Internship Program may be for you!

Where? Various U.S. locations.

When? Summer of 2013. Applications are due by 5pm EST on Monday, October 1, 2012.

Click [here](#) to learn more about the program.

"I'm very fortunate for this internship in bark beetle taxonomy. Close mentorship by Dr. Cognato has greatly increased my knowledge of this taxonomically challenging group of beetles. I hope for the opportunity to use this expertise in my future career."

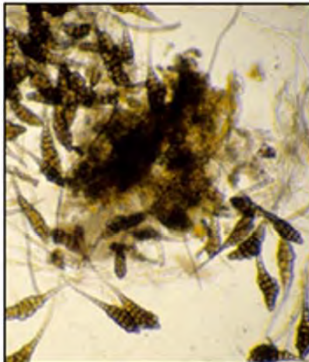
Nick Barc,
Michigan State University



Photo top left by Lyle Buss, University of Florida

Photo bottom left from Division of Plant Industry Archive, Florida Department of Agriculture and Consumer Services, www.bugwood.org, #5267090

<http://entnemdept.ifas.ufl.edu/hodges/SIP/index.html>



University of Florida | IFAS | myUFL

UNIVERSITY of FLORIDA ENTOMOLOGY and NEMATOLOGY DEPARTMENT

[Webmaster](#) | [E-mail](#) | [Location](#) | [f](#) [t](#)

PO Box 110620 | Bldg. 970 Natural Area Drive | Gainesville, FL 32611

PHONE: 352.392.1901 | FAX: 352.392.0190

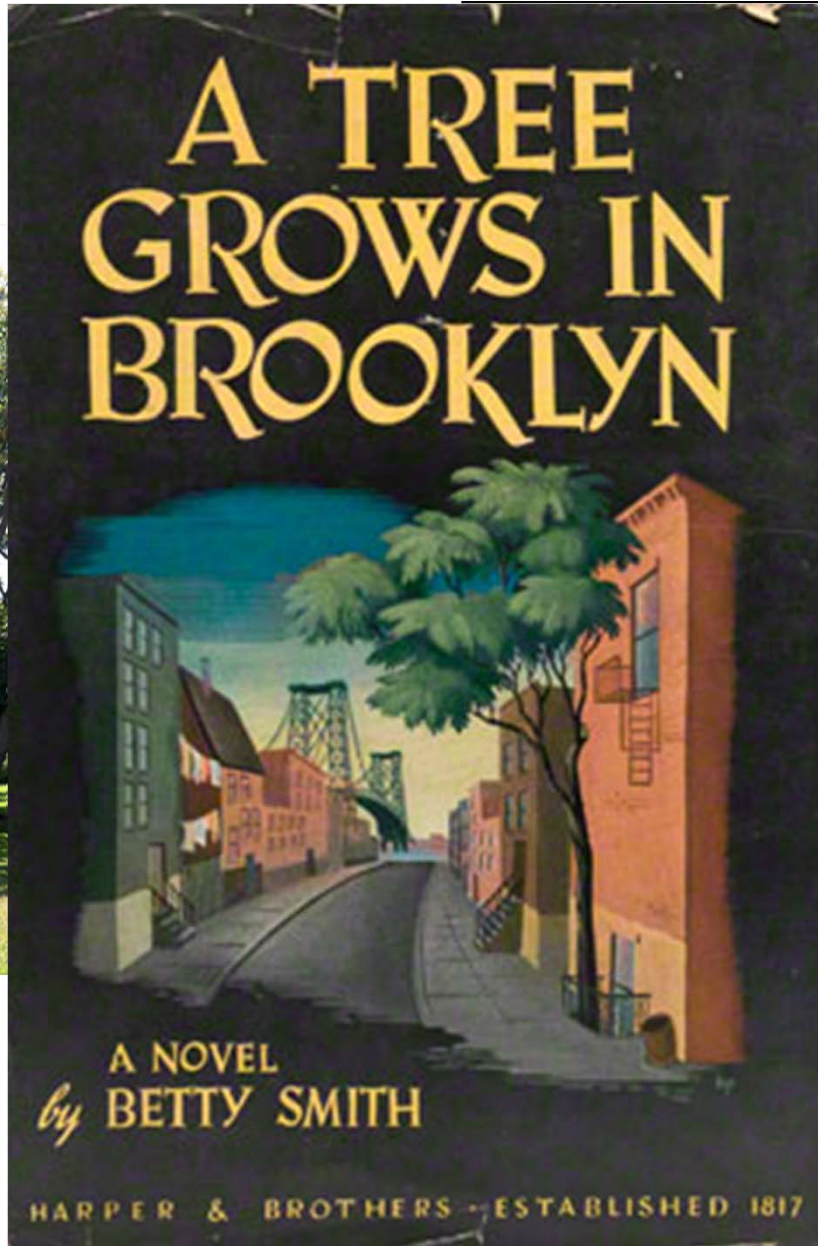
© 2010 UF/IFAS Entomology and Nematology Department. All rights reserved.

