Chemical Ecology of *Xyleborus glabratus* and Implications for Monitoring and Management

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Conference on Laurel Wilt Disease and Natural Ecosystems: Impacts, Mitigation, and the Future

16-18 June 2015

Applied Approach

- Target = Adult female RAB, host-seeking flight
- Dispersal flight is risky
  - Harsh environment (dessication, wind, rain)
  - Predation (e.g. dragonflies)
  - No food available
- Strong selection
  - Reliable cues to find host trees quickly
  - Optimize timing of flight to minimize risks

Chemical Ecology of RAB

- Initial research on RAB attractants
  (Hanula et al. 2008, Hanula & Sullivan 2008)
  - No evidence of pheromones (aggregation or sex)
  - No long-range attraction to fungal symbionts
  - No attraction to ethanol (standard lure for ambrosia beetles)
- Conclusions:
  - RAB can attack healthy, unstressed trees
  - Host tree volatiles = primary attractants
  - α-copaene, calamenene are likely attractants from redbay; but cost/availability make them impractical for field lures
  - Manuka and phoebe oils identified as attractive baits for RAB

ARS - Miami,FL

Research - RAB attractants (2009-pres.)

Multidisciplinary approach

- Field tests (forest sites)
- Lab tests (Archbold Biol. Station, Lake Placid)
  - Behavioral bioassays (attraction, boring preferences)
  - Electrophysiology techniques to measure olfactory responses
- Chemical sampling & analysis (Miami)
  - Volatile collections
  - Gas chromatography-mass spectrometry (GC-MS)

1. Method for Capture of Live RAB

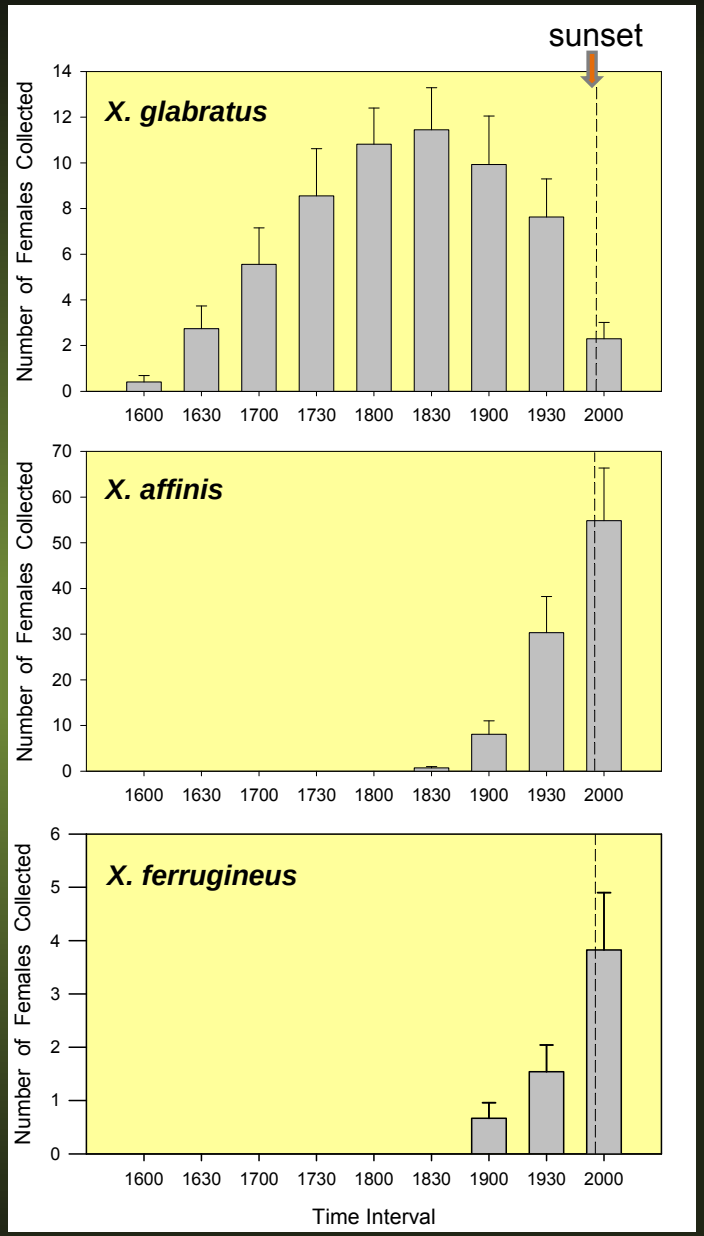
General Method

- Late afternoon (~4:00 pm)
- Lay cotton sheet on ground
- Bait = Freshly-cut host wood
- “Lure in” ambrosia beetles
- Collect with soft brush

Kendra et al. 2012 FL Entomol.

Unique Observation: Temporal Separation of Beetle Species

- Multiple species attracted to wood volatiles
- *X. glabratus* flies earlier
  (27 collection dates, Apr-Oct 2011)
- Useful method for obtaining RAB in host-seeking behavior, the perfect stage to evaluate attractants
  - Lab bioassays
  - Electrophysiology studies



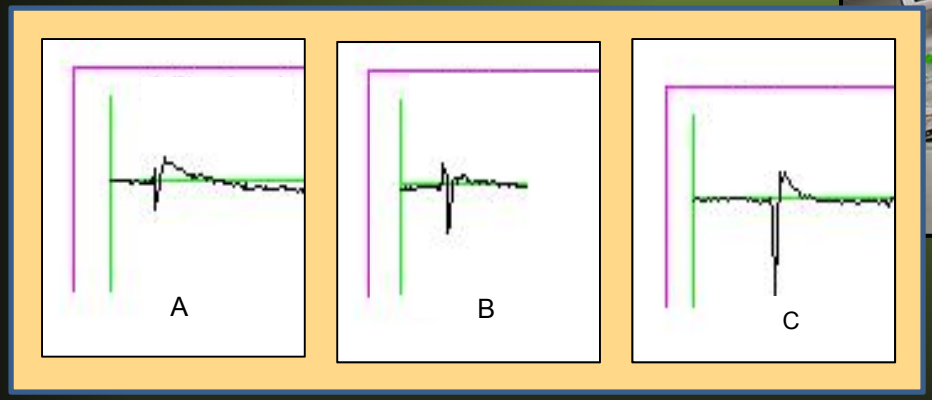
Kendra et al. 2012 Environ. Entomol.

2. Electroantennography (EAG)

Technique to measure olfactory response of antennal receptors (How well does an insect smell a particular chemical?)

