

Host Chemistry and Redbay Ambrosia Beetle Attraction

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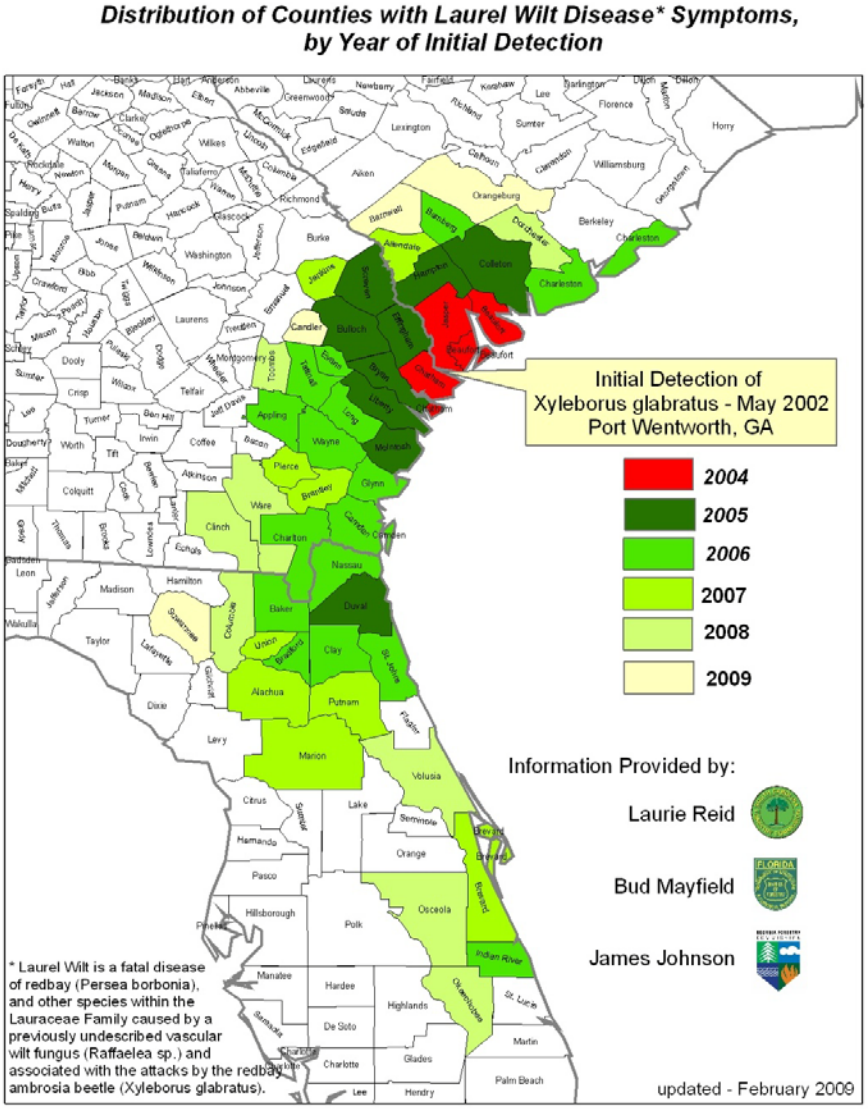
The Invasive Beetle, *Xyleborus glabratus*

- Native to Asia: likely introduced to US in solid wood packing material
- Spread from initial site of detection at ~20 miles/year
- Colonizes trees of family Lauraceae
- Releases vascular wilt fungal pathogen (LWD) and feeds on “ambrosia”
- Attacks trees of Lauraceae in native range in Asia



Distribution of Lauraceae

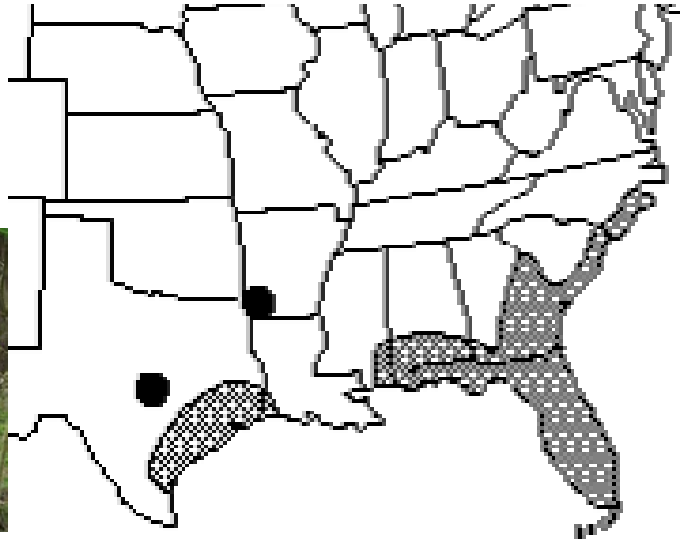
- >2000 species, 55 genera
- Majority of species in subtropics in Asia and Brazil
- 9 genera in US
  - *Cassytha filiformis* (s. tip of FL)
  - ***Cinnamomum camphora*** **(introduced)**
  - *Licaria triandra* (s. tip of FL)
  - ***Lindera*** (3 sp.)
  - ***Litsea aestivalis***
  - *Nectandra coriaceae* (s. FL)
  - ***Persea*** (3 sp.)
  - ***Sassafras albidum***
  - *Umbellularia californica*

The Host Species

- In SE US, 5 woody species in family Lauraceae susceptible to LWD
  - *Persea borbonia/palustris*
  - *Sassafras albidum*
  - *Lindera melissifolia* (E)
  - *Litsea aestivalis* (state-threatened)
  - *Cinnamomum camphora* (introduced)
- What do these species have in common?

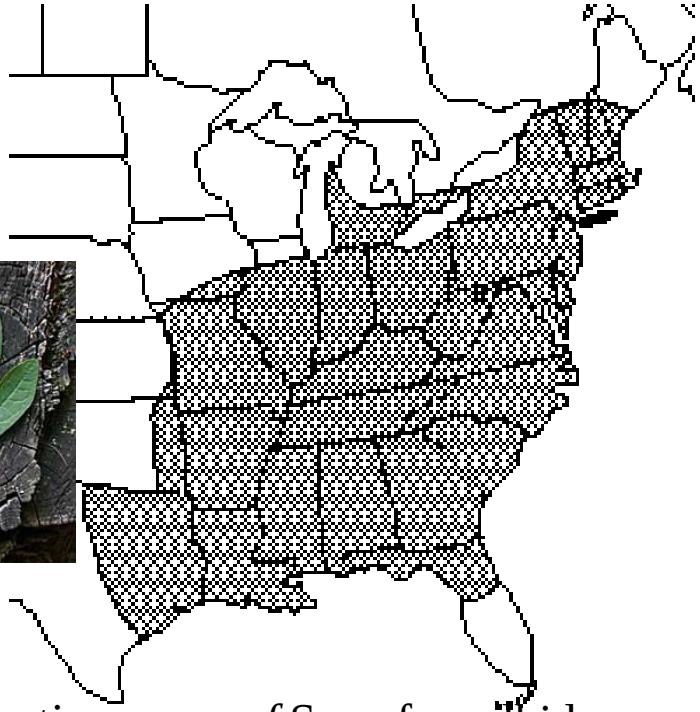
Distribution of Common Lauraceae

- *Persea borbonia*
  - Medium tree (15m)
  - Variety of habitats from dry to wet



The native range of Persea borbonia

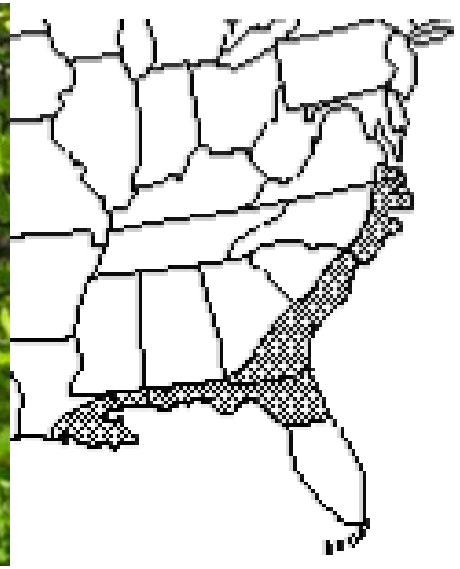
- *Sassafras albidum*
  - Clonal tree
  - Weedy colonizer



The native range of Sassafras albidum

Distribution of Rare Lauraceae

- *Litsea aestivalis (*Pond spice - multi-state threatened)
  - Shrubs to 3m
  - Margins of swamps, depressions



The native range of Listea aestivalis

- *Lindera melissifolia (*Pondberry- federally endangered)
  - Low shrub
  - Low woods, depressions, pond and sink margins

