Chemical and Canine Analysis as Complimentary Techniques for the Identification of Active Odors in an Invasive Agricultural Pest in the USA

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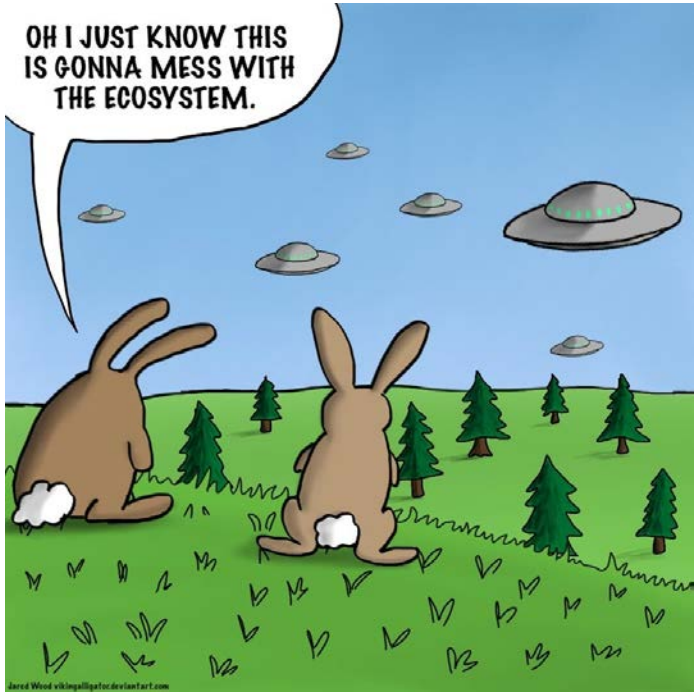
Outline

- Background
  - Environmental substance detection
  - Laurel wilt disease
- Chemical analysis
  - VOC identification through HS-SPME-GC-MS
- Column vent development
- Canine trials
- Conclusions

Effects of invasive species to the US

- Over 50,000 alien species in the US costing $120 billion per year
  - There are about 750,000 native species
- 42% of species on the threatened or endangered lists are at risk
- Invasive crop plant pathogens
  - $21.5 billion total losses per year
- Total plant pathogens
  - $33 billion total losses per year



Pimental, D.; Zuniga R.; Morrison, D. *Ecological Economics* **2005**, 52.3.

Canine detection and environmental protection

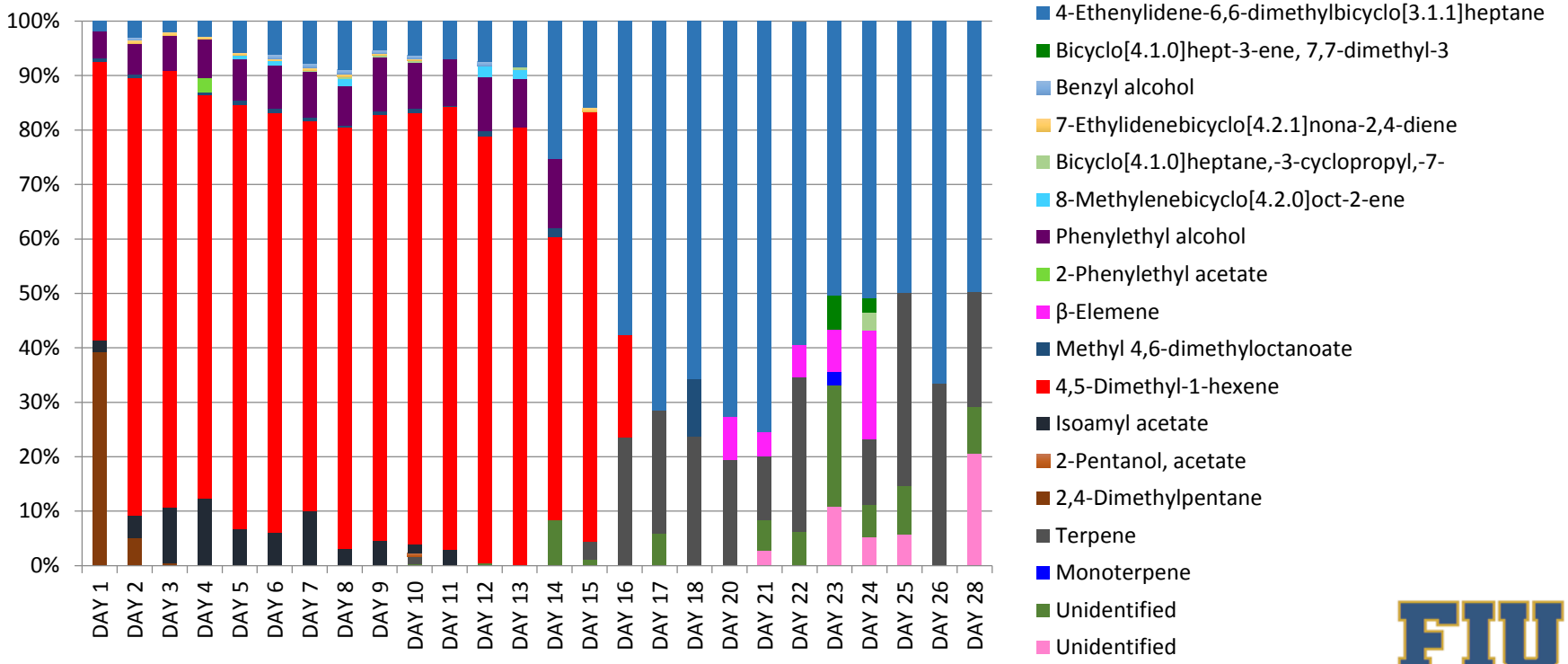
- USDA APHIS has utilized the Beagle Brigade since 1984 to decrease the number of invasive or harmful species entering the United States
- Recent applications to track endangered or invasive species
  - For example: whales, brown tree snakes, tigers, pine bark beetles, …
- They can't stop everything!
  - Volume of activity
  - Lack of safe and efficient training aids