- Mount antenna between 2 electrodes
- Place under purified air flow
- Deliver chemical sample
- Measure receptor potential

EAG lab at Archbold Biol. Station

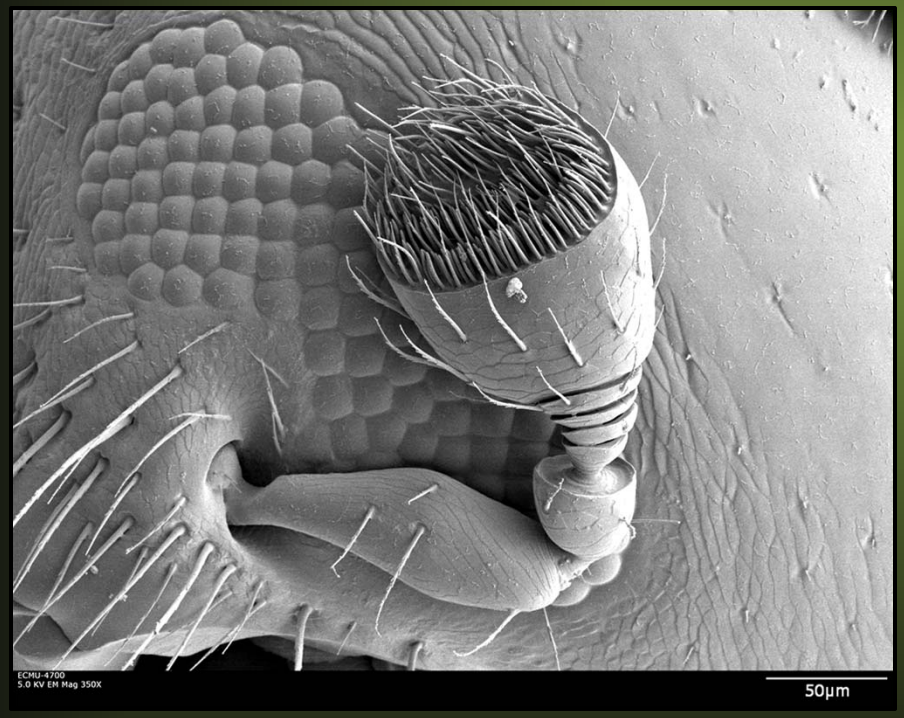


Sample EAG recordings from single RAB antenna

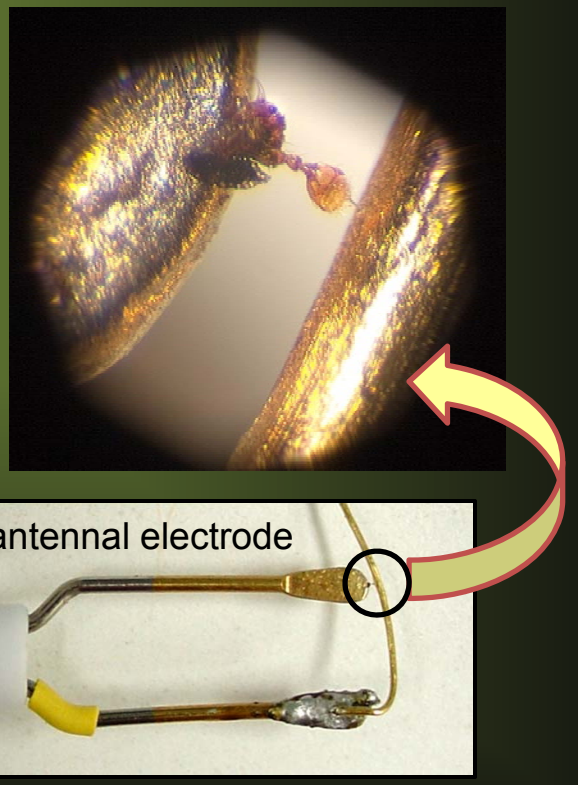
EAG Technique

Major challenge: small size of RAB antenna (0.3-0.4 mm length)

- Used a gold, 2-pronged electrode
- Electrode modified by attaching a flexible gold wire to one prong



Scanning electron micrograph of RAB antenna

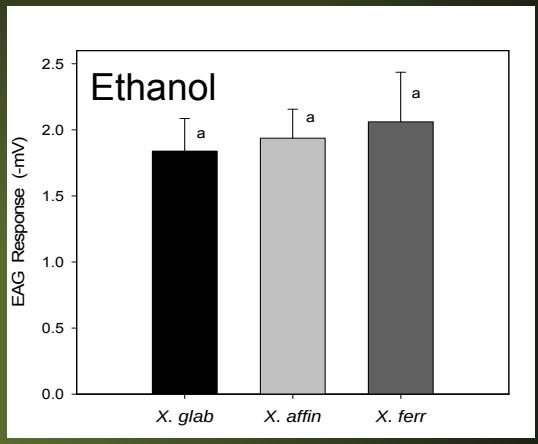


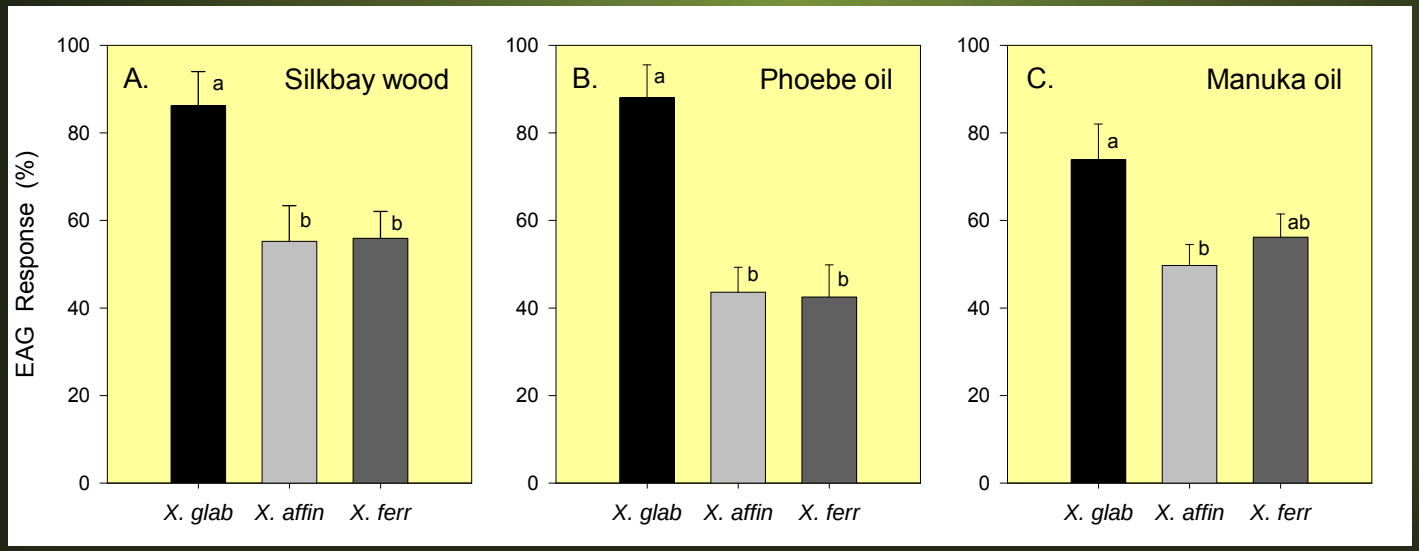
Validation of New EAG Methods

Comparative studies with 3 species:

