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Microbial community composition and wood digestion in the gut of the Asian Longhorned Beetle

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Asian Longhorned Beetle (ALB) (*Anaplophora glabripennis*)

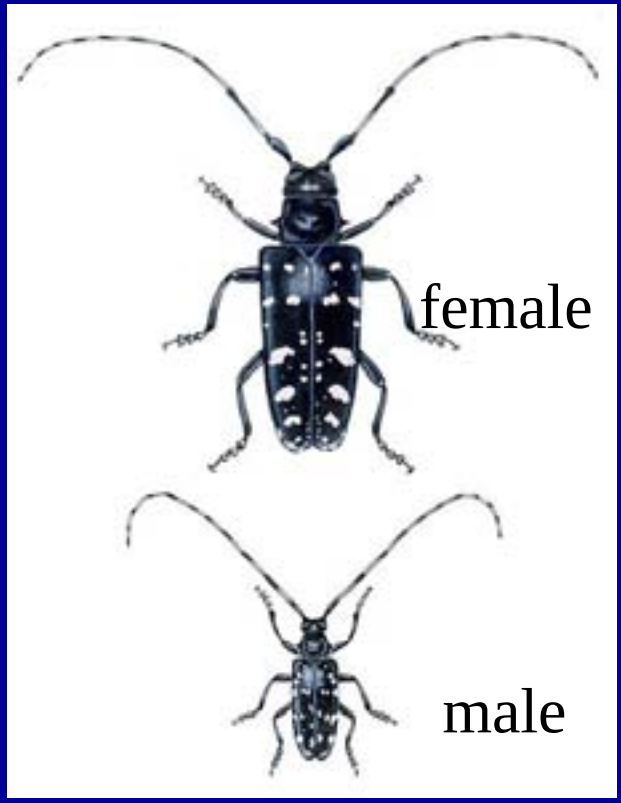
Wood feeding beetle

Adults

- Black with white markings
- Long antennae with black & white bands

Larvae

- Worm-like, burrowing into the heartwood of host trees
  - Populus
  - Acer
- Unknown how they are able to develop in woody tissue