The native range of Lindera melissifolia

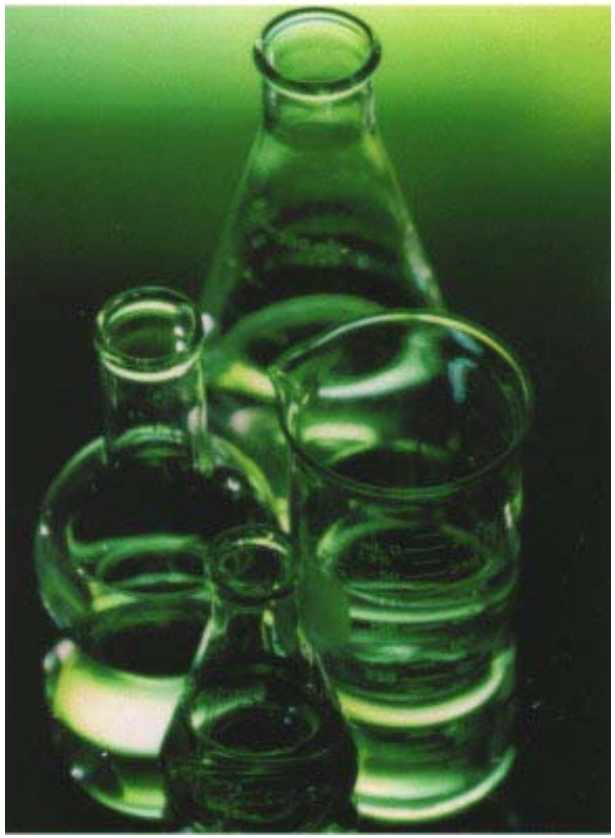
Objectives

- Part I: To determine volatile chemicals present in healthy vs. infested wood of five Lauraceous species in GA

- Part II: To test the response of *Xyleborus glabratus* to volatiles common to the host species (as determined in Part I) in a series of field trials

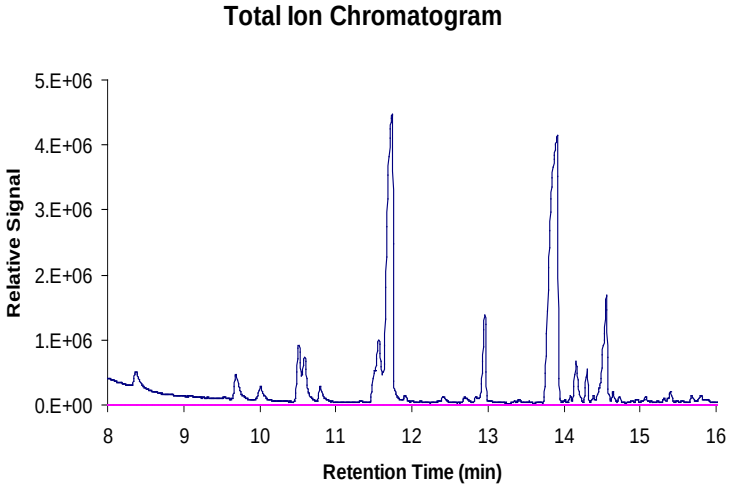
Part I: Methods

- Located and collected specimens (~10 of each) healthy and infested
- Cut ~ 0.3m segments of stems, bagged and return to lab
- Refrigerated until extraction: usually < 24 h, but up to 5 days
- Shaved and weighed ~1g inner bark from stem
- Placed in 125 mL flask with 20mL methylene chloride with 6.6 ppm *m*-xylene as internal standard
- Extracted for two weeks at room temperature