X. glabratus, X. affinis, and *X. ferrugineus*

- Ethanol = standard reference chemical
- EAG responses to test chemicals could then be expressed as a percentage relative to ethanol





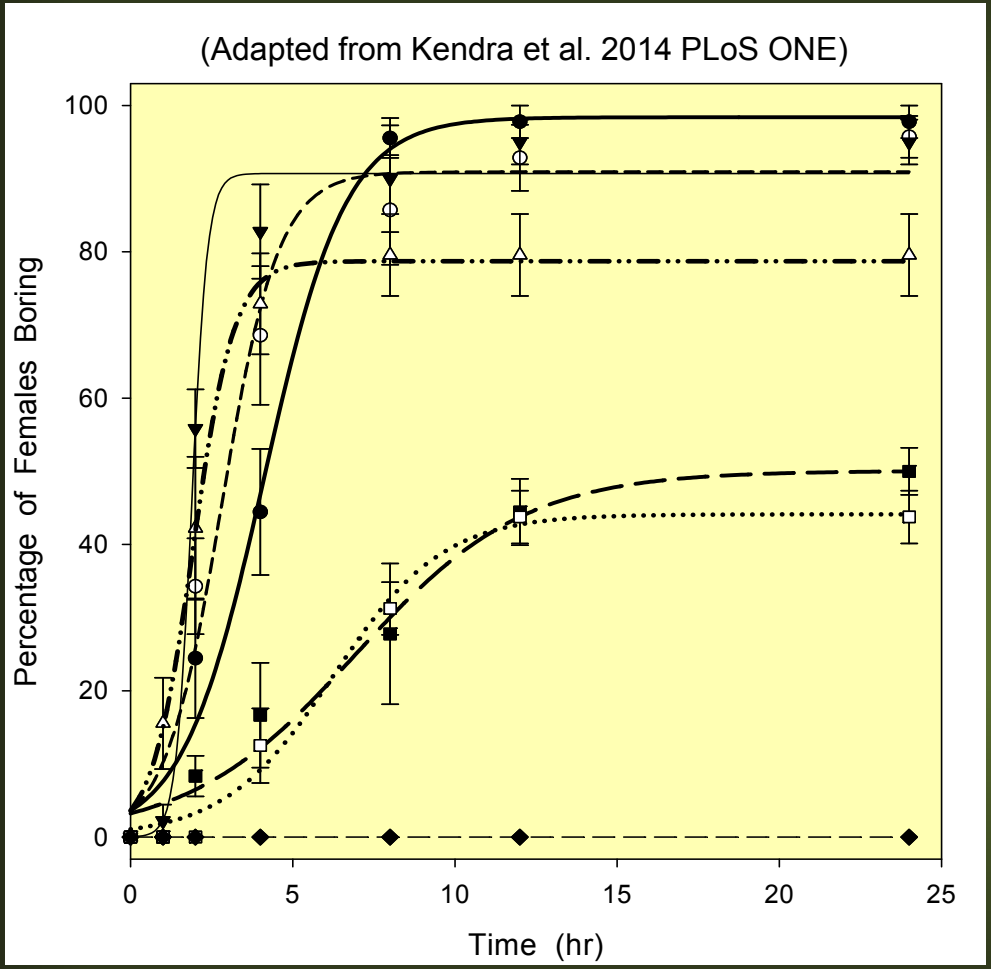
Kendra et al. 2012. Environ. Entomol.

3. Bolt Boring Bioassay

- No-choice tests to assess RAB boring behaviors
  - 1-gallon bucket
  - 1 bolt plus 10-15 female RAB
  - Recorded # RAB boring and location on bolt after 2, 4, 6, 8, 24 hr
    (Positive boring = half of the body inserted)
  - Replicated 5x

Kendra et al. 2013 FL Entomol.

Composite Results – Bioassays



Silkbay (99%)

Swampbay/Redbay (91%)

Avocado (80%)

Camphor tree (50%)

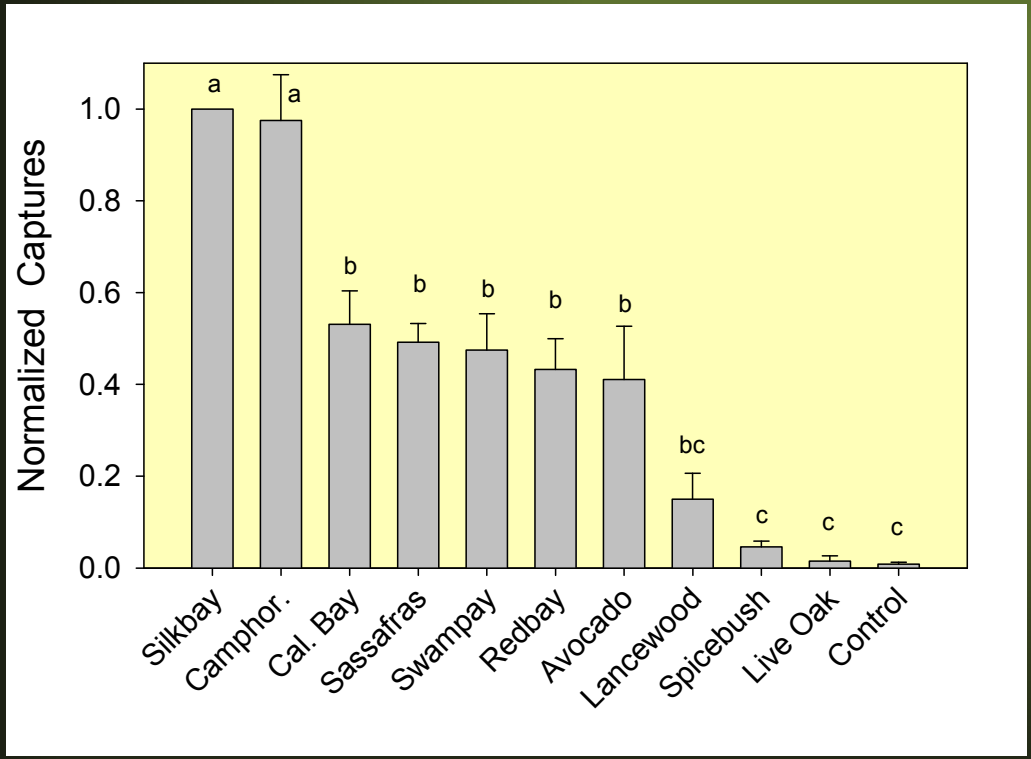
Lancewood (44%)

Live Oak (0%)

>70% boring on cut end; trees most susceptible to attack after pruning or injury.