Asian Longhorned Beetle Lifecycle

Adults and ovipostion scars

Emergence holes

Adult emerging from tree

Larva in tree

Pupal chamber in tree

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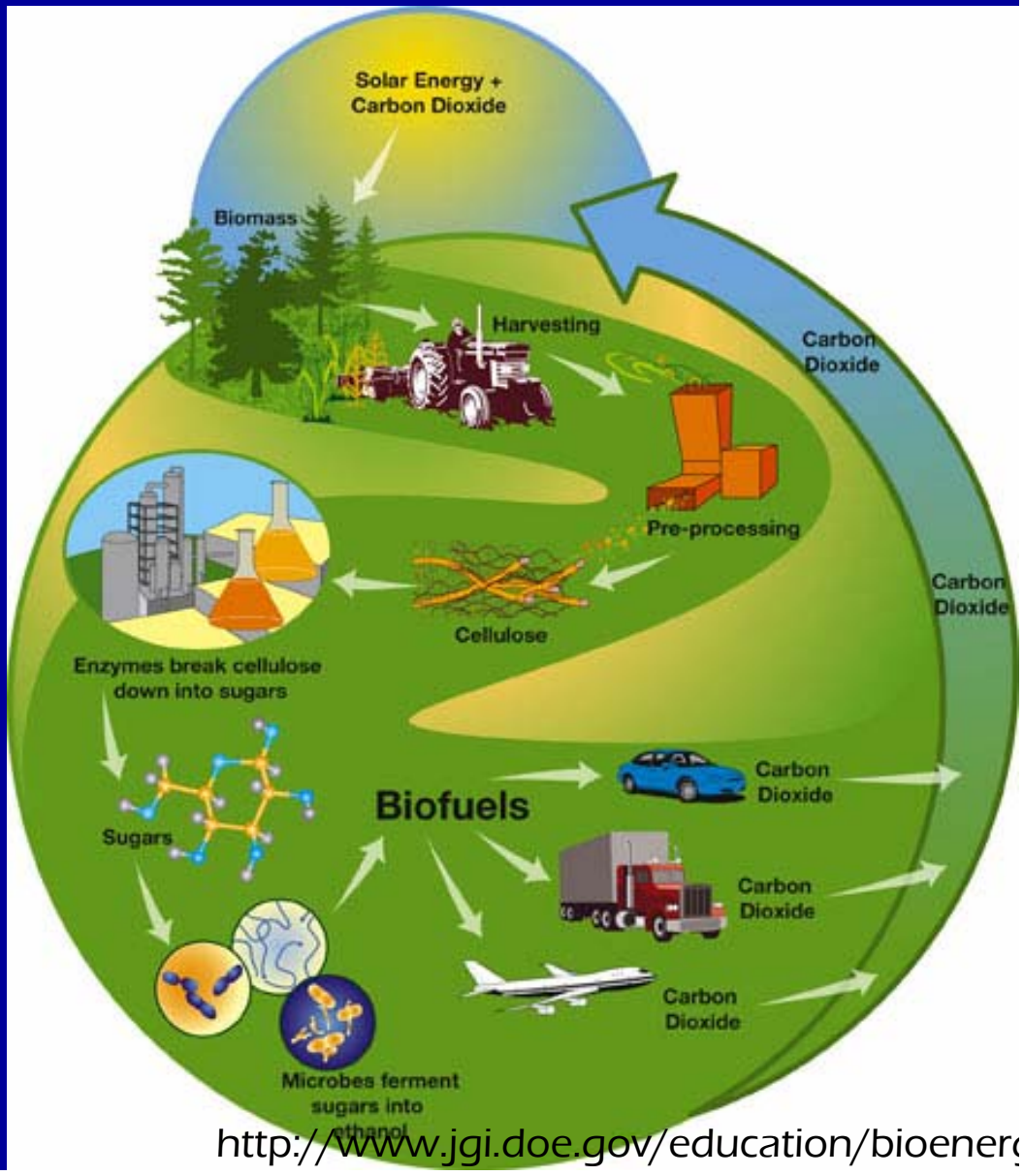
Diagram by Michael Bohne

ALB and Biofuels

Larval ALB

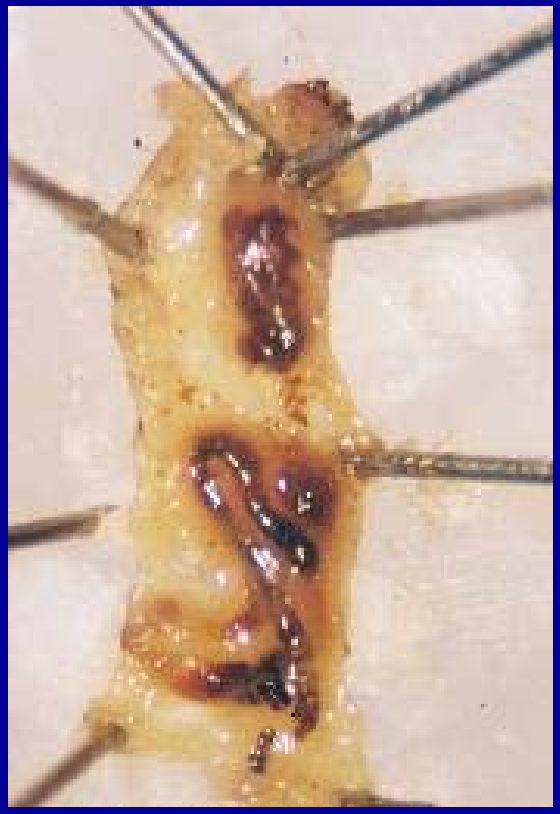
- Feeds on *living wood*
- Grows and develops feeding *only on non-decaying woody tissue*

