The Effect of *Sirex* spp. Woodwasps and Their Fungal Associates on Alabama Forest Health

Forest Health Dynamics Laboratory
School of Forestry and Wildlife Sciences – Auburn University

Background: *Sirex* Wasps

- *Sirex noctilio* is a woodwasp associated with *Amylostereum* spp. fungi, white rot of wood
- Females of all *Sirex* spp. oviposit mixture of eggs, fungal mycelia, and venom into trees
- *S. nigricornis* native to Alabama

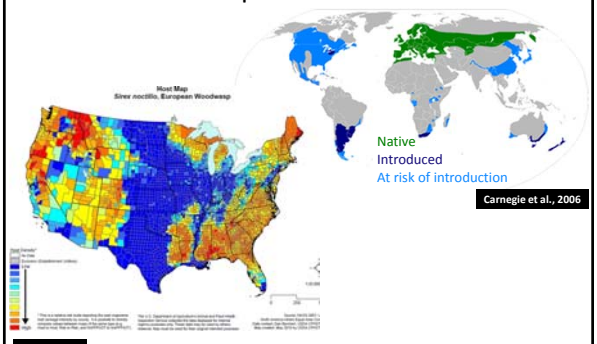

NEDPN


Gerard Trichies


FHDL


FHDL

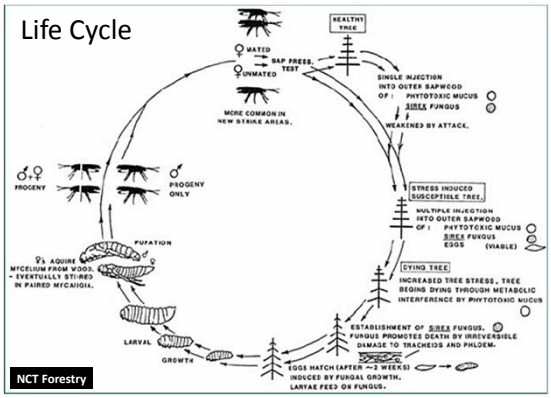
Host Map and Distribution



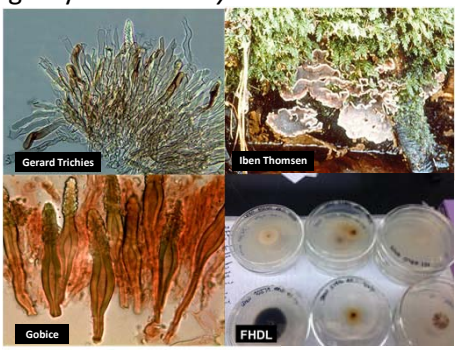
Carnegie et al., 2006

USDA APHIS

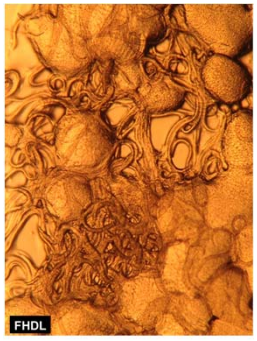
Life Cycle

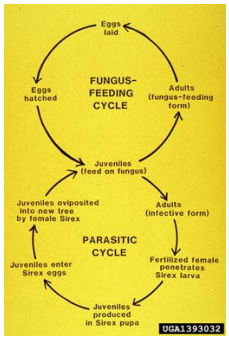


Fungal Symbiont: *Amylostereum areolatum*



Biological Control Agent- *Deladenus siricidicola*





Inoculating Trees with *Deladenus siricidicola*

Objectives

1) Monitor and survey native and potentially non-native woodwasp populations in various latitudes across Alabama
2) Determine the length of Siricid flight seasons
3) Collect and identify bark and ambrosia beetles caught in *Sirex* traps to determine if emergence overlaps temporally as potential competitors

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Flight Phenology

Trapping Survey

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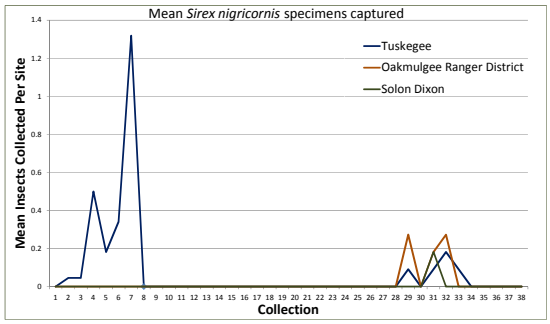
Flight Phenology