4. Comparative Field Tests

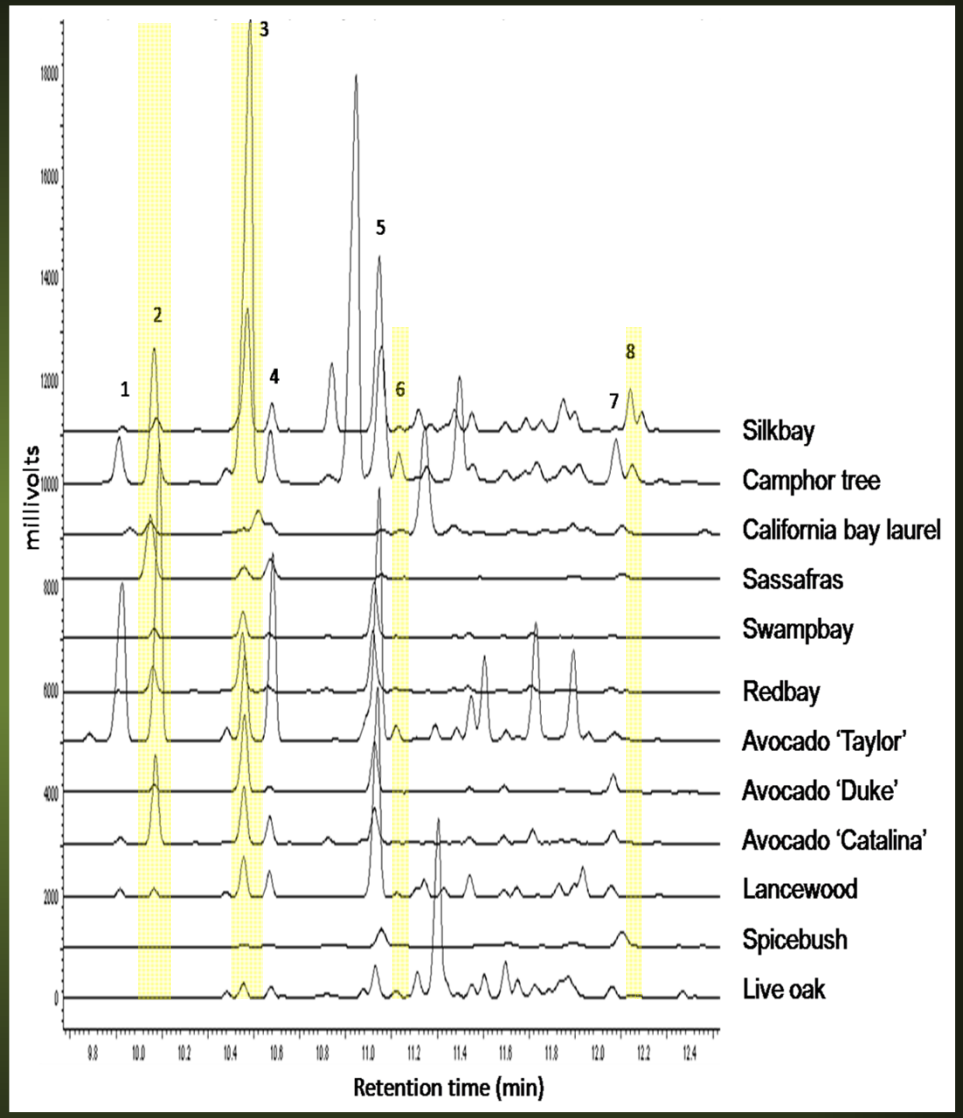
- Replicated field tests with freshly-cut wood bolts
- Silkbay most attractive = positive control; captures normalized
- Determined relative attraction of 9 species within Lauraceae



Kendra et al. 2014 PLoS ONE

5. Chemical Analysis

- Emissions of terpenoids from bolts correlated with RAB captures in field
- 4 sesquiterpenes
  - Major
    - α-cubebene (peak 2)
    - α-copaene (peak 3)
  - Minor
    - α-humulene (peak 6)
    - calamenene (peak 8)



Kendra et al. 2014 PLoS ONE

Current Hypothesis - Host Location, Acceptance

Long-range: RAB in flight attracted to the odor plume from host trees.
Terpenoids: α-copaene, α-cubebene, α-humulene, calamenene (Hanula & Sullivan 2008, Kendra et al. 2011, 2014); eucalyptol (Kuhns et al. 2014); other monoterpenes (Martini et al. 2015)

Mid-range: RAB uses visual cues to find hosts of appropriate diameter (Mayfield and Brownie 2013)

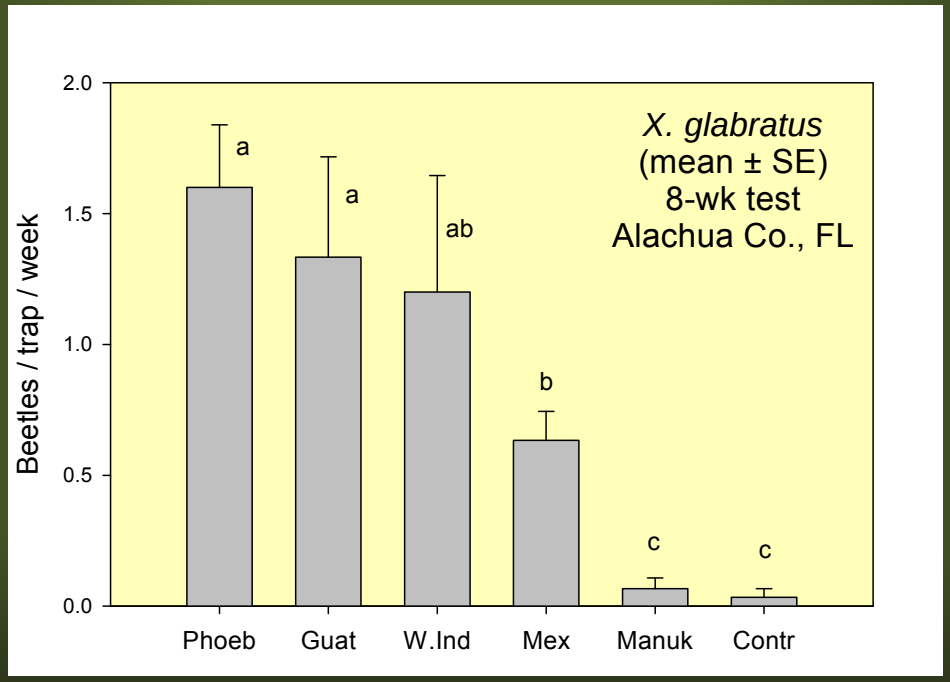
Short-range: RAB detects (1) terpenoid gradients to find best site for attack (avocado: α-copaene and α-cubebene) (Niogret et al. 2013), and (2) fungal odors = food attractants (Hulcr et al. 2011, Kuhns et al. 2014)

On contact: RAB 'taste' and 'feel' the wood to confirm suitability (eucalyptol, Kuhns et al. 2014)

Summary: Host must smell, look, taste, and feel 'right' before boring initiated.