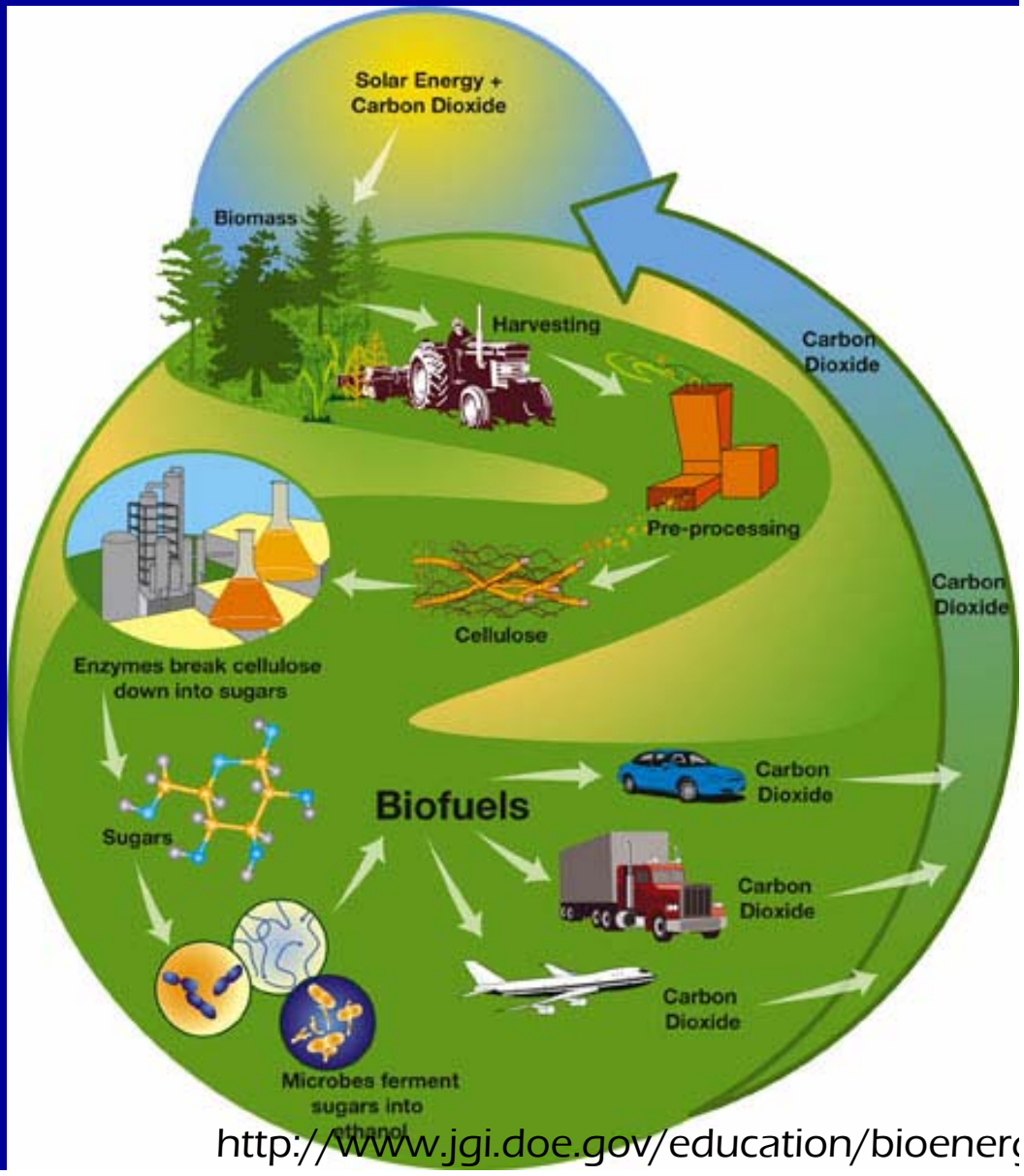


http://www.jgi.doe.gov/education/bioener

ALB and Biofuels

Larval ALB Gut