Results



Collections from 9/18/14 to 2/25/16

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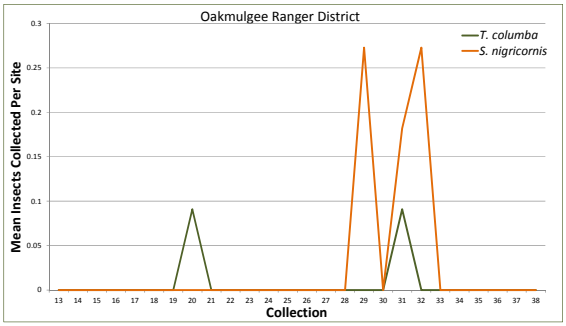
Flight Phenology

Results



Collections from 3/7/15 to 2/20/16

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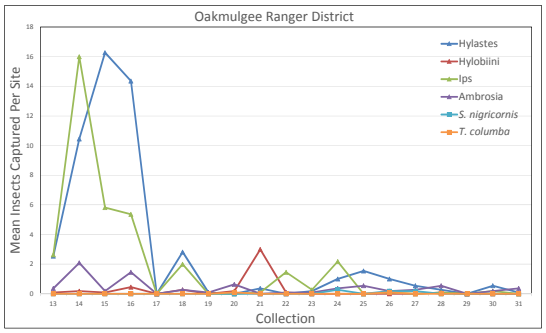
Forest Health Dynamics Laboratory

Flight Phenology

Results



Collections from 3/7/15 to 1/20/16

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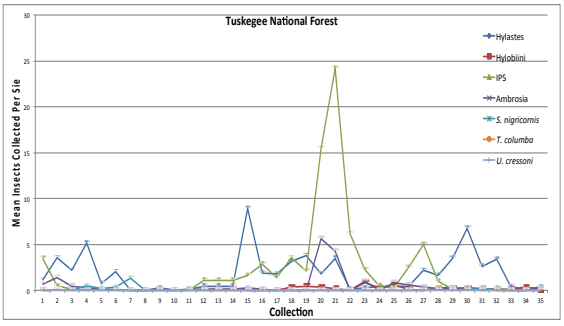
Flight Phenology

Results



Collections from 9/18/14 to 2/25/16

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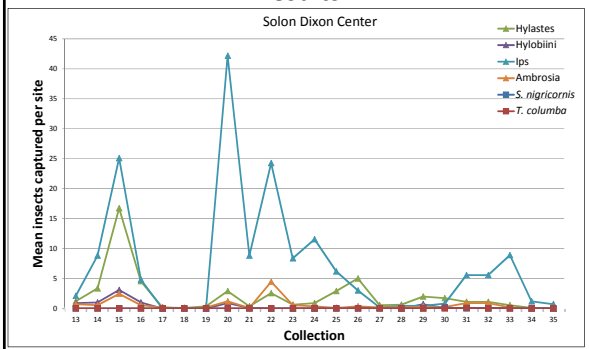
Flight Phenology

Results



Collections from 3/11/15 to 1/28/16

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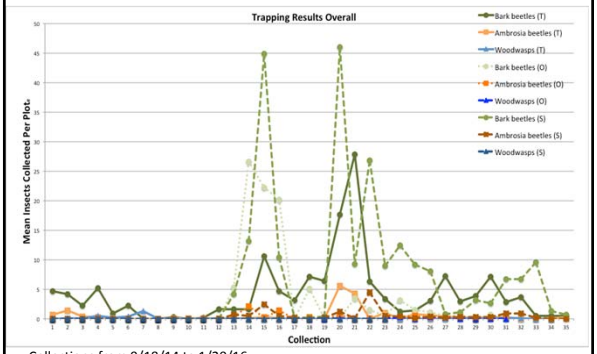
Results



Collections from 9/18/14 to 1/20/16

Conclusions

- Mean *S. nigricornis* capture in Tuskegee National Forest was higher in 2014 than 2015
  - *S. nigricornis* and *Tremex columba* had similar emergence periods, with the exception of the early emergence trapping of *T. Columba* in Talladega National Forest
  - Little likelihood of competition occurring as *T. columba* is a pest of deciduous hardwood trees
  - *T. columba* emergence could overlap temporally with *S. noctilio*, if in same locality