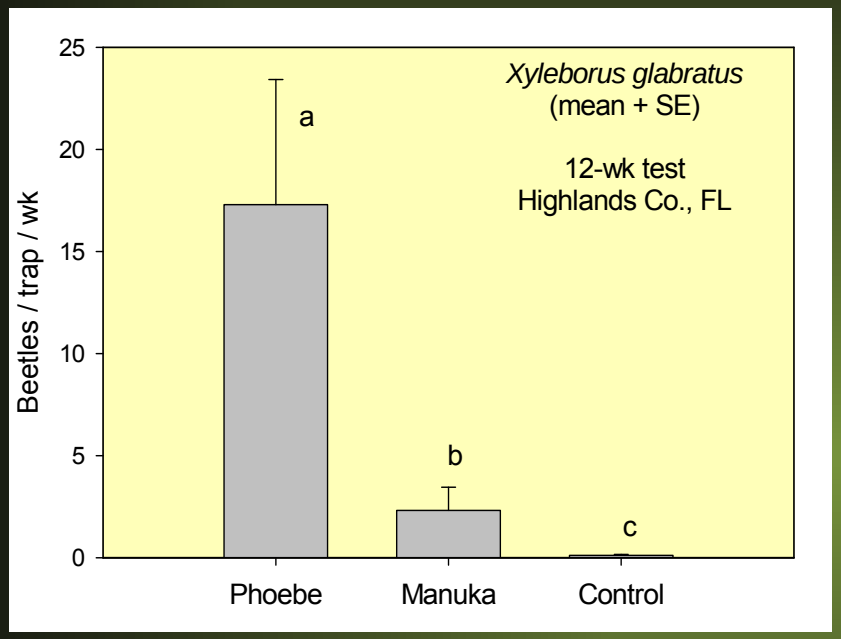
Development of Field Lures for RAB

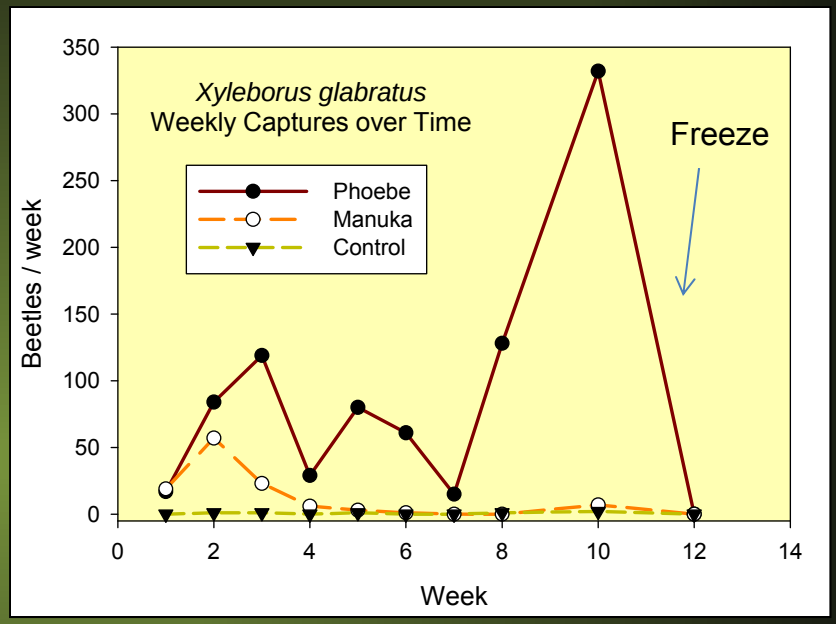
- First lures = phoebe oil, manuka oil (Hanula & Sullivan 2008)
- However, tests in FL (2009-2010) indicated that manuka lures not very effective



(Kendra et al. 2011. J. Chem. Ecol.)

Follow-up Tests Confirmed Results





(Kendra et al. 2012. J. Econ Entomol.)

- Phoebe caught 6X more RAB
- Manuka lost attraction after 3 wk
- Unfortunately, phoebe lures no longer available, manuka lures only option for RAB monitoring

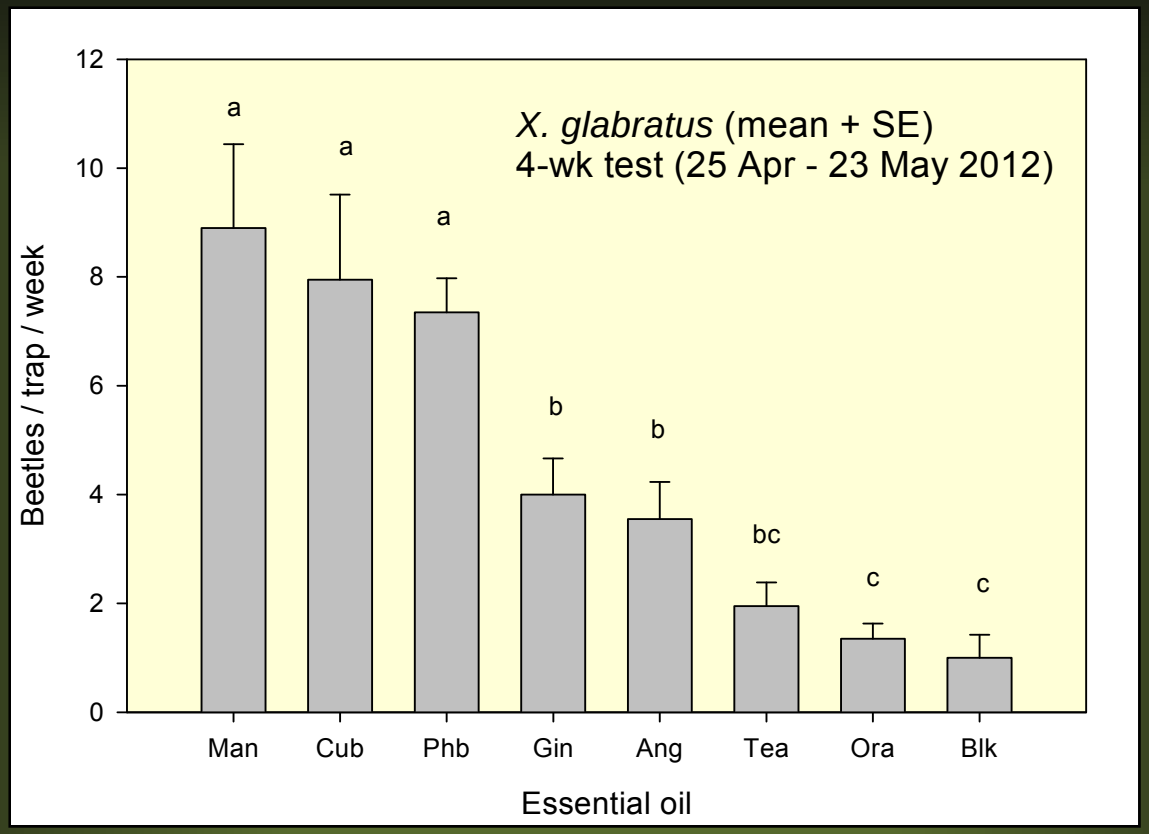
Evaluation of Other Essential Oils

Field tests in 2012 compared 7 essential oils for RAB attraction:

(Homemade lures – 2 ml oil)