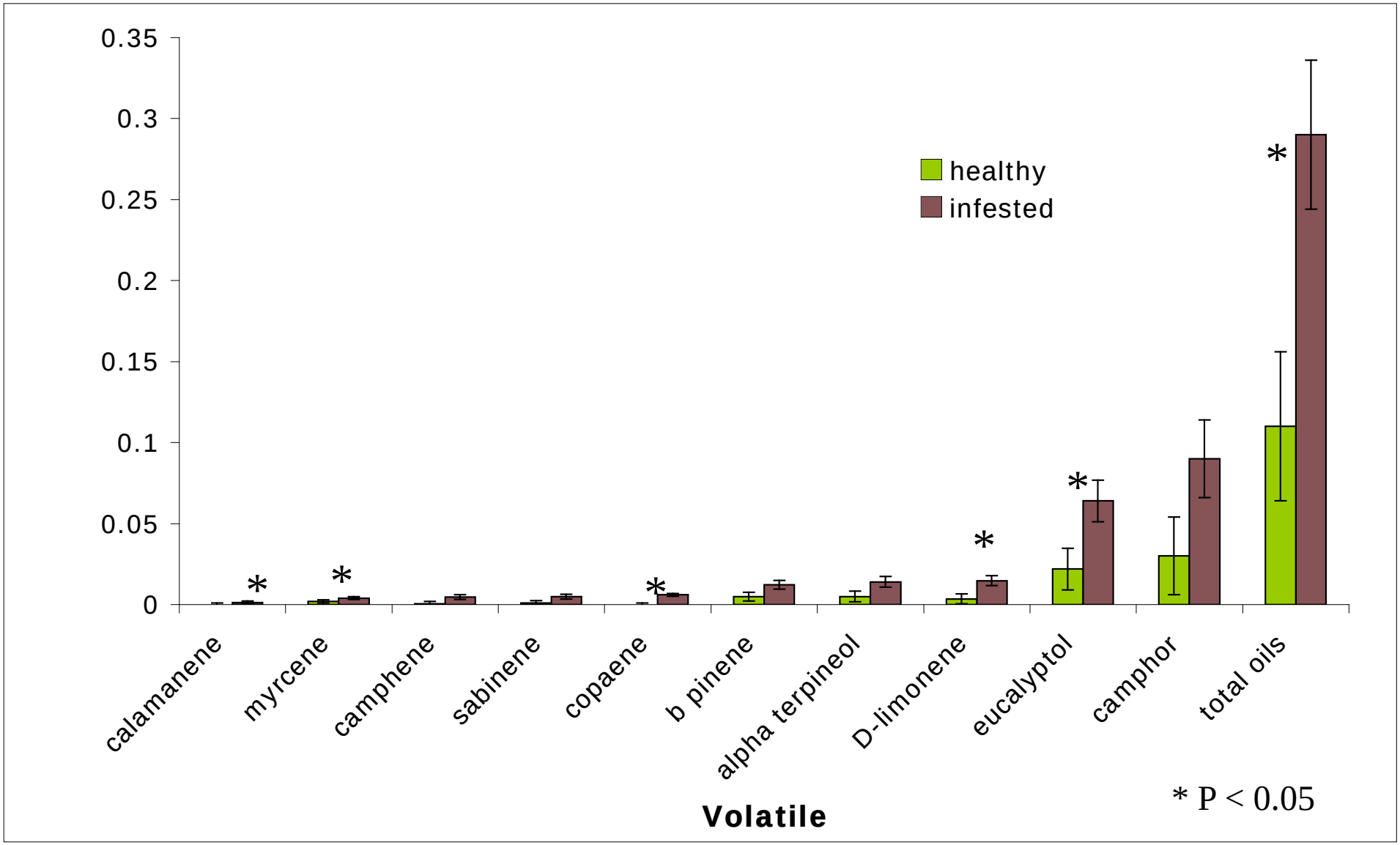


Methods

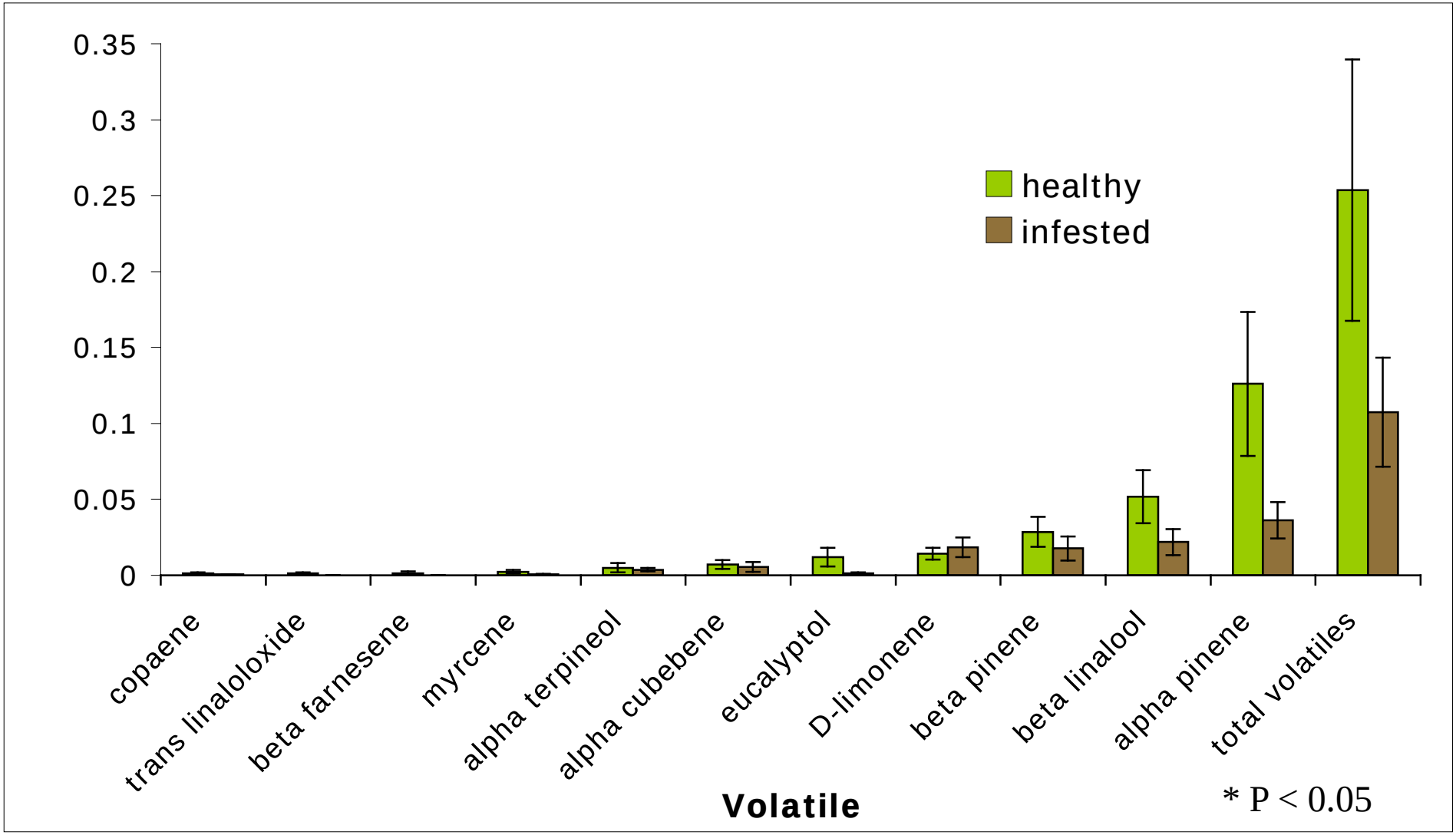
- Samples blown down and run on Gas Chromatograph Mass Spectrometer (GC/MS) with autosampler
- Determine volatile chemicals present: both quantity and type
- Major peaks quantitated electronically for retention times of 20 and less.
- Peaks identified by comparison to authenticated compounds
- Used ANOVA to compare mean concentrations of important compounds in healthy and infected trees within species



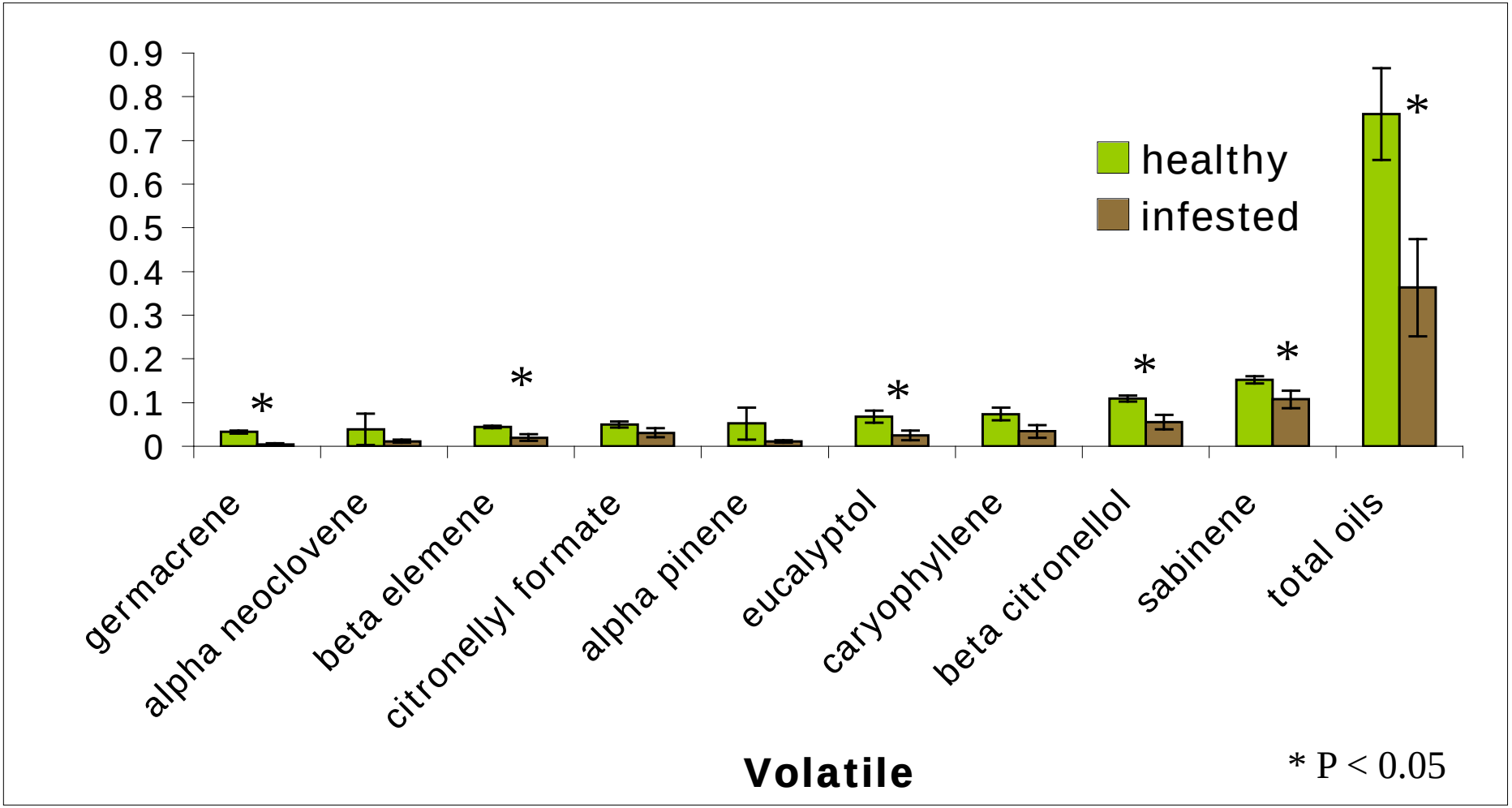
Volatile Concentrations in Healthy vs. Infected *Persea borbonia*



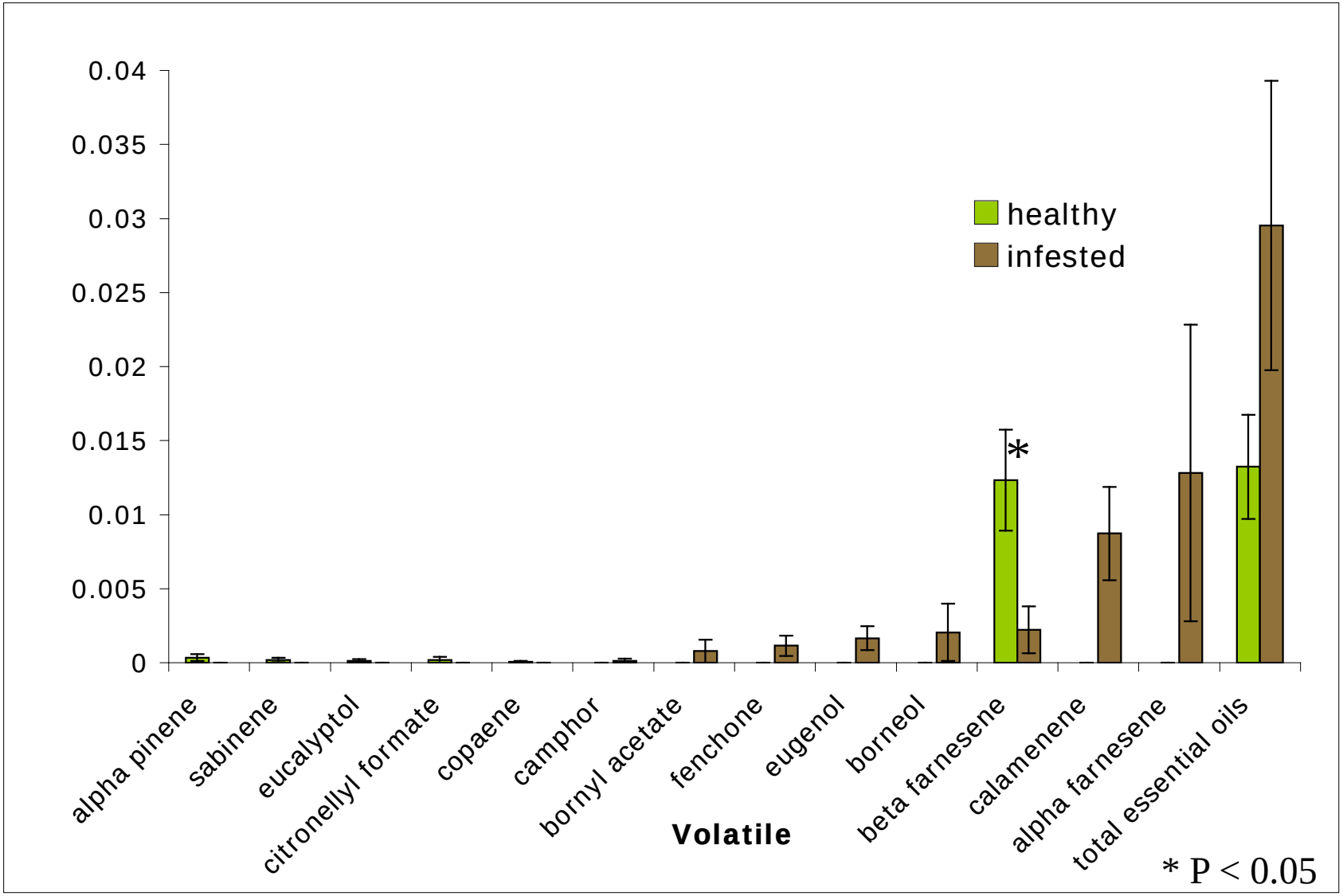
Volatile Concentrations in Healthy vs. Infected *Sassafras albidum*