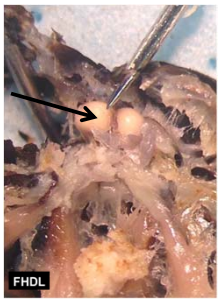
Conclusions

- Peak flight season for bark and ambrosia beetle species occurred earlier in the year than *S. nigricornis* peak emergence
- Bark and ambrosia beetle populations would peak before predicted *S. noctilio* populations

Objectives

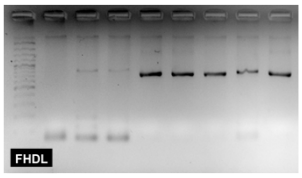
1) Determine the species of *Amylostereum* and *Deladenus* associated with *S. nigricornis* in Alabama
2) Examine the relationships that *Deladenus* spp. has with the associated *Amylostereum* spp.
3) Determine species of nematode associated with *T. columba* specimen

Woodwasp Dissection



Methodology

DNA Extraction:
Sirex (legs)
Amylostereum (mycangia)
Deladenus (*Sirex* eggs)



Polymerase Chain Reaction

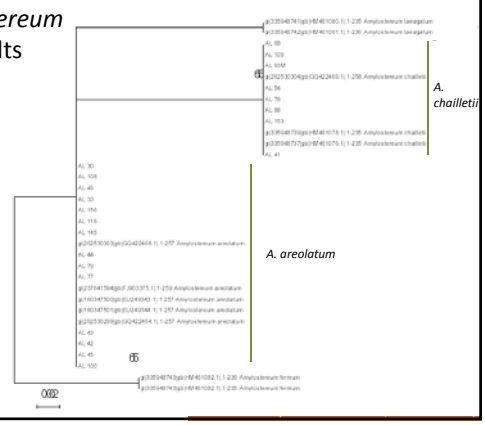
Sequencing

Molecular Analyses

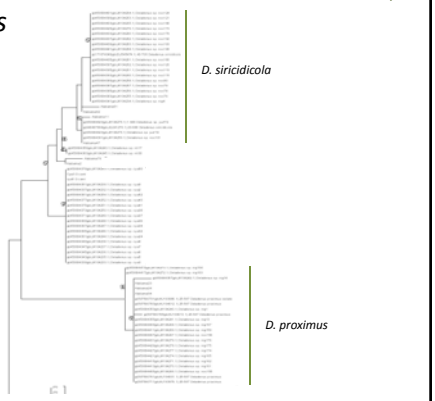
Aligned sequences

Drew Phylogenetic trees

Amylostereum results



Deladenus results



Conclusions

- *S. nigricornis* was found to carry *A. areolatum* and *D. siricidicola*, normally associated with *S. noctilio*
- *T. columba* was found to be parasitized by *D. siricidicola*
- *D. siricidicola* was shown to be in association with both *A. chailletii* and *Cerrena unicolor* (*T. columba*)

Objectives

1) To determine how 10 different terpenes commonly produced by southern pine trees affect the growth of *Amylostereum* spp. fungi isolated from 10 locations worldwide
2) To determine how growth rates are affected by direct or indirect contact of terpenes

Background: Tree Defense

Once attacked by *S. noctilio,* a tree begins to exhibit defensive behavior

- Constitutive defense: bark thickness
- Induced defense: oleoresins, terpenes

Chemicals emitted have different functions within a tree

- Stress chemicals: α-Pinene, β-Pinene
- Defense chemicals: camphene, myrcene, limonene, phellandrene, 4AA

Study Design

Atmospheric Trial

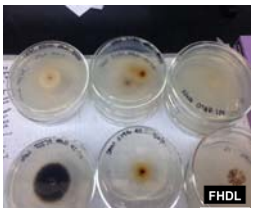
- Atmospheric chambers (paint cans)
- Glass petri dishes, no lids
- Examined how vaporized terpenes affect fungal growth

Tactile Trial

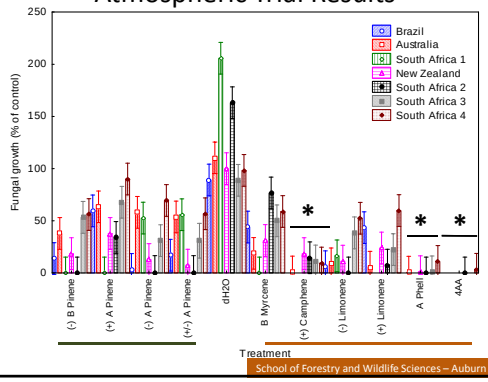
- Fungal isolates in direct contact with tepenes on media
- Examined how direct contact of terpenes affects fungal growth