- Manuka
- Phoebe
- Cubeb
- Ginger root
- Angelica seed
- Tea tree
- Valencia orange

(Agricultural Research 2012)



(Kendra et al. 2013. Am. J. Plant Science)

Cubeb oil identified as a new RAB attractant

(from berries of tailed pepper *Piper cubeba*)

Field Tests 2013

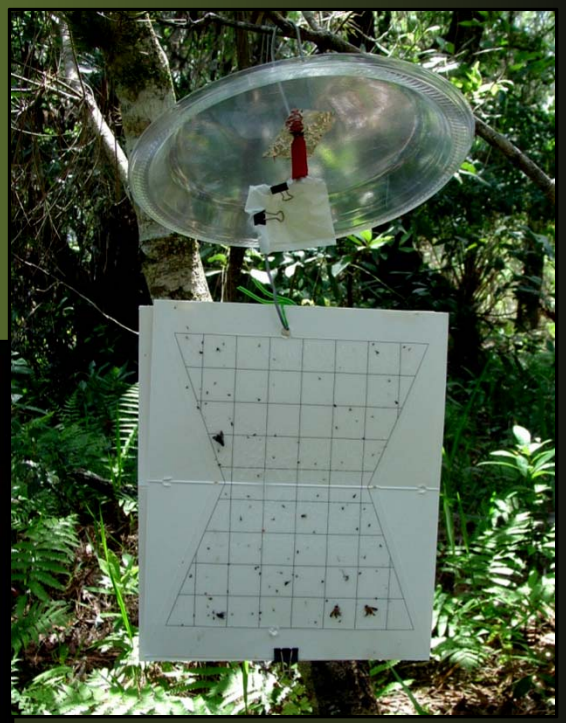
Collaboration with Synergy Semiochemicals Corp. (BC, Canada)

New lure = distilled cubeb oil in bubble lure

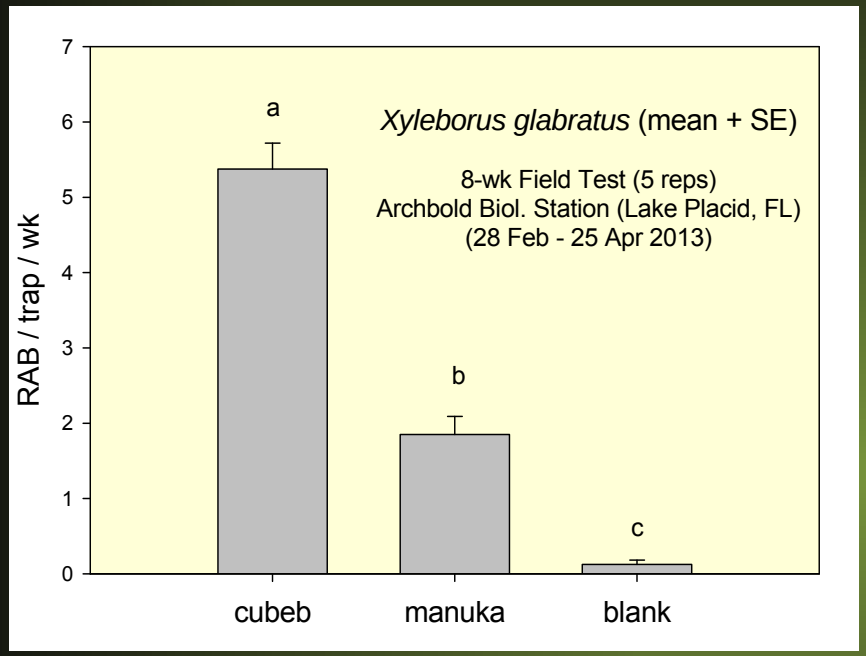
(enriched in sesquiterpenes)

Field tests to compare efficacy and longevity of commercial lures:

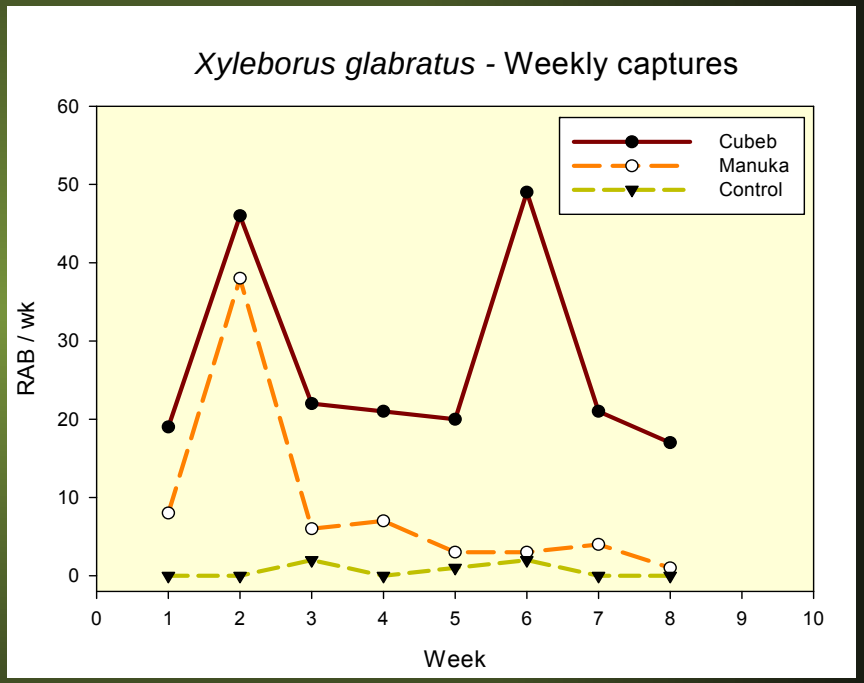
- Cubeb bubble lure
- Manuka lure
- Phoebe lure
- Unbaited control



Test 1 (Highlands County, FL)