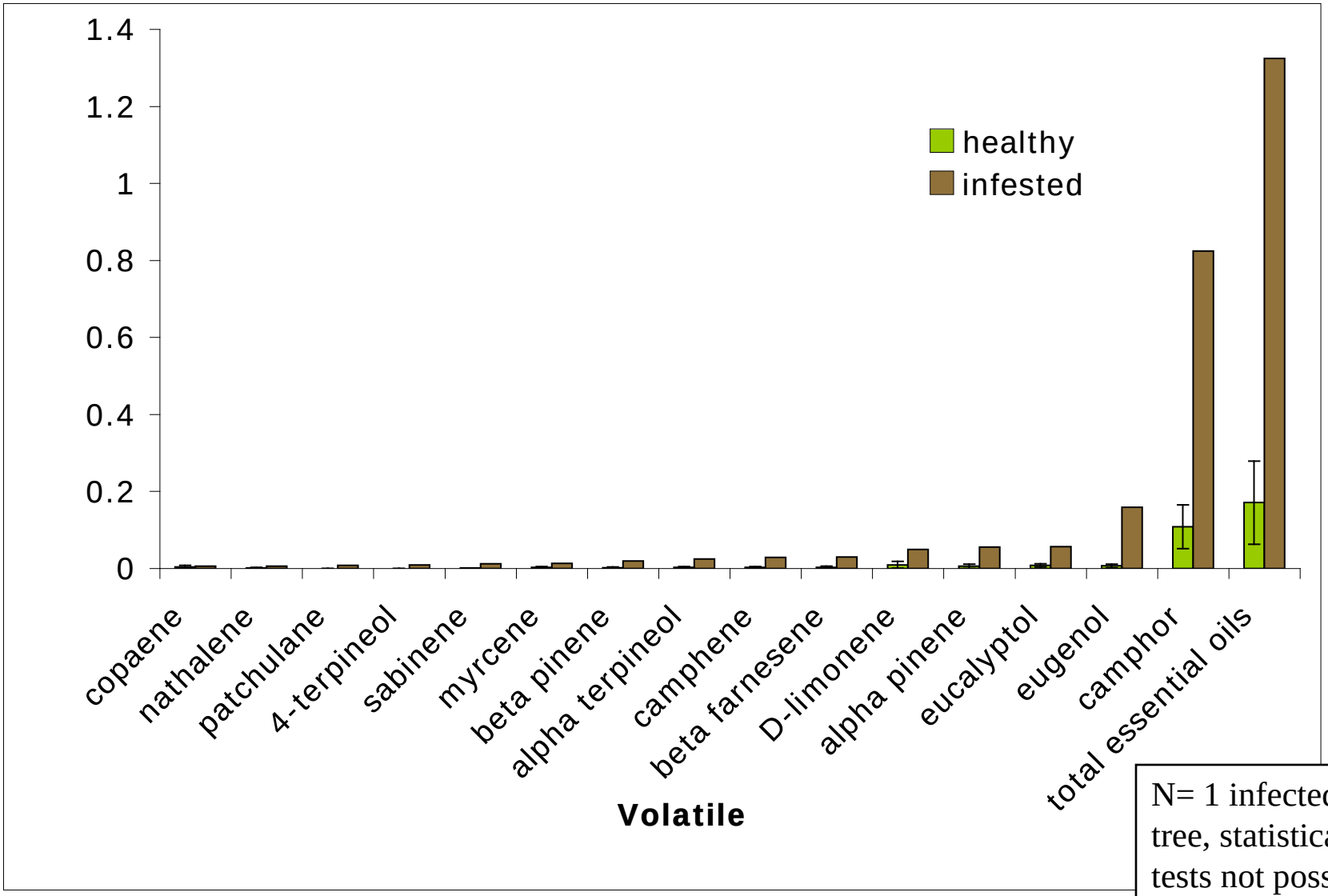
Volatile Concentrations in Healthy vs. Infected *Lindera melissifolia*



Volatile Concentrations in Healthy vs. Infected *Litsea aestivalis*



Volatile Concentrations in Healthy vs. Infected *Cinnamomum camphora*



N= 1 infected tree, statistical tests not possible

Similarity in volatile composition

compound	Redbay		Sassafras		Lindera		Litsea		Camphor		# types
	infected	*healthy*	*infected*	*healthy*	*infected*	*healthy*	*infected*	*healthy*	*infected*	*healthy*	
4-terpineol	x	x		x	x				x		5
alpha cubebene	x		x	x	x				x	x	6
alpha farnesene	x		x				x			x	4
alpha pinene	**x**	**x**	**x**	**x**	**x**	**x**		**x**	**x**	**x**	**9**
alpha terpineol	**x**	**x**	**x**	**x**	**x**	**x**			**x**	**x**	**8**
beta farnesene	x				x	x	x	x	x	x	7
beta linalool	x	x	x	x	x						5
beta pinene	x	x	x	x		x			x	x	7
borneol	x	x					x				3
bornyl acetate	x						x				2
calamenene	**x**		**x**		**x**		**x**				**4**
camphene	x	x							x	x	4
camphor	**x**	**x**	**x**				**x**		**x**	**x**	**6**
copaene	**x**		**x**	**x**	**x**	**x**		**x**	**x**	**x**	**8**
D-limonene	**x**	**x**	**x**	**x**	**x**	**x**			**x**	**x**	**8**
eucalyptol	**x**	**x**	**x**	**x**	**x**	**x**		**x**	**x**	**x**	**9**
fenchone	x						x				2
myrcene	**x**	**x**	**x**	**x**	**x**	**x**			**x**	**x**	8
p-cymene	x	x	x	x	x				x	x	7
sabinene	x	x			x	x			x	x	6
# cmpds	20	13	13	11	13	9	7	4	14	14	

Similarity in volatile composition

Compound	Redbay		Sassafras		Lindera		Litsea		Camphor		# types
	I	*H*	*I*	*H*	*I*	*H*	*I*	*H*	*I*	*H*	
alpha pinene	**x**	**x**	**x**	**x**	**x**	**x**		**x**	**x**	**x**	**9**
alpha terpineol	**x**	**x**	**x**	**x**	**x**	**x**			**x**	**x**	**8**
beta farnesene	x				x	x	x	x	x	x	7
beta linalool	x	x	x	x	x						5
calamenene	**x**		**x**		**x**		**x**				**4**
camphor	**x**	**x**	**x**				**x**		**x**	**x**	**6**
copaene	**x**		**x**	**x**	**x**	**x**		**x**	**x**	**x**	**8**
D-limonene	**x**	**x**	**x**	**x**	**x**	**x**			**x**	**x**	**8**
eucalyptol	**x**	**x**	**x**	**x**	**x**	**x**		**x**	**x**	**x**	**9**
myrcene	**x**	**x**	**x**	**x**	**x**	**x**			**x**	**x**	8
p-cymene	x	x	x	x	x				x	x	7
sabinene	x	x			x	x			x	x	6

Summary Findings from Part I

- Infested redbay have significantly more volatiles than healthy redbay
- Healthy *Lindera* have significantly more volatiles than infested *Lindera*
- Total % weight of volatiles in inner bark range from 0.03 - 1.4%
- Eucalyptol, D-limonene, alpha pinene, alpha terpineol, myrcene, copaene common to at least 8 of 10 sample groups
- Calamanene, camphor in 4 of 5 infected tree species

Part II: Aliens in a New Land: How do Asian Ambrosia Beetles Find Their Hosts?