Materials and Methods

Isolation | Inoculation and Growth | Measurements



Atmospheric Trial Results



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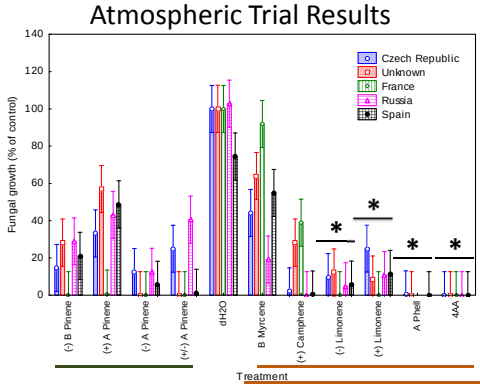
Terpene Study

Atmospheric Trial Results



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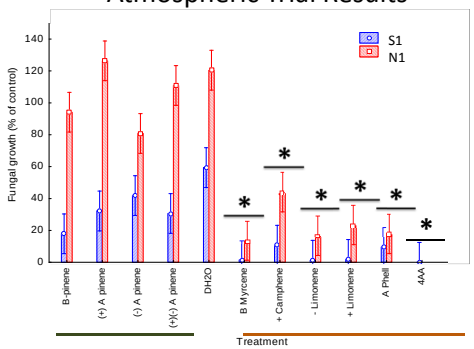
Terpene Study

Atmospheric Trial Results



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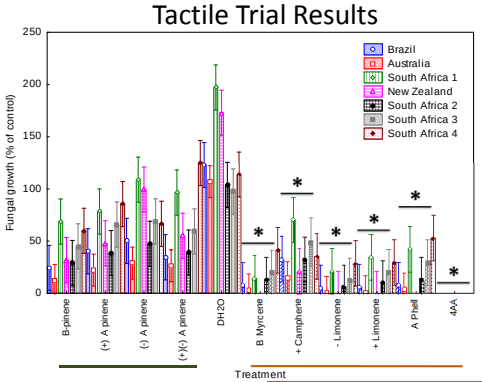
Terpene Study

Tactile Trial Results



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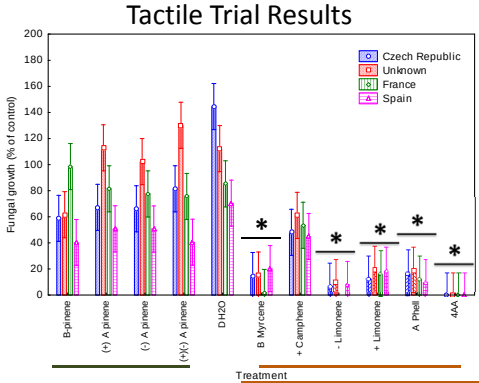
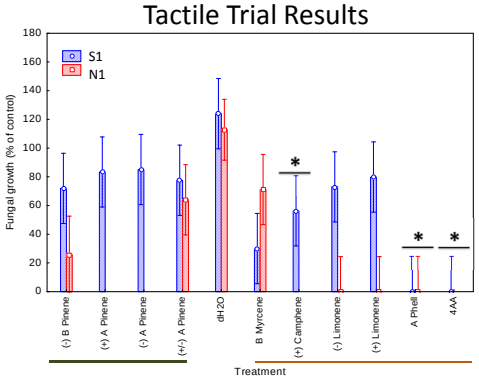
Tactile Trial Results



Tactile Trial Results



Conclusions

Overall

- The Northern Hemisphere collected isolates were slower growing compared to the fungal isolates from the Southern Hemisphere
- β-Myrcene significantly increased growth of *A. areolatum* isolates for the atmospheric trial, but not the tactile trial
- The compounds α-Phellandrene and 4AA resulted in nominal growth of *A. areolatum* isolates

Conclusions

Atmospheric Trial

- (+)α-Pinene and β-Myrcene resulted in the highest percentage of fungal growth compared to that of the control in *A. areolatum* isolates
- Isolate N1 of *A. chailletii* had higher growth rates than S1 when exposed to (+) α-Pinene, (-) α-Pinene, and (+/-) α-Pinene

Tactile Trial

- β-Myrcene resulted in significantly less growth than the control in *A. areolatum* isolates
- Isolate N1 of *A. chailletii* had lower growth rates than S1