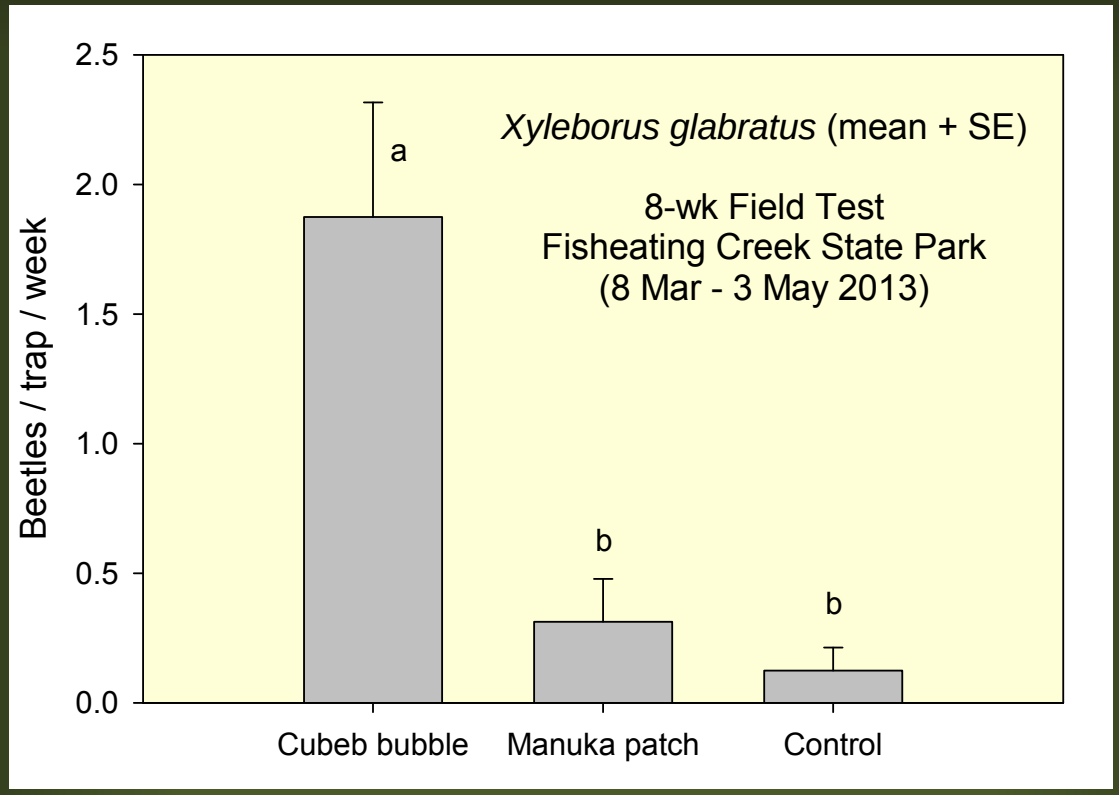


(Kendra et al. 2014. J. Pest Science)



- Cubeb bubble lure captured 3 times more RAB than manuka

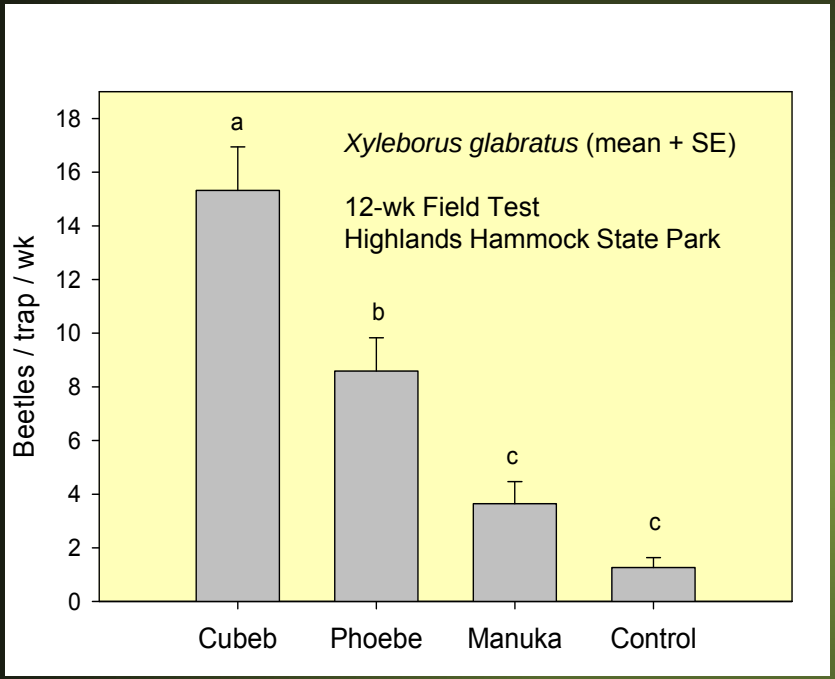
Test 2 (Glades County, FL)

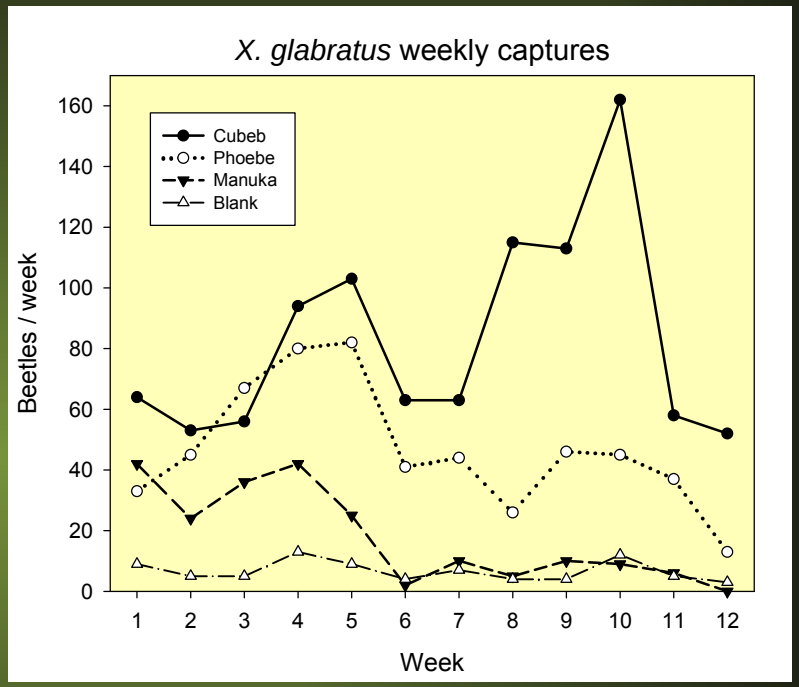


(Kendra et al. 2014. J. Pest Science)

- At very low population levels, cubeb bubble lure captured 6 times more RAB than manuka

Test 3 (Highlands County, FL)