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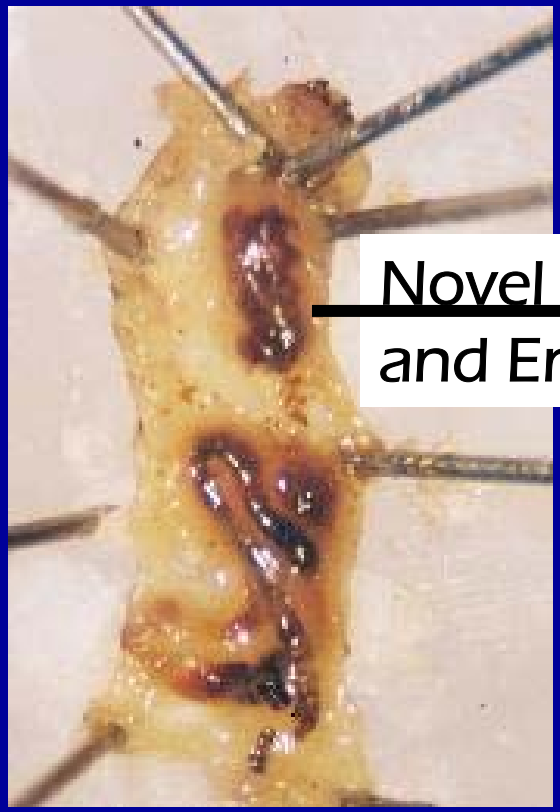
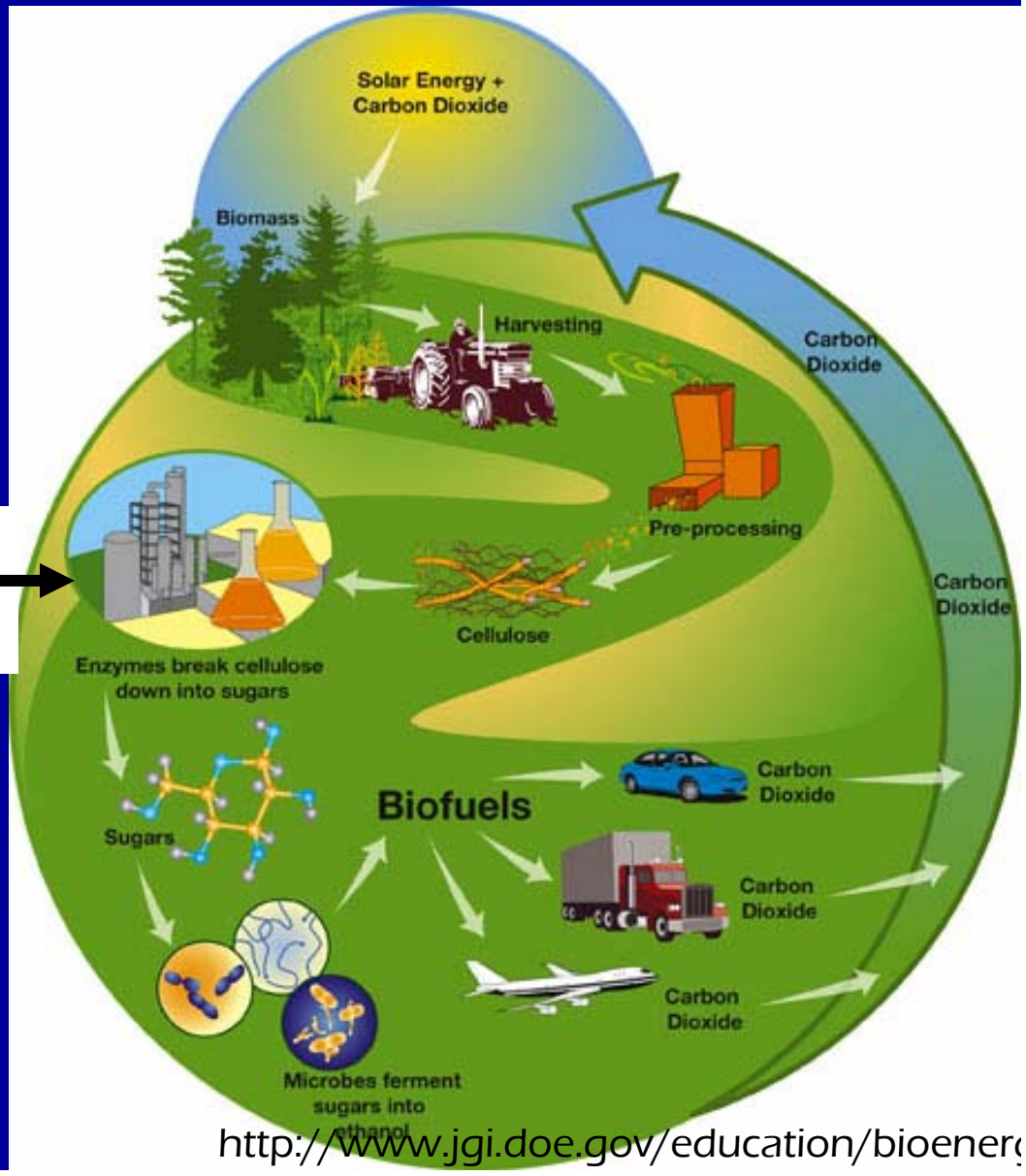
ALB and Biofuels