Ambrosia beetles, symbiotic fungi, and tree diseases

- Dieback of avocado and other hosts in Israel and California
 - Pest/vector: *Euwallacea fornicatus* (complex?)
 - Pathogen: Undescribed *Fusarium* sp. in *F. ambrosium* clade. No symbiotic *Raffaelea*?
- Redbay laurel wilt
 - Pest/vector: *Xyleborus glabratus*
 - Pathogen: *Raffaelea lauricola*. No symbiotic *Fusarium*?
 - Threat to avocado
- Verticillium wilt of *Ailanthus*
 - Insect (vector?): *Euwallacea validus*
 - Pathogen: *Verticillium nonalfalfae*
 - Symbionts: Another undescribed sp. *F. ambrosium* clade, undescribed *Raffaelea* sp.

If the beetle and its symbionts aren't killing the trees, then who cares?

- **To understand a situation gone wrong, it's useful to understand what's normal first**
- **One beetle and one fungus appear to**
- **The fungi and their hosts may hybridize**
- **Hosts may exchange fungal symbionts**
- **Fungal symbionts may interbreed**
 - **We have evidence for this**



“Tree of Heaven”

“Ghetto Palm”

Verticillium wilt on *Ailanthus*

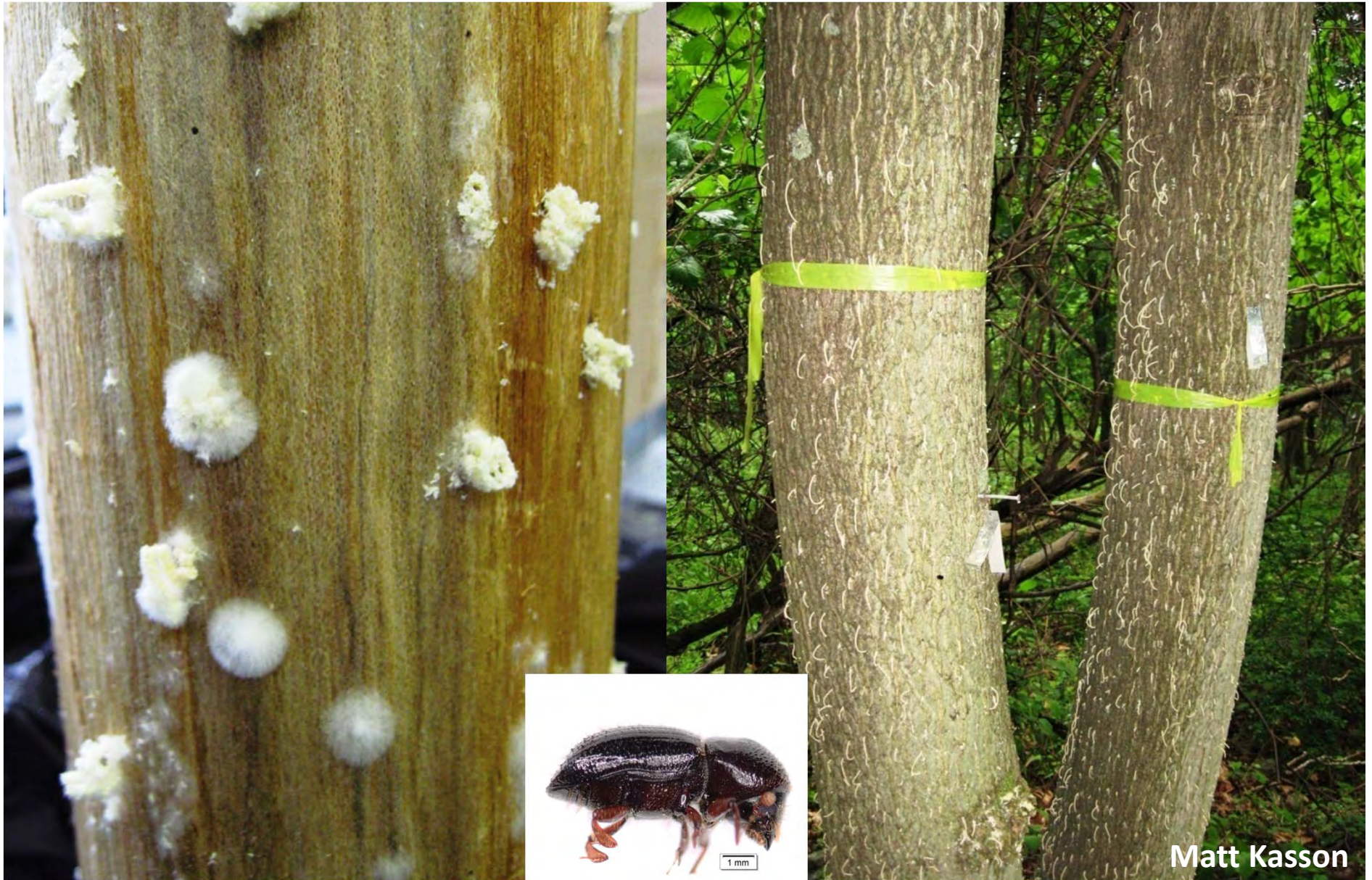
- Discovered in a stand of otherwise mixed hardwood/coniferous forest in S-Cen PA
- Since found in multiple foci in PA and surrounding states
- Pathogen first identified as *V. albo-atrum*, later refined as *V. nonalfalfae*
- Possible biological control agent?