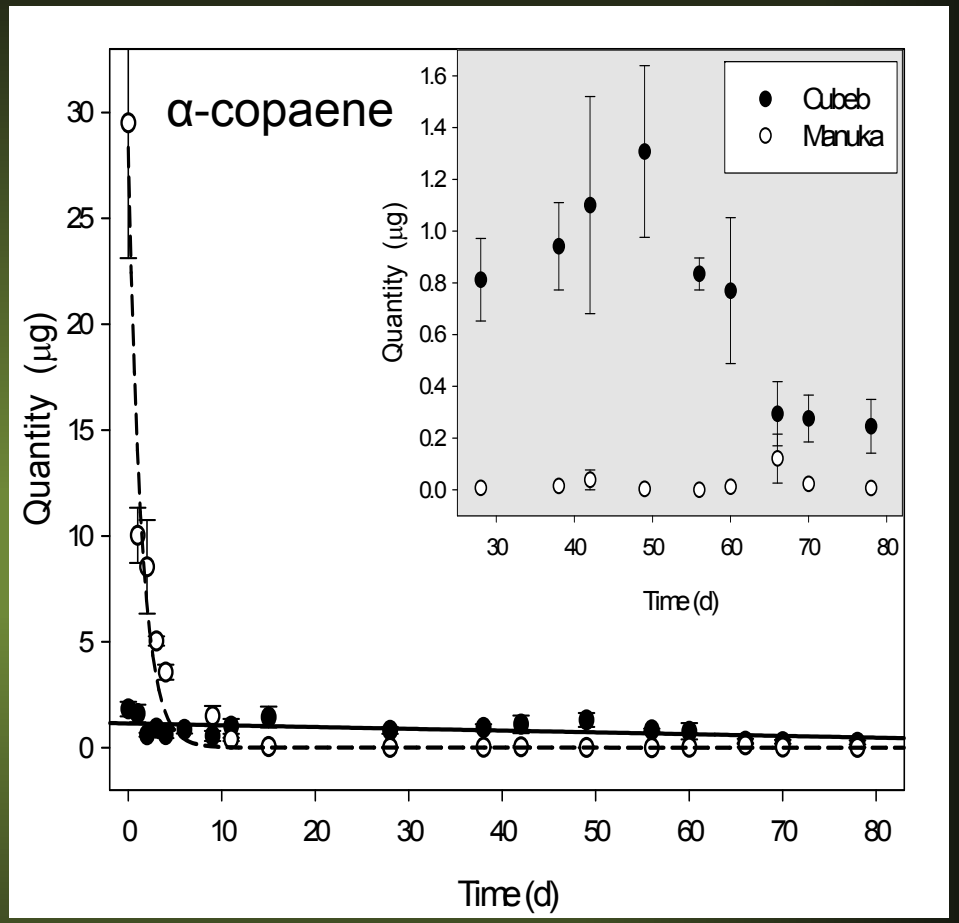
(Kendra et al. 2015. J. Econ. Entomol.)

- Cubeb > phoebe > manuka
- Cubeb field life of 3 months

Lure Emissions

- Manuka:
  High initially, expon. decay
  (large surface area: volume ratio)

- Cubeb:
  Low initially, slow, steady release
  (smaller surface area: volume ratio)



(Kendra et al. 2015. J. Econ. Entomol.)

Conclusions

Cubeb bubble lure

- More attractive than the manuka lure
- Has a field life of at least 3 months, due to extended low release of sesquiterpenes
- Best product currently available for detection of RAB

  (and less expensive than the manuka lure)

However, cubeb lure contains a complex mixture of terpenoids; specific attractive chemicals had not been confirmed.

Further Improvement of RAB Lure

(Collaboration with Synergy)

Goal = Elucidate the primary attractants in cubeb oil.

- Used fractional distillation to separate whole oil into 17 fractions (based on chemical boiling point)
- Fractions formulated as bubble lures
  - EAG analyses
  - 2-choice bioassays
- Only 2 fractions were significantly attractive to RAB; both were high in α-copaene and α-cubebene
- Decided to focus on α-copaene first

New Lure Evaluations

Two prototype lures prepared

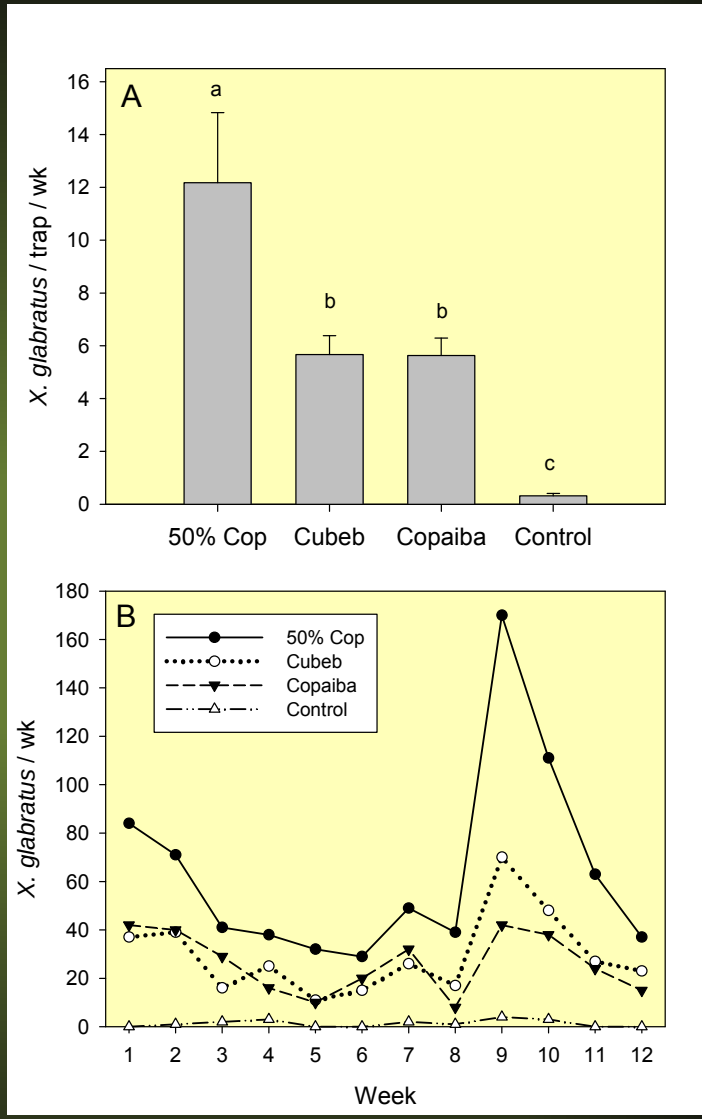
- Copaiba oil
  (9% α-copaene, no cubebene)
- Proprietary oil product
  (50% α-copaene)

12-wk field test

- Compare new lures to cubeb lure
  (10% α-copaene, 10% α-cubebene)

Conclusions

- (-) α-copaene = primary attractant
- Increased copaene = improved lure



Kendra et al. J. Pest Sci. (in review)

THANK YOU!

Sources of Funding

- USDA National Plant Disease Recovery System
- Florida Avocado Administrative Committee

Recent Publications

- Kendra, P. E., W. S. Montgomery, J. Niogret, E. Q. Schnell, M. A. Deyrup, and N. D. Epsky. 2014. Evaluation of seven essential oils identifies cubeb oil as most effective attractant for detection of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). J. Pest Sci. 87: 681-689.

- Kendra, P. E., W. S. Montgomery, J. Niogret, G. E. Pruett, A. E. Mayfield III, M. MacKenzie, M. A. Deyrup, G. R. Bauchan, R. C. Ploetz, and N. D. Epsky. 2014. North American Lauraceae: Terpenoid emissions, relative attraction, and boring preferences of redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). PLoS ONE 9 (7): e102086.

- Kendra, P. E., J. Niogret, W. S. Montgomery, M. A. Deyrup, and N. D. Epsky. 2015. Cubeb oil lures: Terpenoid emissions, trapping efficacy, and longevity for attraction of redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae). J. Econ. Entomol. 108: 350-361.

- Kendra, P. E., W. S. Montgomery, M. A. Deyrup, and D. Wakarchuk. Improved lure for redbay ambrosia beetle developed by enrichment of α-copaene content. J. Pest Sci. (in review).