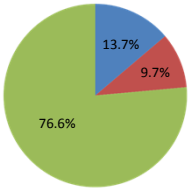
Objectives

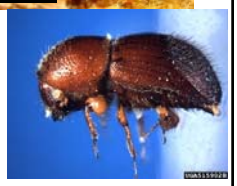
1) To determine how isolates of native and non-native *Amylostereum* might compete with blue stain pathogens *Leptographium terebrantis* and *L. procerum*
2) To determine the relationships between isolates of *Amylostereum* used in the competition assay by testing for Vegetative Compatibility Groupings (VCGs)

Potential Competitors

Potential Competitors

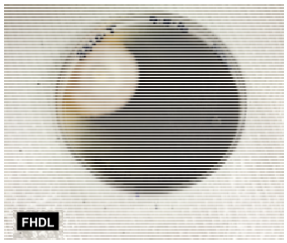
Total Captured Insects of Concern 2014



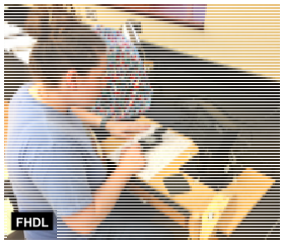


Materials and Methods

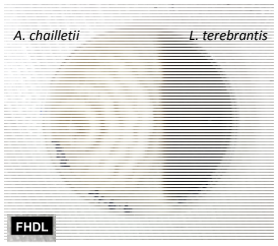
Inoculation and Growth

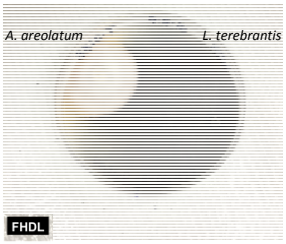


Measurements



Amylostereum vs. *Leptographium*





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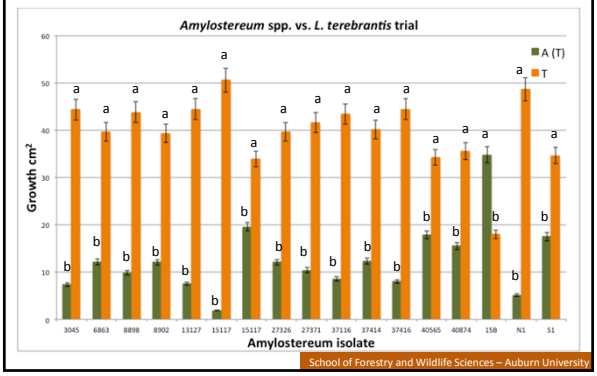
Competition Study

Results



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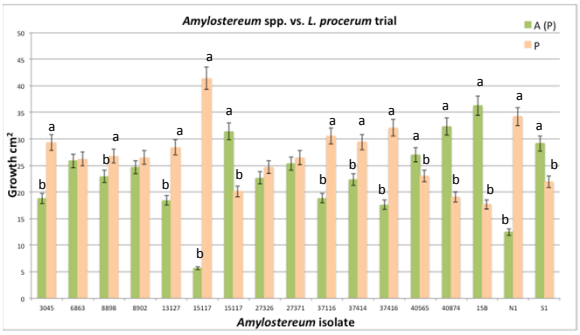
Competition Study

Results



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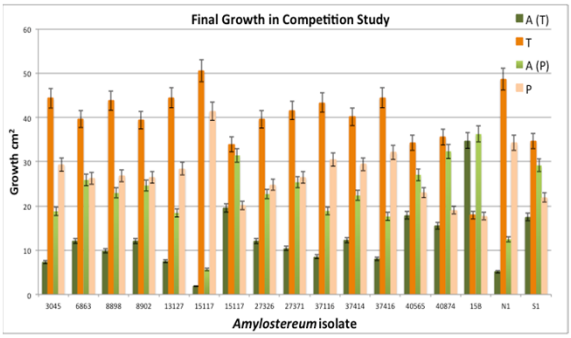
Competition Study

Overall results



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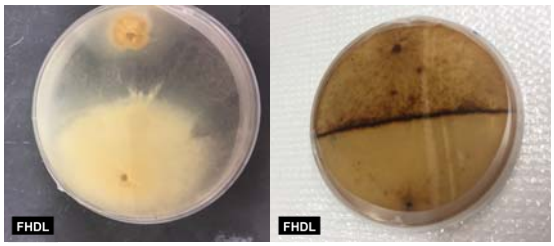
Vegetative Compatibility Groupings