Larval ALB Gut



Novel Bacteria and Enzymes



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Goal of Project

- Determine how ALB is able to grow and develop in the inner wood of wide variety of hardwood trees
- Wood degradation for ethanol production
  - Role of gut microbial community
  - Detoxification & lignocellulose degrading enzymes produced in gut
  - Relationship between gut community composition, gut enzymes, and host range

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Approaches

Compare changes between life stages and development on different host trees

Gut microbial community analysis

Lignocellulolytic enzyme activity

Gut proteome profiling

Focusing on mid-aged larvae on preferred host tree species

Localization of microbial symbionts within gut

ALB/microbiota gut transcriptome sequencing

Gut microbial metagenome sequencing (DOE-JGI project)

Effect of Host Tree on Gut Community Composition in ALB

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Gut Community Analysis: Objective

Based on host tree preferences:

- Sugar Maple, Preferred host
- Pin Oak, Secondary host
- Callery Pear, Resistant tree species
- Antimicrobial artificial diet

How is the gut microbial community affected by host tree?

Is host suitability correlated with microbial community composition?

Gut Community Analysis: Methods

Collection Technique:

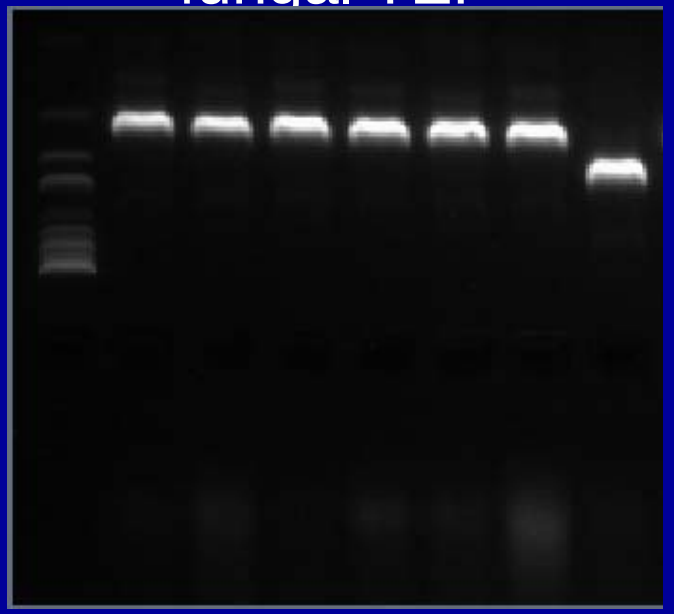
Collect larvae reared on host tree of interest or artificial diet

Remove gut aseptically

Gut Community Analysis: Methods

Extract total genomic DNA from gut samples

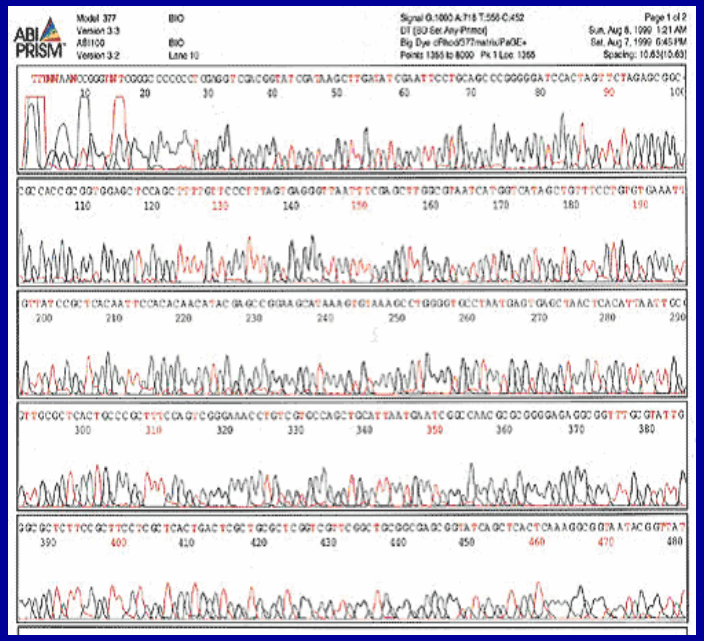
PCR amplify 16s rRNA gene (530F and 1392R primers) or fungal TEF