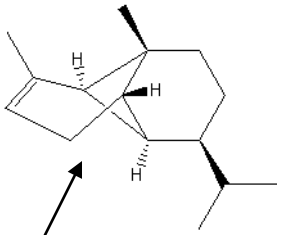
Objective: Part II

To test the response of *Xyleborus glabratus* to volatile compounds common to the host species (as determined in Part I) in a series of field trials.

Beta-pinene

camphor

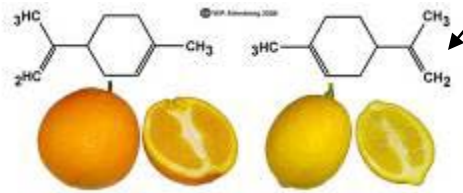
copaene



Eucalyptol

linalool

D-limonene



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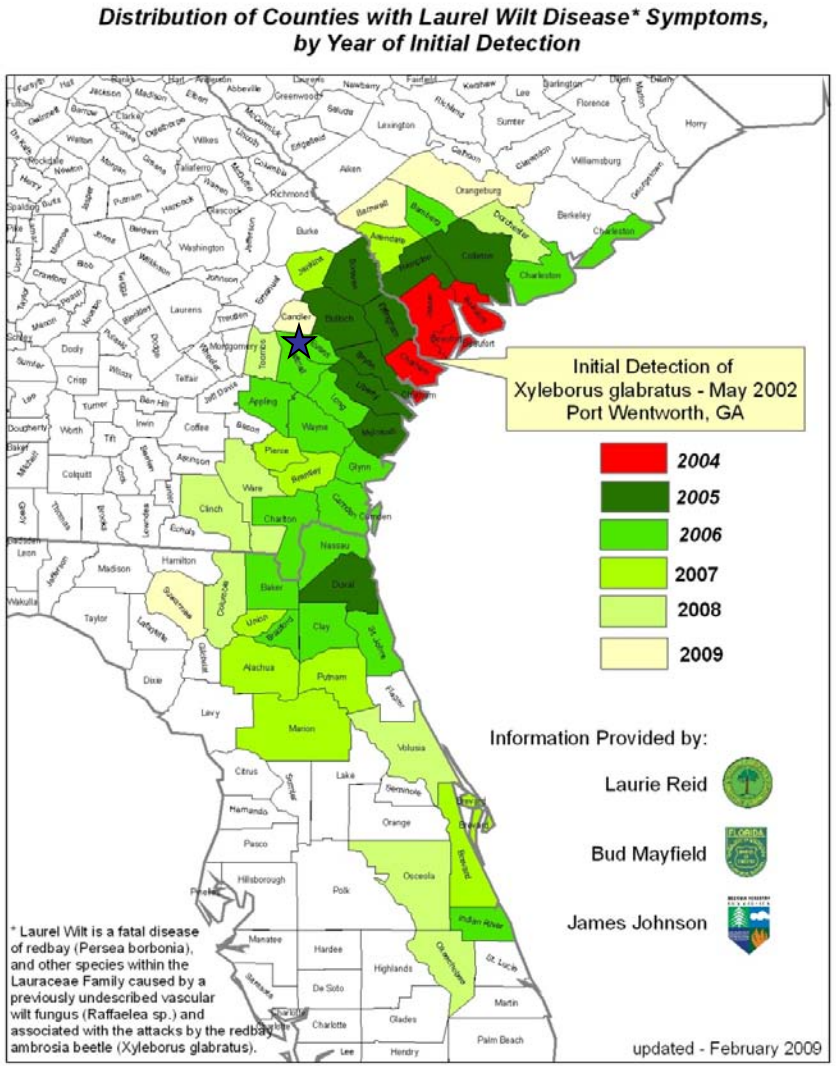
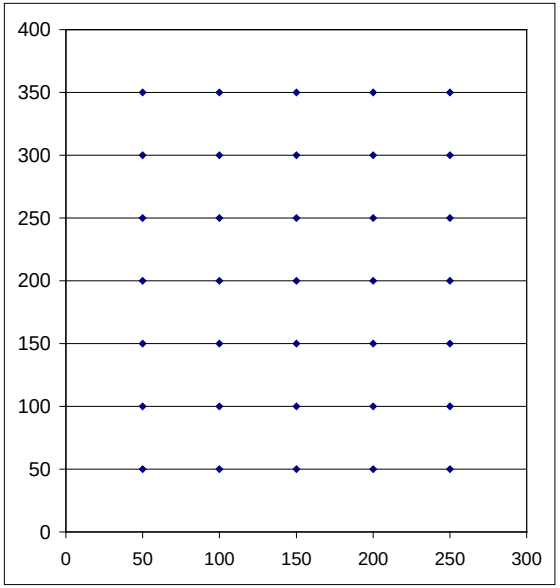
Study Site



- Privately owned site in Evans County, GA
- Massive redbay mortality due to Laurel Wilt Disease

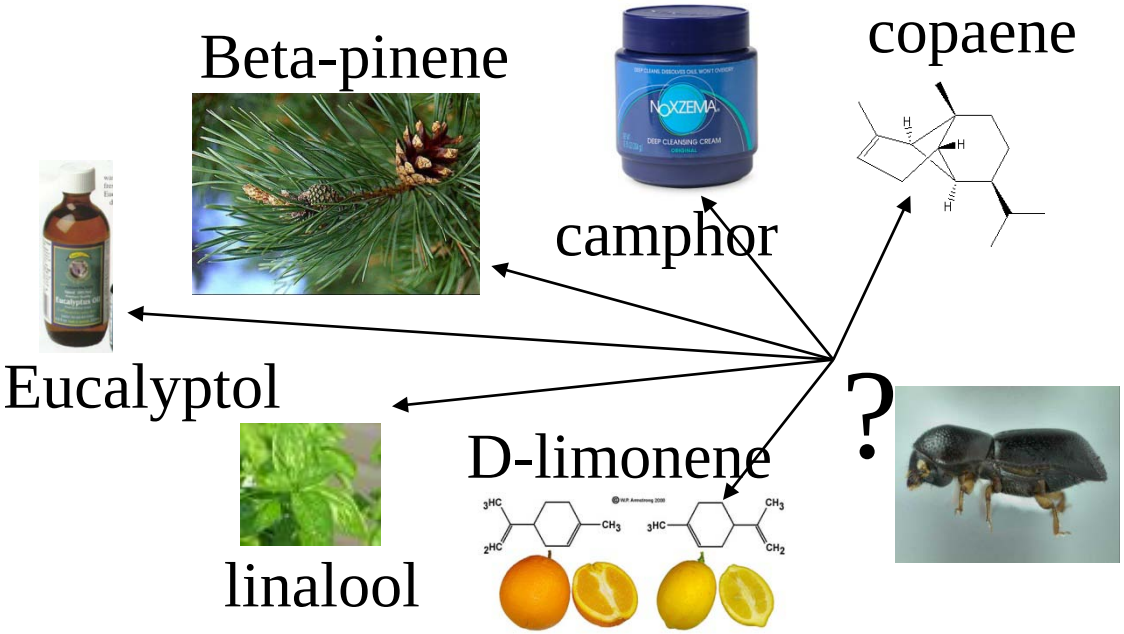
Experimental set-up

- Five foot PVC pipes at 50 meter intervals in 200 x 300 m grid: 35 traps (5 reps/ttmt)
- Sticky traps: laminated graph paper spread with tanglefoot
- Baited with vial loaded with solution in field, capped, with wick for elution
- Traps hung on post
- Collected two weeks from the day of set up