An aerial photograph of a dense forest. The majority of the trees are green, but there is a prominent, roughly rectangular area in the center where the trees are dead, appearing as a brownish-grey canopy. This area is surrounded by healthy green trees, illustrating the spread of a pathogen.

E. Validus on *Ailanthus*

- Four sites in PA, averaging ~66 km apart
- Both naturally infected as well as 25 trees inoculated with *Verticillium* pathogen
- *E. validus* infestations began within weeks after wilt symptoms appeared
- All inoculated trees were heavily infested within one year, as well as additional diseased and dying trees

'Euwallacea validus' on dead and dying *Ailanthus*



Matt Kasson

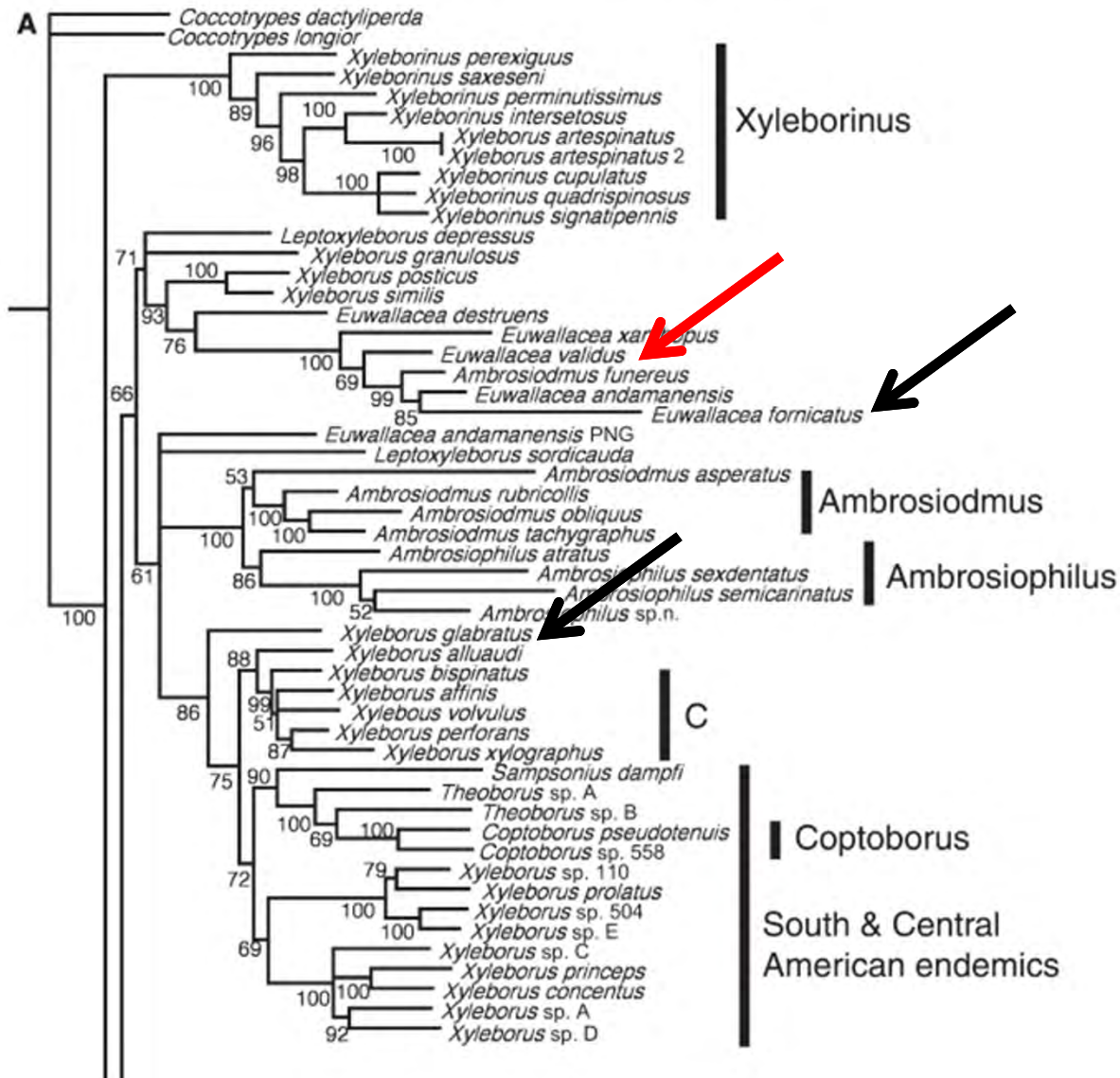
***Euwallacea validus* (Eichhoff)**

Rabaglia et al.