Gut Community Analysis: Methods

Clone PCR product into bacterial plasmid

Sequence random subset of clones from each gut and determine taxonomy (Ribosomal Database Project)



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Gut Community Analysis:

	Pear	Oak	Maple
Unclassified Enterobacteriaceae	X		X
Unclassified Bacteria	X		
Genus Enterobacter	X		
Genus Curtobacterium	X		
Genus Raoultella	X		
Genus Rhodococcus	X	X	
Genus Brevibacterium	X		X
Genus Cellulomonas		X	
Genus Sphingobacterium		X	
Genus Agrobacterium		X	
Genus Acinetobacter		X	
Genus Ochrobactrum		X	
Genus Brachybacterium		X	X
Genus Microbacterium		X	X
Unclassified Microbacteriaceae		X	X
Genus Enterococcus		X	X
Genus Bordetella		X	X
Genus Stenotrophomonas		X	X
Genus Enterobacter		X	X
Unclassified Cyanobacteria			X
Unclassified Brucellaceae			X
Unclassified Rhizobiales			X
Unclassified Xanthomonadaceae			X
Genus Streptomyces			X
Unclassified Streptomycetaceae			X
Unclassified Enterobacteriaceae	X	X	X

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Identification of fungal species in ALB gut: *Fusarium solani*

- All samples contained a single fungal species (determined by EF1-α sequence):
  - *Fusarium solani*
    - Known to produce cellulases and lignin peroxidases
    - Related species are plant pathogenic
  - ALB galleries collected from NYC were also infested with the same species of fungus
  - Very interested in this species role in gut community

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Gut Community Analysis: Conclusions

- ALB gut community dramatically affected by host tree
  - Suitable hosts: similar gut profiles
  - Resistant host: greatly reduced microbial diversity
- Current Research
  - screening field collected larvae from NYC and China
  - vertical transmission of bacteria within insect stages

Effect of Host Tree on Cellulase Activity in ALB

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Wood Digestion in ALB

- ALB gut microbial community composition changes when larvae feed in different host tree species
- Microbiota may provide essential enzymes for wood digestion:
  - Cellulases:
    - Endoglucanases
    - Exoglucanases
    - Beta-glucosidases
  - Lignin degradation and detoxification

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Wood Digestion in ALB

- ALB gut microbial community composition changes when larvae feed in different host tree species
- Microbiota may provide essential enzymes for wood digestion:
  - Cellulases:
    - Endoglucanases
    - Exoglucanases
    - Beta-glucosidases
  - Lignin degradation and detoxification
- Question: Is host tree-related changes in gut community associated with differences in cellulase enzyme activity?