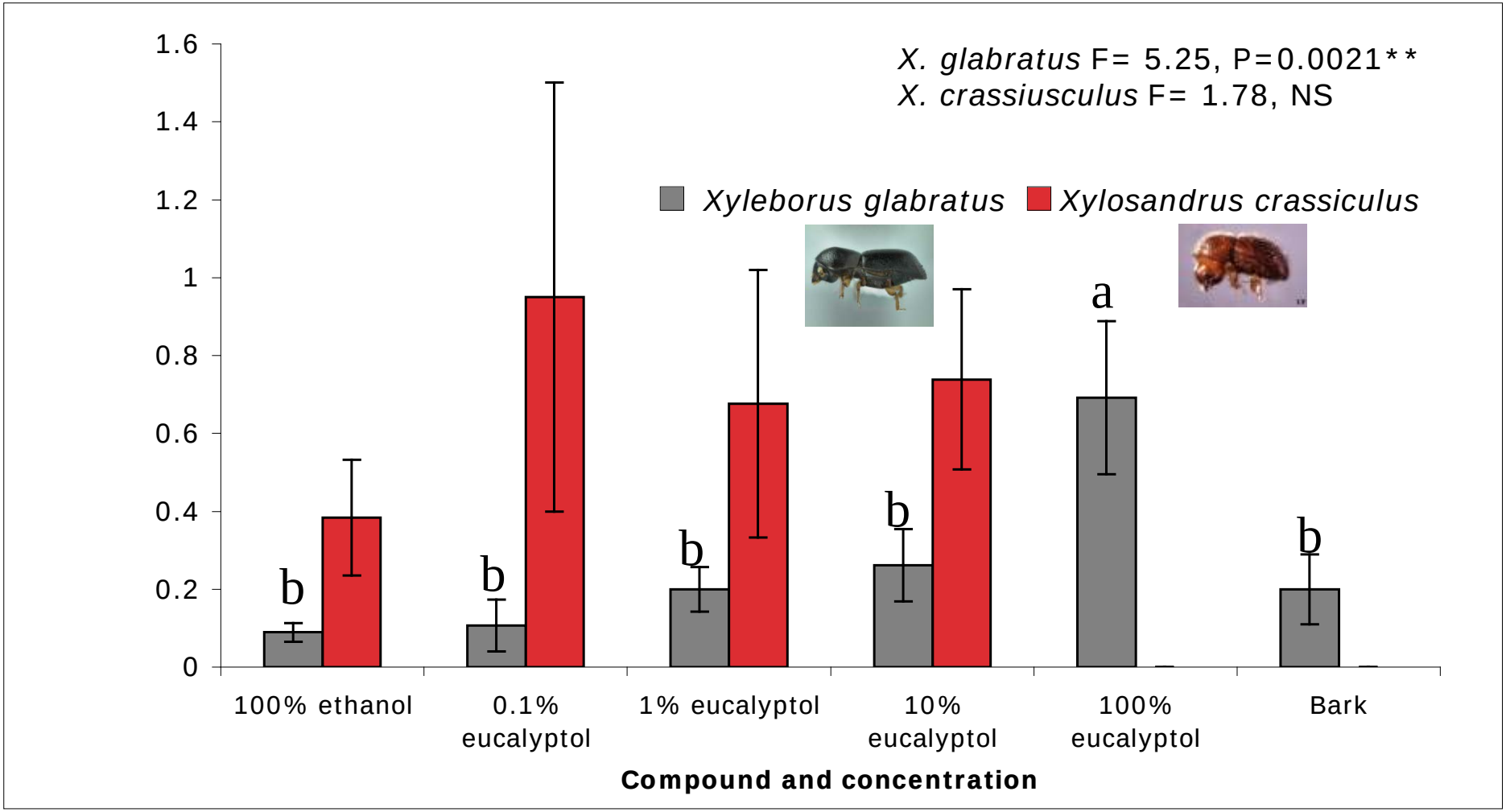
Experimental set-up

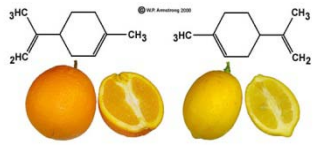
- Volatile compounds tested:
  - Eucalyptol
  - D-Limonene
  - Beta pinene
  - Beta linalool
  - Camphor
  - Manuka oil (copaene/calamenene)
- Controls:
  - Water
  - Bark
  - 100% Ethanol
- October - July 2007 - 2008



- Volatiles in concentrations of 0.1% - 100%
- Diluted with ethanol

Eucalyptol Trial





D-Limonene Trial

X. glabratus F= 1.45, NS
X. crassiusculus F= 1.07, NS

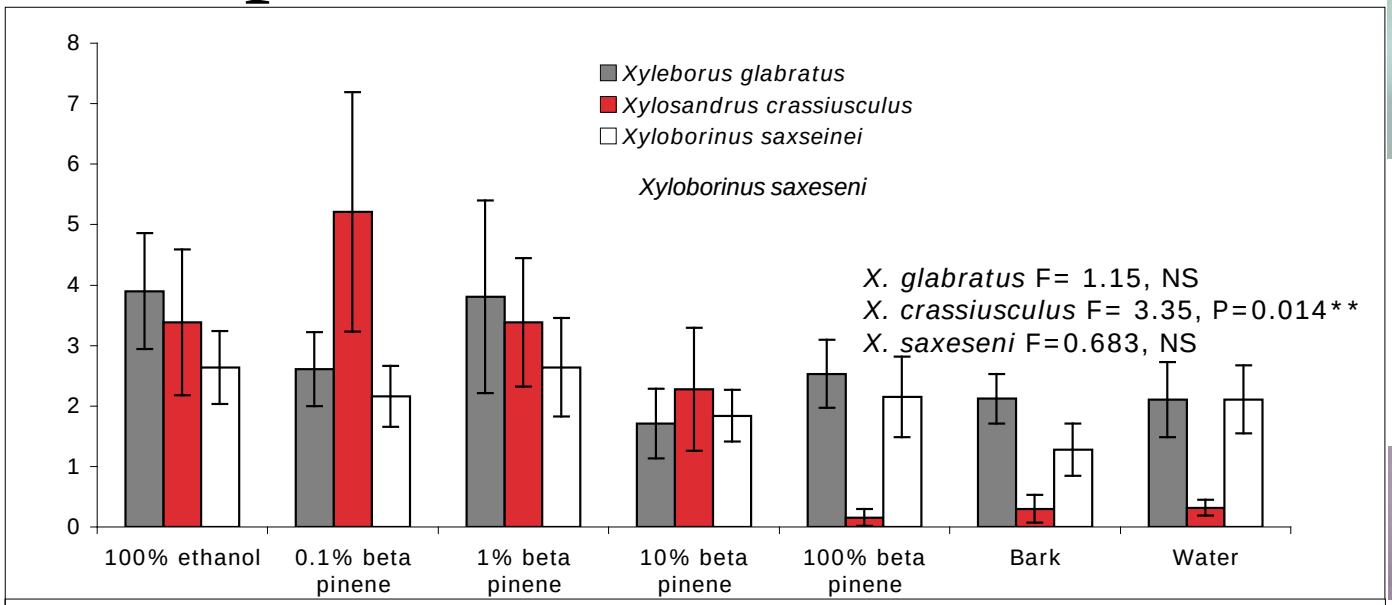
Xyleborus glabratus *Xylosandus crassiusculus*