- **Introduced, originally from Japan/East Asia**
- **= *Xyleborus validus***
- **Wide host range in Asia**
 - Only dying or dead hosts
- **First detected on Long Island, NY in 1976**
- **Now widespread in mid-Atlantic**
 - Known on *Ailanthus*, black oak, yellow poplar

Phylogeny of haplo-diploid, fungus-growing ambrosia beetles (Curculionidae: Scolytinae: Xyleborini) inferred from molecular and morphological data

ANTHONY I. COGNATO, JIRI HULCR, STEPHANIE A. DOLE & BJARTE H. JORDAL



Mycangia

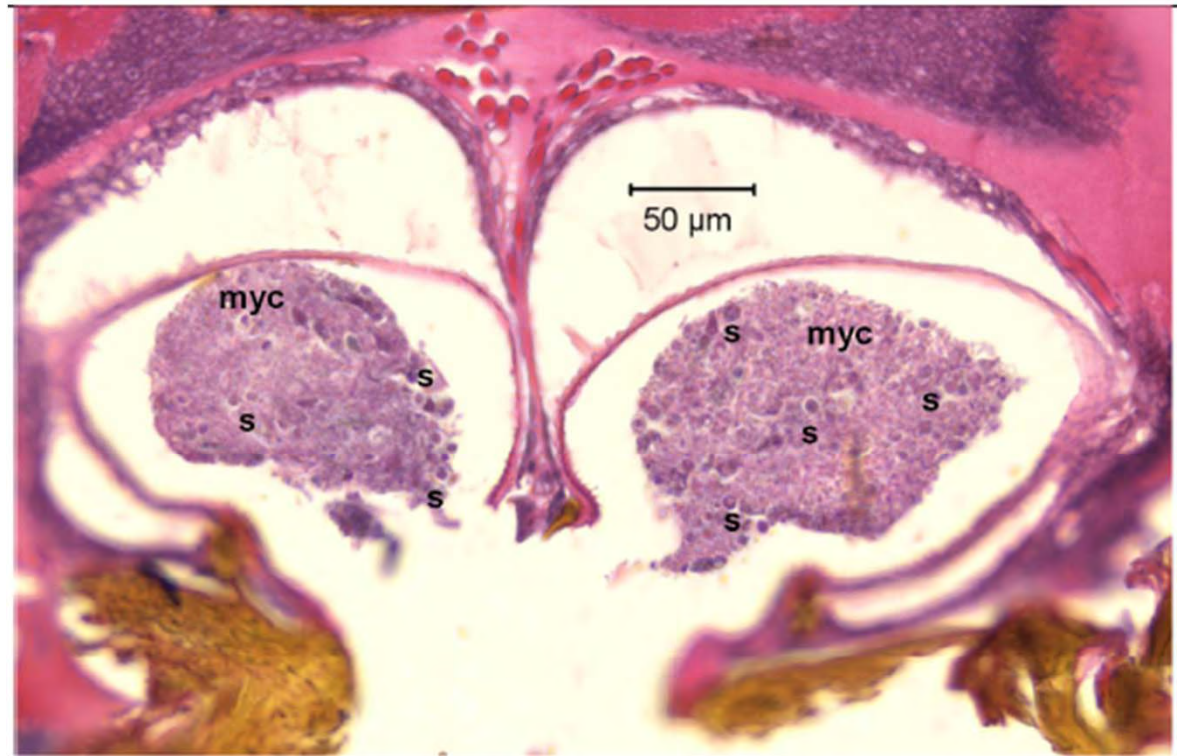
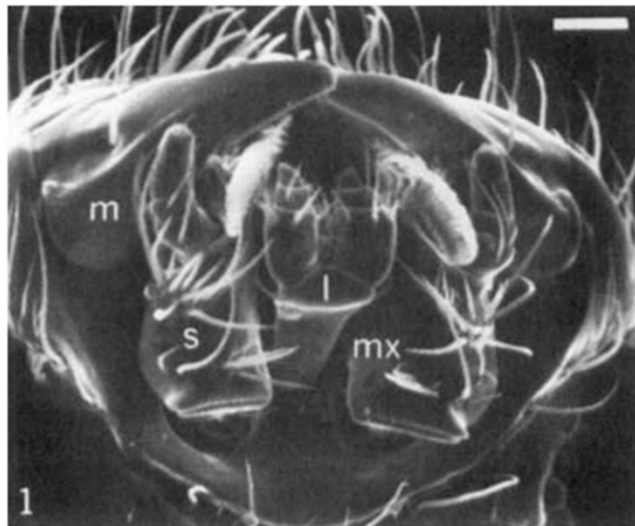


Figure 1. Transverse section through the head of *Erwallacea validus* showing paired mandibular mycangia (myc) containing fungal spores (s).