Cellulase Activity in ALB: Methods

Larvae reared in host tree

Let Feed

Extract larvae from tree

Cellulase Activity in ALB: Methods

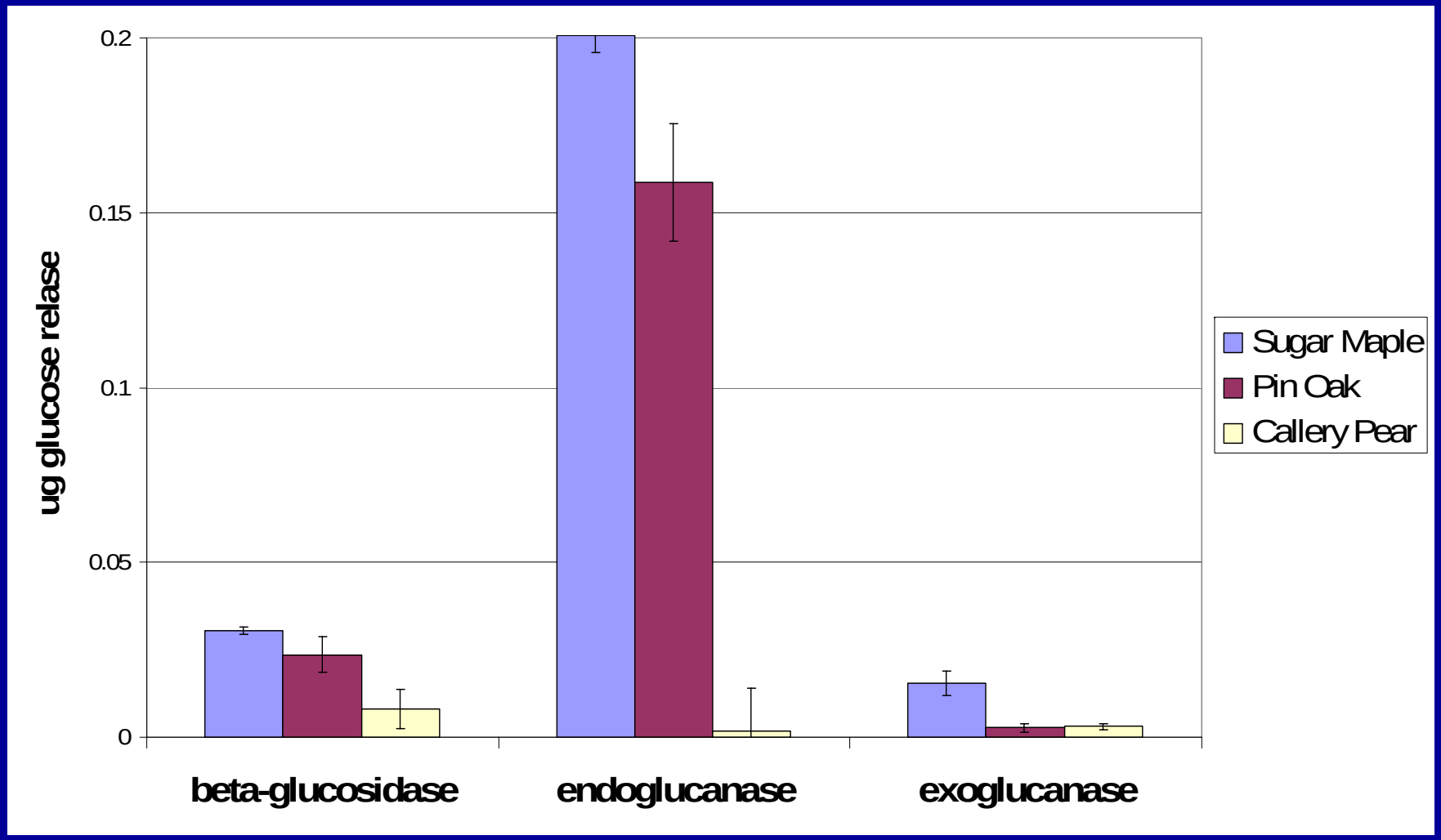
Dissect Guts

Combine Gut Extract + Cellulose Substrate

Incubate

Determine amount of reducing sugar liberated (DNS Assay)

Gut Cellulase Activity in ALB grown in different hosts



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Cellulase Activity in ALB: Conclusions

- Gut of larval ALB produces complete cellulase complex
  - Host tree affects cellulase activities
    - Preferred hosts: highest enzyme activity
    - Resistant host: greatly reduced enzyme activity
  - Correlation with gut microbial community diversity
    - **Microbes are critical for wood degradation**

Current and Future Work:

- Isolation of specific cellulase enzymes
- Peroxidase activity and lignin degradation
- Enzyme activities through insect development
  - Identify most “active” stages for wood degradation