Inoculation → Scoring

VCG | No VCG



	3309	3310	6863	8898	8902	13827	27371	37116	37414	37416	40565	40874	S1	N1	15B	15102	27326
3309	--																
3310	0	--															
6863	0	1	--														
8898	0	1	1	--													
8902	0	0	0	0	--												
13827	1	0	0	0		--											
27371	0	0	0	0	1	0	--										
37116	0	0	0	0	0	0	0	--									
37414	0	0	0	0	0	0	0	1	--								
37416	0	0	0	0	0	0	0	1	1	--							
40565	0	0	0	0	0	0	0	0	1	1	--						
40874	0	0	0	0	0	0	0	0	1	1	1	--					
S1	0	0	0	0	0	0	0	0	0	0	0	0	--				
N1	0	0	0	0	0	0	0	0	0	0	0	0	1	--			
15B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--		
15102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--	
27326	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	--

Conclusions

Competition Study

- The isolate of *L. terebrantis* had significantly higher growth rates than most isolates of *Amylostereum* (all except 15B), while the results from the *L. procerum* trial varied much more
- *L. procerum* is known as a mild pathogen, and was outgrown by *L. terebrantis* against every isolate tested
- *A. chailletii* isolate 15B from a female *S. nigricornis* in Auburn, Alabama outcompeted both the *L. terebrantis* ($p<0.0001$) and *L. procerum* ($p<0.0001$) in all replications of the study

Conclusions

Vegetative Compatibility Groupings

- The two isolates from Alabama that are molecularly confirmed as *A. chailletii* isolates 15B did not form a VCG with S1 or N1 (all from Alabama)
- Isolates S1 and N1 from Alabama formed a VCG

Overall Conclusions

- No invasive *S. noctilio* were captured through the duration of the surveys
- Bark and ambrosia beetle peak flight periods were earlier in the year than *S. nigricornis*, suggesting they could reduce substrate for *S. nigricornis*
- *S. nigricornis* was found to carry *A. areolatum* and *D. siricidicola*, two species typically associated with *S. noctilio*

Overall Conclusions

- *D. siricidicola* was found to be non-genera specific as a parasite, leaving questions over whether it could be used as a biological control agent in the United States
- Defense chemicals: α-Phellendrene and 4-AA significantly reduced growth rates of hyphae in comparison to a dH_2O control.
- Stress chemicals: α-Pinene and β-Pinene did not have an adverse affect on hyphae growth rates. β-Myrcene affected growth of hyphae differently when in direct or indirect contact.

Overall Conclusions

- Overall, *A. areolatum* from abroad was a poor competitor for *Leptographium* spp., while a native isolate of *A. chailletii* (15B) outcompeted both *Leptographium* spp.
- The isolate of *A. chailletii* (15B) did not form a VCG with other *A. chailletii* isolates from Alabama
- If *S. noctilio* was introduced into Alabama forests, it would likely have enough competition that it would not be an economically damaging pest as it has been in the Southern Hemisphere

Future Work

- Further trapping should be conducted in a wider variety of habitats for more than one or two flight seasons to gain a true understanding of *S. nigricornis* population patterns.
- Rearing *S. noctilio* larvae in wood material that has been infected with *Leptographium* spp, *Ophiostoma* spp., and other fungal pathogens associated with the bark and ambrosia beetles found in the southeast
- Further studies could be conducted to determine why β-Myrcene affects hyphae differently when directly in contact, as opposed to an atmospheric environment.

Future Work

- Further work on clone (15B) of *A. chailletii* needs to be conducted to determine if it could become a potential problematic pathogen in the southern pine ecosystem.
- Studies should determine whether or not the *D. siricidicola* associated with *S. nigricornis* is capable of sterilization, because if sterilization is occurring, native populations of the non-pest host could be affected.
- More research should also be directed at *T. columba*, and its relationship with *D. siricidicola*

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Dr. Scott Enebak

Insect Collections
US Forest Service Foresters
Solon Dixon Center Staff

Graduate Students
Jessica Ahl
Gifty Acquah
Nick Barnwell
Jeff Chieppa
Pratima Devkota
Shrijana Duwadi
Charles Essien
John Mensah
Adam Trautwig

Staff
Tessa Bauman
Sarah Peaden
Dalton Smith

Undergraduate Workers
Jordan Heath
Cody Hartzog
Caleb Killough
Ashton Newman
Chase Seals
Wilson Strickland
Nick Yashko
Cora Yates

FABI
Katrin Fitza
Izette Greyling
Dr. Gudrun Dittrich-Schröder
Dr. Irene Barnes