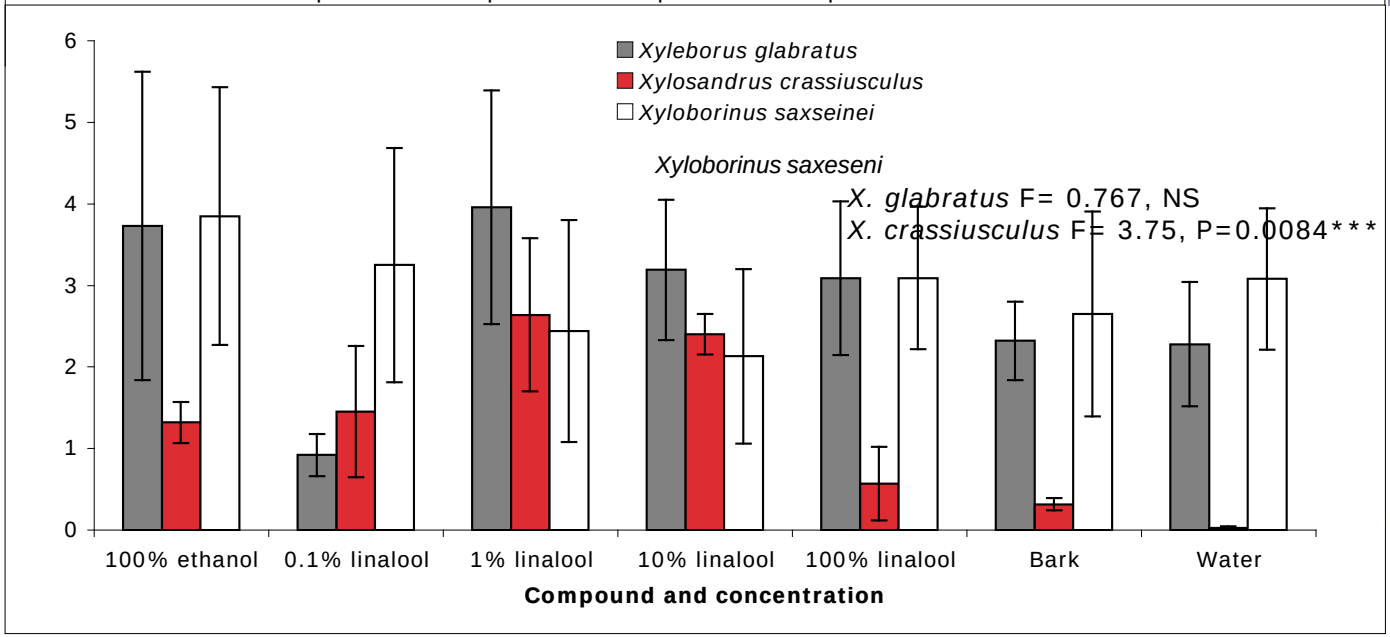
3.5
3
2.5
2
1.5
1
0.5
0

100% ethanol
0.1% limonene
1% limonene
10% limonene
100% limonene
Bark
Water

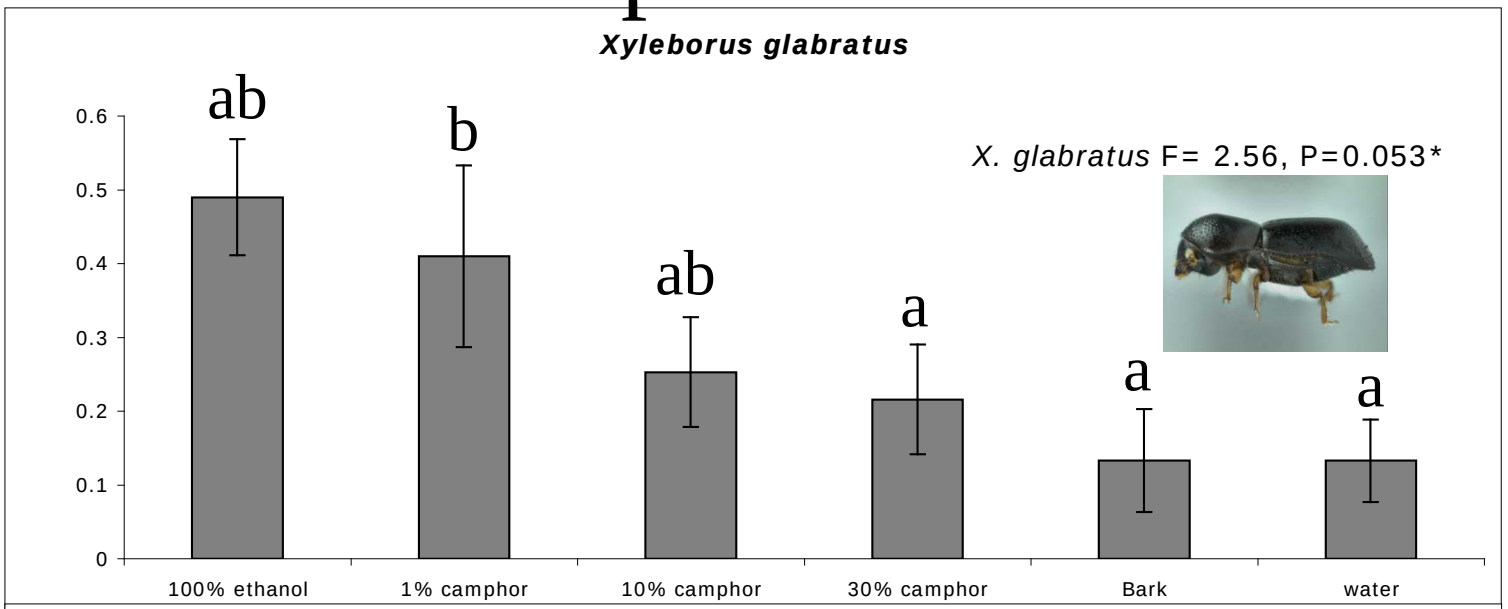
Compound and concentration

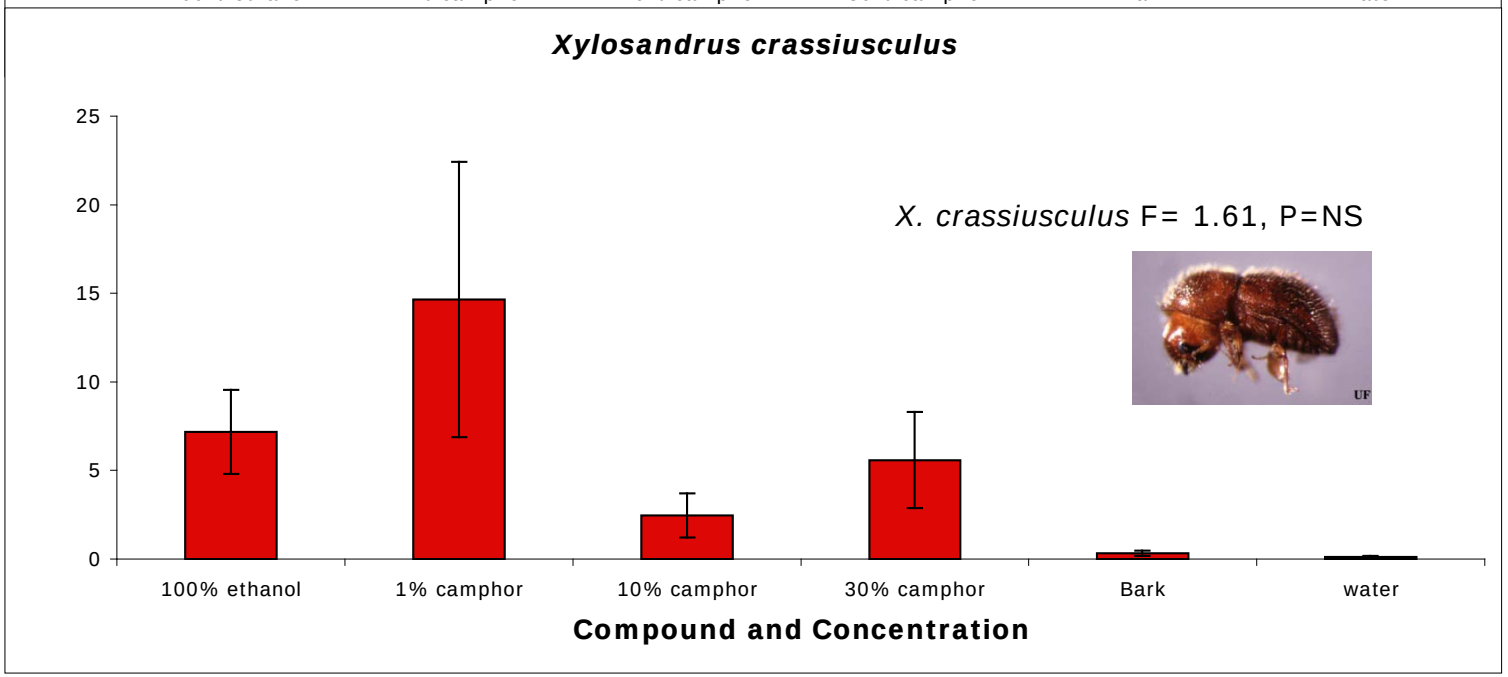
beta-pinenene & beta-linalool



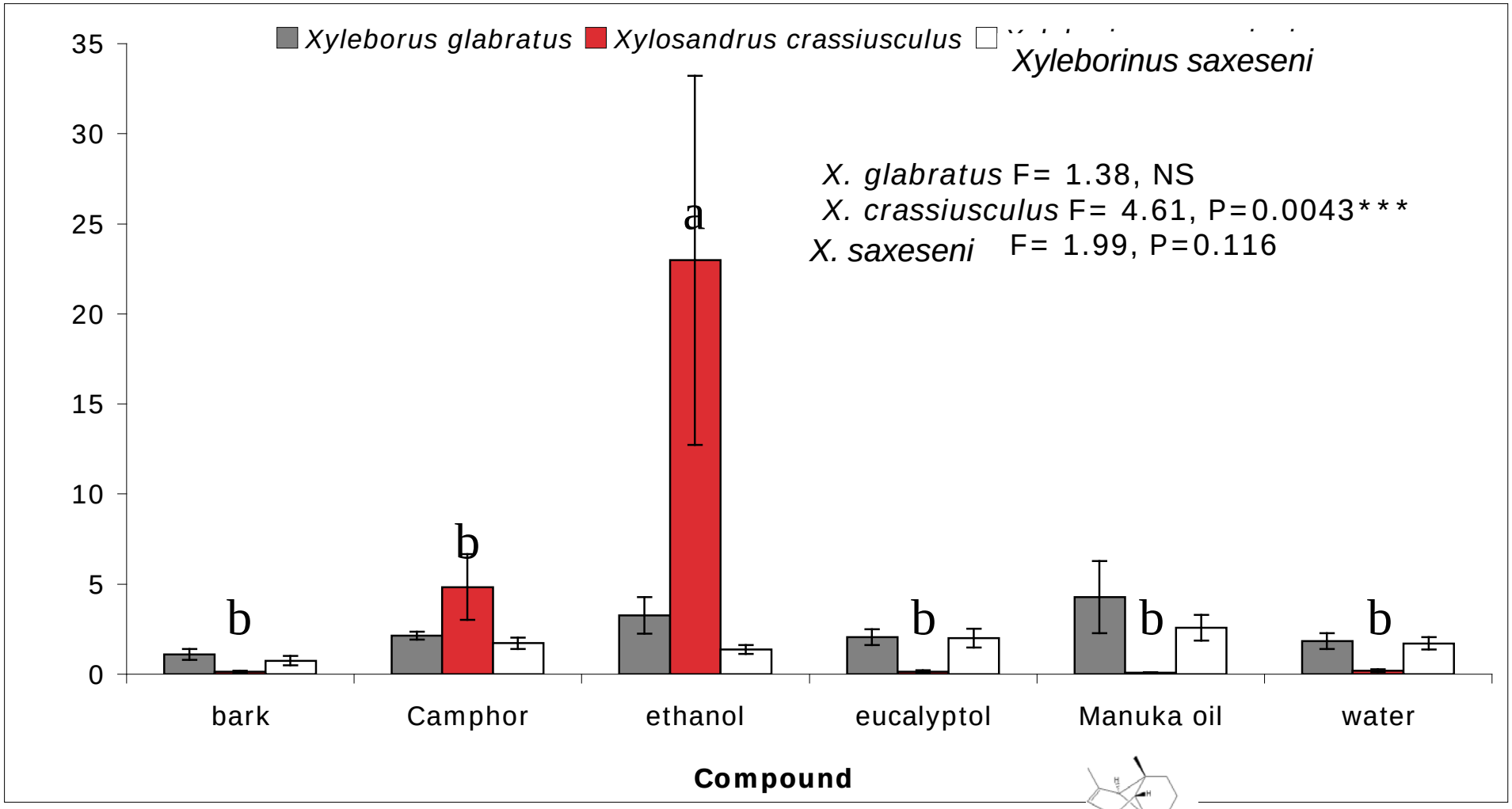


Camphor Trial

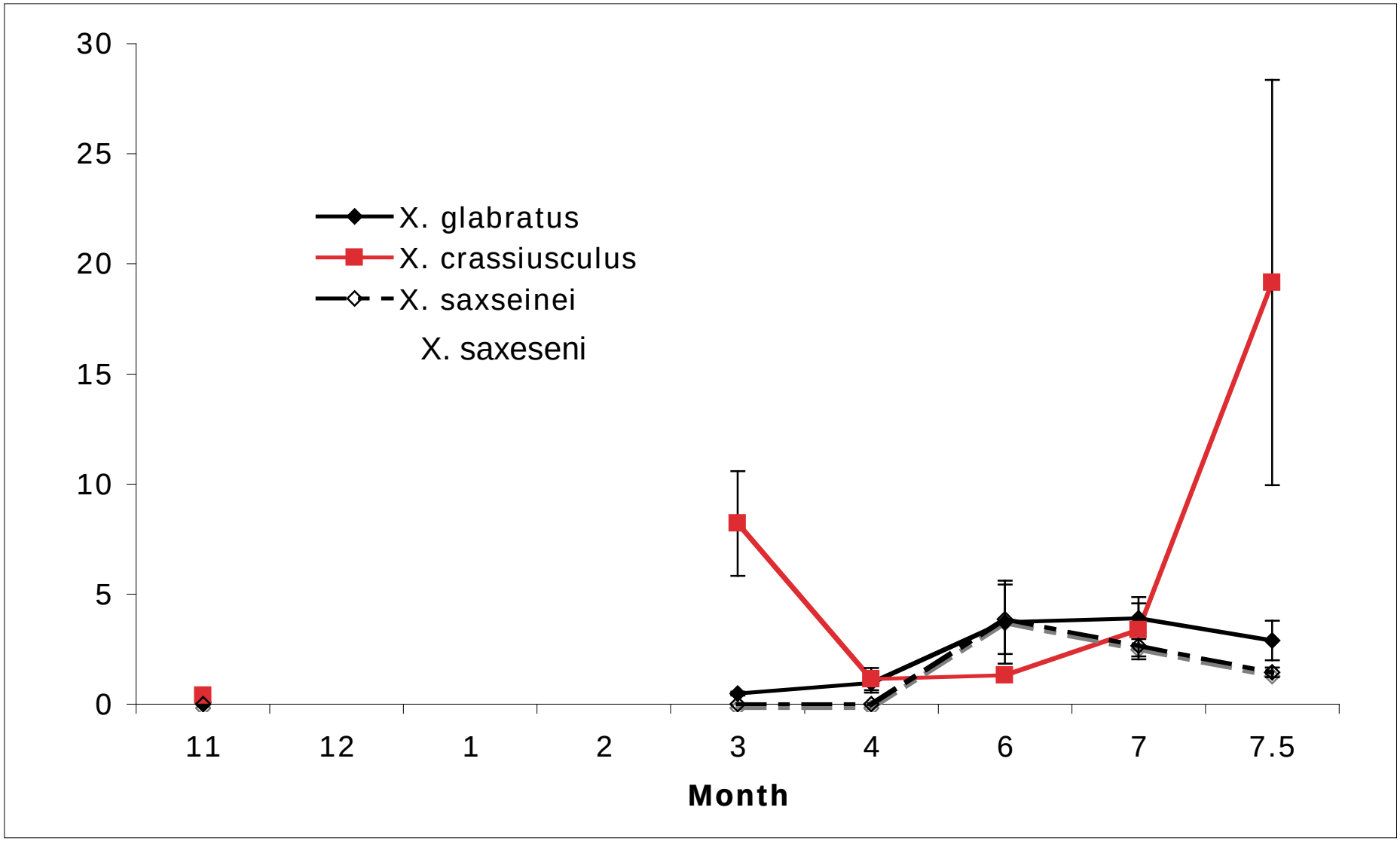




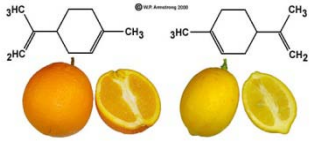
Choice Test

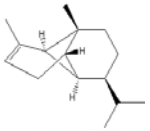

Xyleborus glabratus
Xylosandrus crassiusculus
Xyleborinus saxeseni
X. glabratus F= 1.38, NS
X. crassiusculus F= 4.61, P=0.0043***
X. saxeseni F= 1.99, P=0.116
35
30
25
20
15
10
5
0
a
b
b
b
b
b
bark
Camphor
ethanol
eucalyptol
Manuka oil
water
Compound

Seasonal Population Dynamics



Summary: Part II





			
Eucalyptol	+	0	0
D-limonene	0	0	0
Manuka oil (copaene/ calam.)	+?	0	0
Camphor	-	0	0
B-pinene	0	0	0
B-linalool	0	+	0

Conclusions: Part II

- *X. glabratus* responds positively to eucalyptol (and possibly manuka oil), negatively to camphor, and does not response to any other chemical tested.