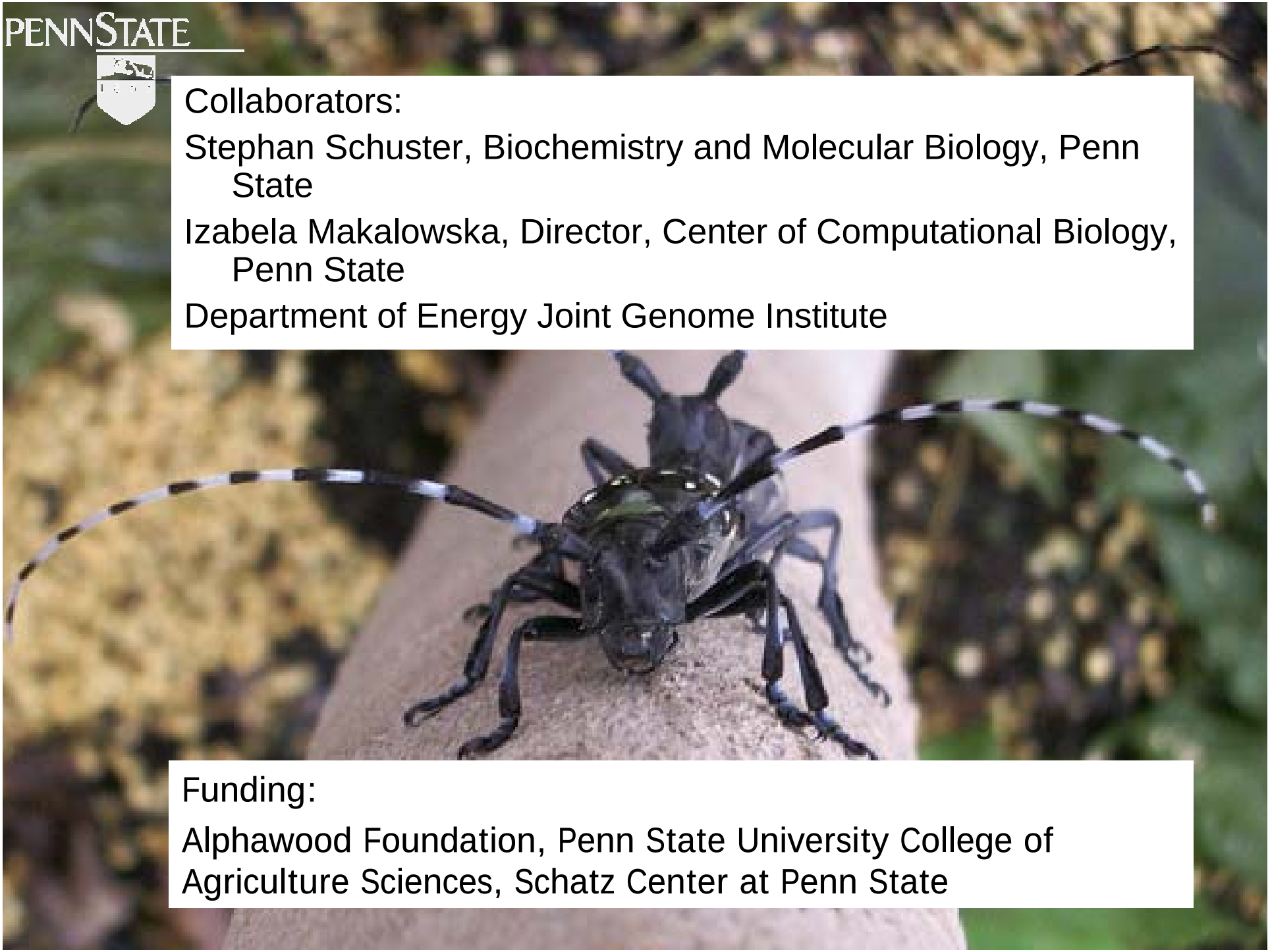
DOE Joint Genome Institute Gut metagenome sequencing

JGI
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

- JGI chose ALB gut metagenome as top priority for sequencing this year
  - Using the larval stage that is feeding on heartwood
- Goals:
  - Gain sequence of novel microbial species
  - Identify enzymes that are produced that play a role in wood digestion (in conjunction with transcriptome and proteome data we are producing)

ALB Webpage
www.alb.cbio.psu.edu

- Info on ALB life history with links to USDA pages
- Sequence data available to public
- Searchable database
- 16s, metagenome, and transcriptome sequences
- Publications
- Project updates and ongoing work



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Department of Energy Joint Genome Institute

Funding:

Alphawood Foundation, Penn State University College of Agriculture Sciences, Schatz Center at Penn State