Fungi associated with head

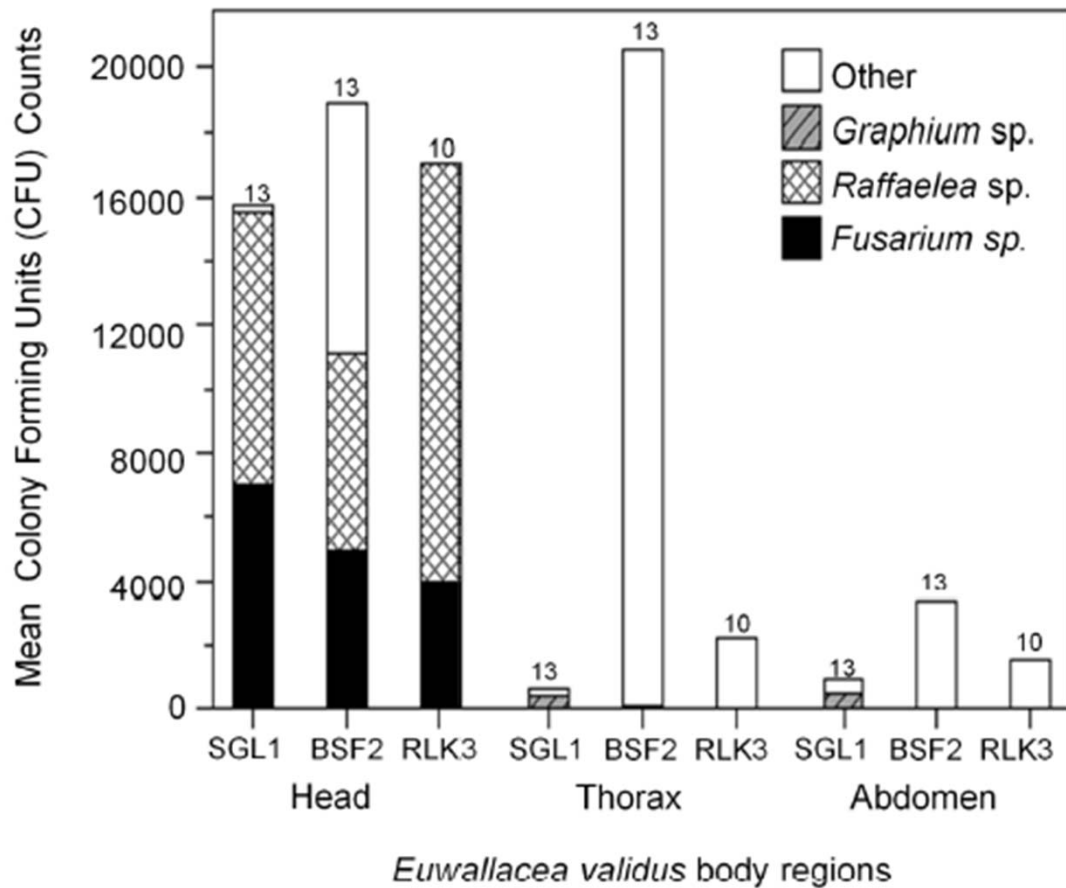
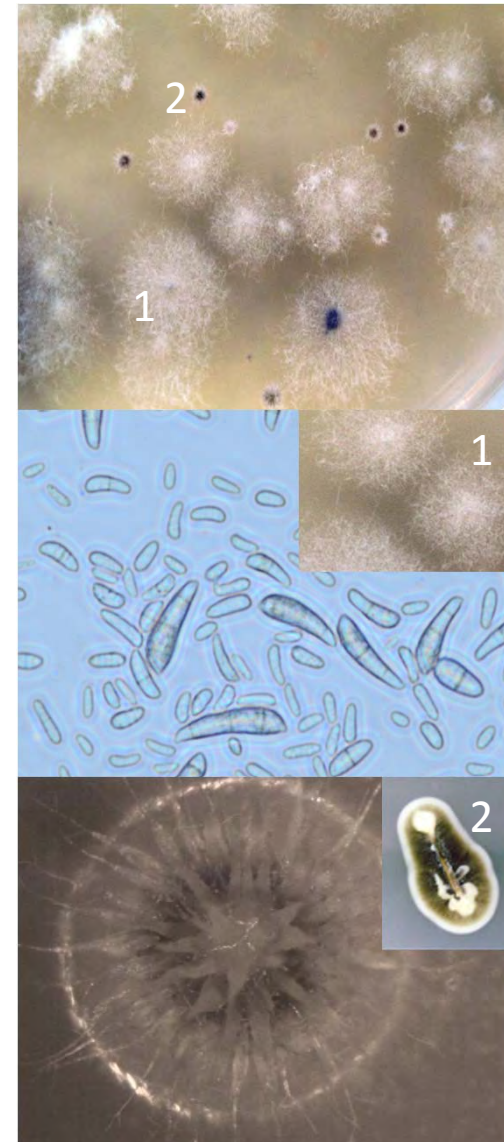