- Volatile chemicals are known to have defensive qualities
  - Camphor can kill termites, mice, bacteria and fungus
- Trees with higher levels of camphor and lower levels of eucalyptol (and possibly copaene) may be better protected from /less detected by *Xyleborus glabratus*
- Eucalyptol and manuka oil trials should be repeated for consistency

Acknowledgements

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  - Juliette Jordan, Wendy Wilson, Monique Copeland, Kimberly Spiegel and JaNee Demery
- NSF- REU program

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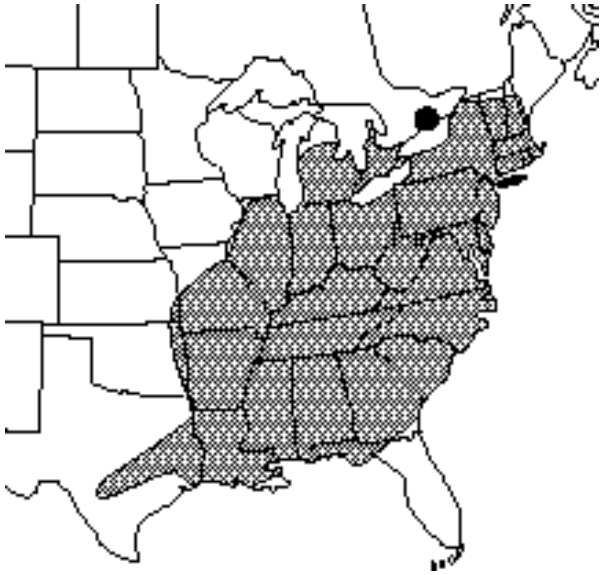
Distribution of other Lauraceae

- *Lindera benzoin (*Spicebush)
  - Shrubs or small trees to 5m
  - Streambanks, low woods, wetland margins, uplands



The native range of Lindera benzoin

- *Lindera subcoriacea*
  - Low shrubs 2m: 4m when shaded
  - Low woods, depressions, pond and sink margins

The native range of Lindera subcoriacea