Figure 2. Mean CFU counts by body region and geographic location for the Asian ambrosia beetle, *Euwallacea validus*. Numbers at top of bars indicate sample size.

Matt Kasson



Putative new species

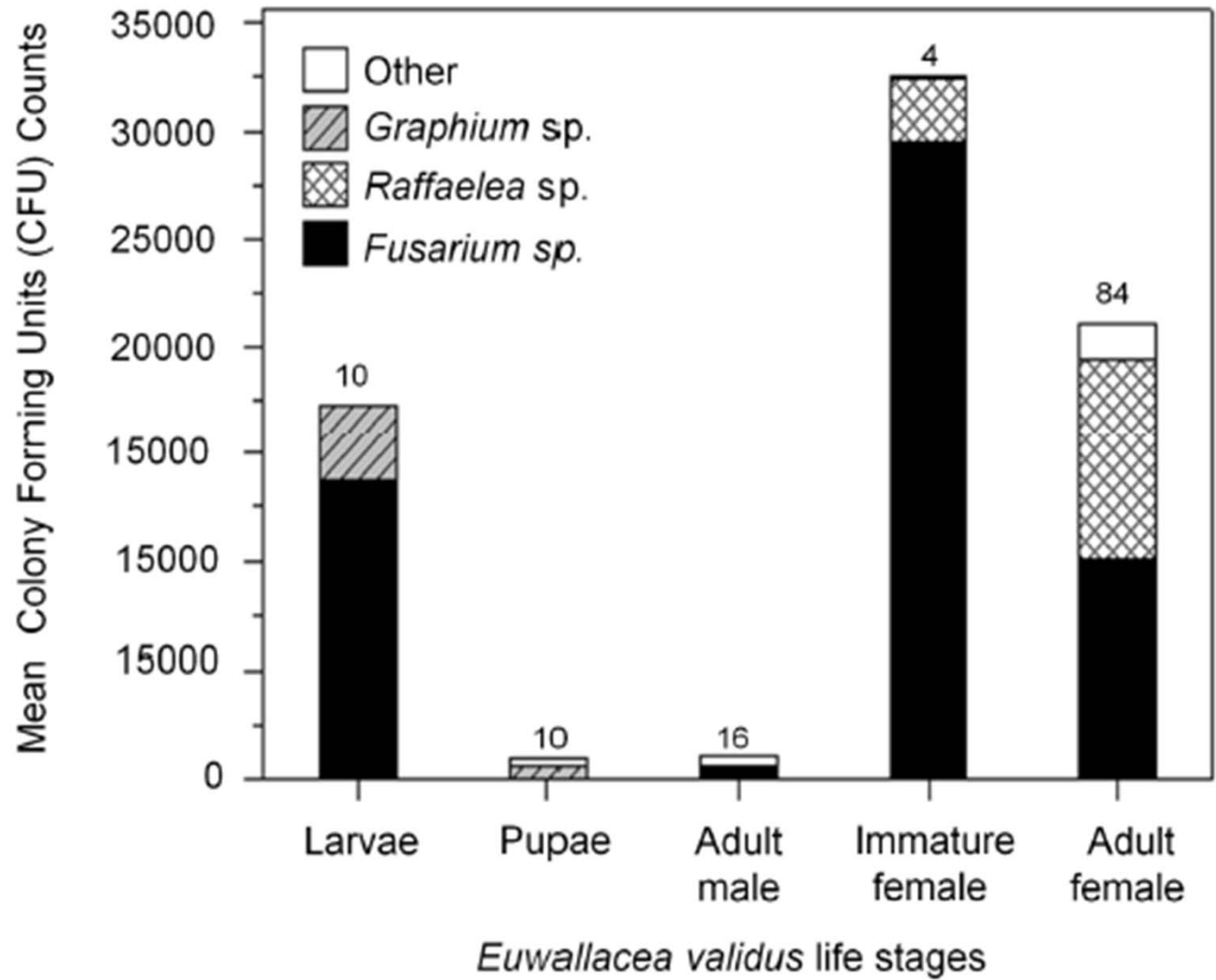
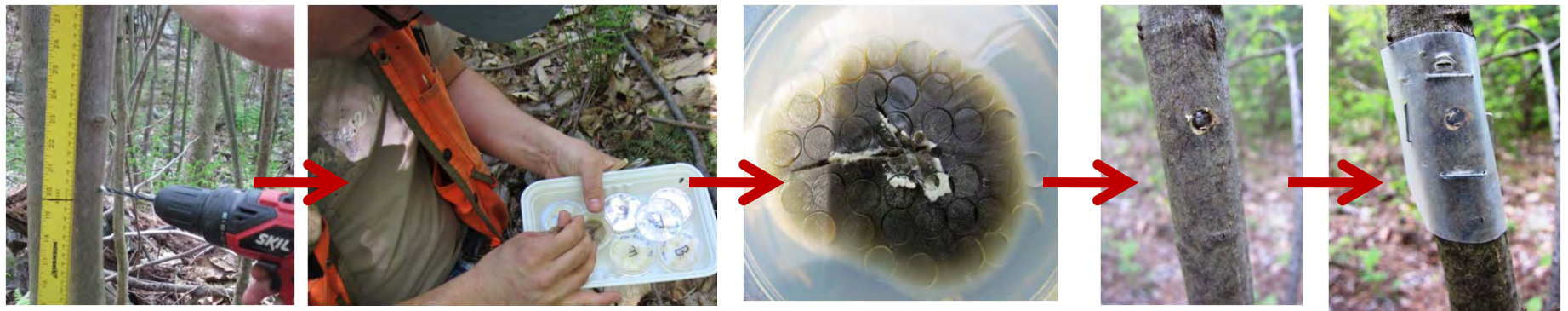
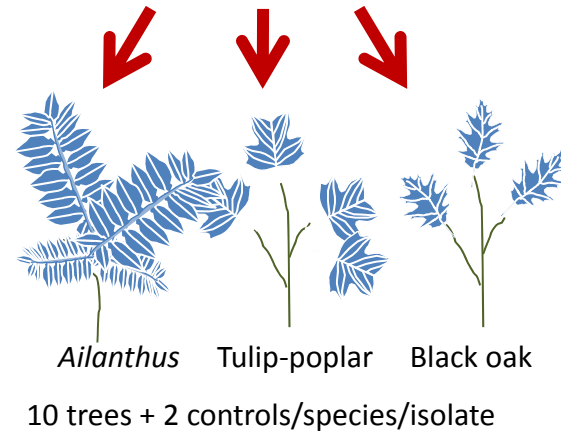
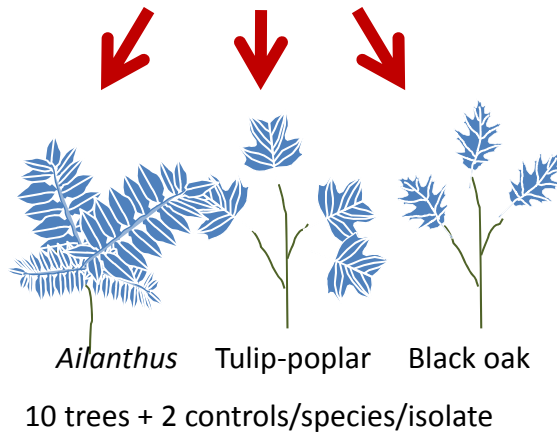
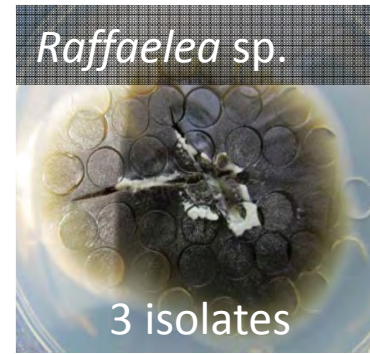
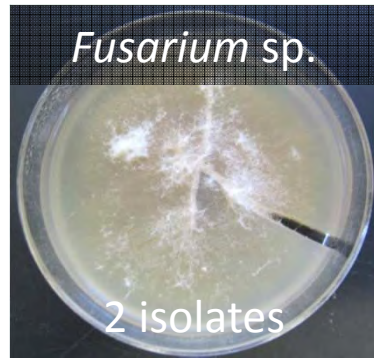


Figure 3. Mean CFU counts by developmental stage for the Asian ambrosia beetle, *Euwallacea validus*. For both adult beetles and immature females, mean CFUs were based on quantification from macerated heads only. For larvae and pupae, CFUs were based on whole body platings.

Numbers at top of bars indicate sample size.

Pathogenicity testing for *E. validus* symbionts



Pathogenicity testing for *E. validus* symbionts and/or fungal associates

- ⦿ No significant differences in necrotic area (cm²) around the inoculation point among controls and inoculation trees for all three test species (data not shown) at 4 months post-inoculation.



Role of Insect in Disease

- **Passive transport?**
- ***V. nonalfalfae* can be isolated from beetles**
- **Beetles can be found on healthy trees where disease and infestations are established**

Phylogenetic analysis of *Fusarium* sp. from *E. validus*

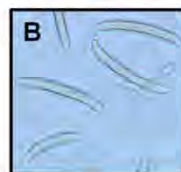
- Ambrosia fusaria represent a strongly supported monophyletic lineage (A) within Clade 3 of the *F. solani* Species Complex
- All *Ailanthus/E. validus* associated fusaria share the same multilocus sequence type
 - Closely related to *Fusarium* from *E. fornicatus*, Israel avocado
- All known fusaria in the *F. ambrosium* clade are derived either from ambrosia beetles or their galleries.
- All known fusaria in the *F. ambrosium* clade produce morphology unique within FSSC
- Members of neighboring clade (incl. from *X. ferrugineus*) produce more typical FSSC morphology

Tea isolates
With *Ailanthus*
EF1 allele

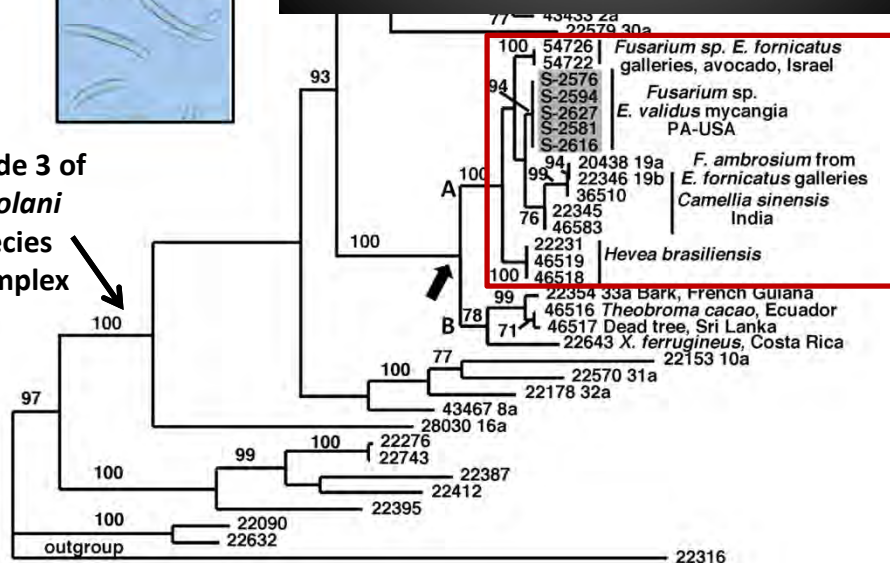
EF-1a, RPB2,
rDNA
3361 bp
627 PIC
2509 steps

— 10 changes

Macroconidia morphology
of two supported groups



Clade 3 of
F. solani
Species
Complex



Conclusions

- ◉ *E. validus* on *Ailanthus* has two unique mycangial fungi
 - ◉ *Fusarium* sp. closely related to *F. ambrosium*
 - ◉ *Raffaelea* sp. (further work is needed to characterize)
- ◉ *Fusarium* isolates from *Euwallacea validus* represent a monophyletic clonal lineage closely related to *F. ambrosium*
 - ◉ Evidence for genetic exchange
- ◉ Mycangial symbionts from at least two *Euwallacea* form a strongly supported monophyletic group within the *F. solani* Species Complex
- ◉ Tree inoculations with *Fusarium* sp. from *E. validus* reveal *Fusarium* and *Raffaelea* not pathogenic on *Ailanthus*, or on co-occurring species in PA forests: black oak, or yellow-poplar
 - ◉ Pathogenic on avocado?

Acknowledgements



Penn State

Eric O'Neal (a.k.a. Black Cohosh)
Eric Rosko, Justin Ross, Chance Yeckley (Field Assistants)
Matt Davis (Davis Lab), Jean Juba and Dylan Short (FRC), Steve Conaway
Suzanne Slack, Annie Ye (Lab assistants)
Dr. Beth Brantley (PSU Mont Alto)
Art Gover (Crop and Soil Sciences)
Jim Savage, Paul Lupo, and Mike Powell (FRL)



PA DCNR (Funding)

Steve Wacker
Donald Eggen (Funding)



PA State Game Commission

David J. Gustafson
Dave Henry



USDA Forest Service (Funding)

Dr. Richard Reardon
Dr. Yun Wu
Dr. Robert Long



PA Bureau of State Parks

Rachel Wagoner
Steven Volgstadt
Andy St. John



US Army Corps
of Engineers®

U.S. Army Corps of Engineers

Jeff Krause



APHIS PPQ

Grace O'Keefe