Current training aids

- Common training aids used for invasive biothreats:
  - Nests, burrows, scat, carcasses, feathers, live flora or fauna
- Not possible for many invasive species
  - Cost
  - Short shelf-life
  - Difficulty of obtaining and containing the odor
  - Legality of obtaining and transporting
  - Rarity of species
  - Questions of safety
- Mimic training aids are generally unavailable
  - Only possible if the odorant is known!

Difficulties with biological targets

- Mimic training aids are only possible if the odorant is known
- Biologically dynamic training aids are extra challenging!



Simon et al. *J Chrom A* (2017)

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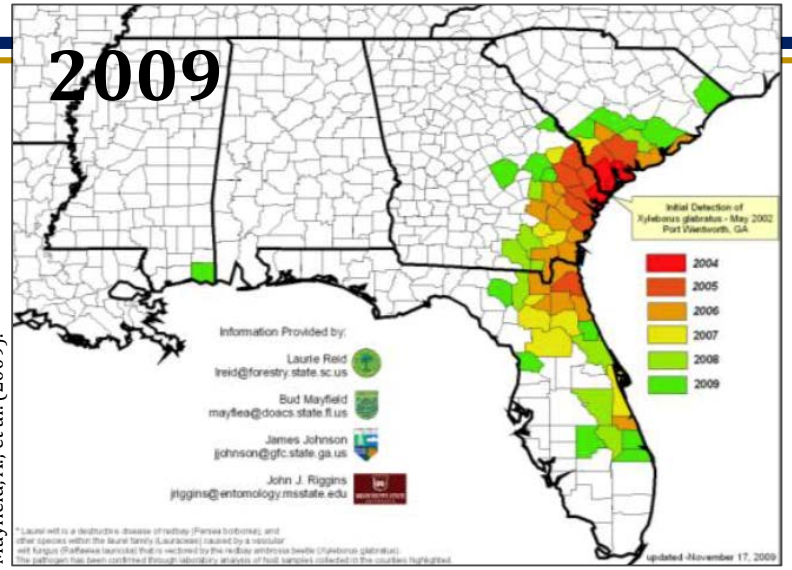
The question

- How do we rapidly identify the odorant(s) of an environmental/agricultural target substance so that we can train and deploy reliable, safe canine teams?
- Proof of concept: laurel wilt disease

Raffaelea lauricola

- An invasive fungus causing laurel wilt disease in Lauraceae trees carried by the redbay ambrosia beetle
- Has spread through eight states and has killed over 12,000 trees in Miami-Dade County, Florida plus about half a million wild trees
- Kills trees in 4-6 weeks

Laurel wilt distribution



Mayfield, A.; et al. (2009).

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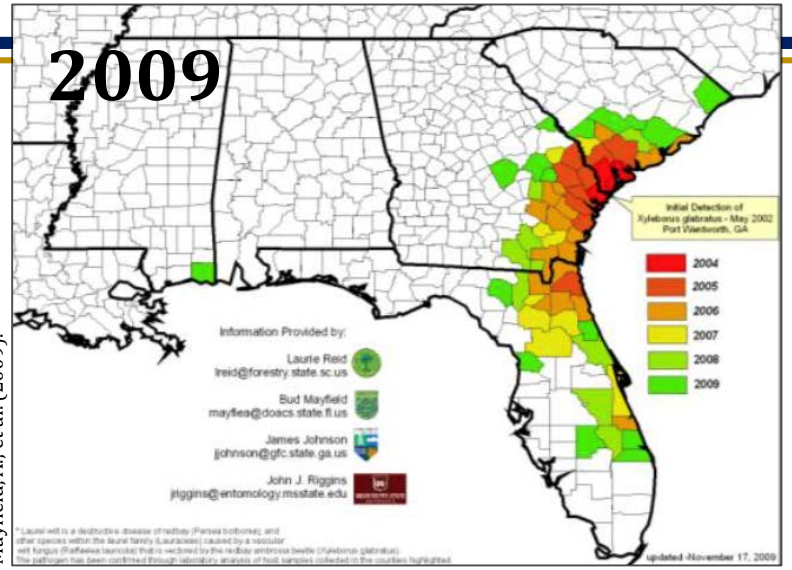
Laurel wilt distribution



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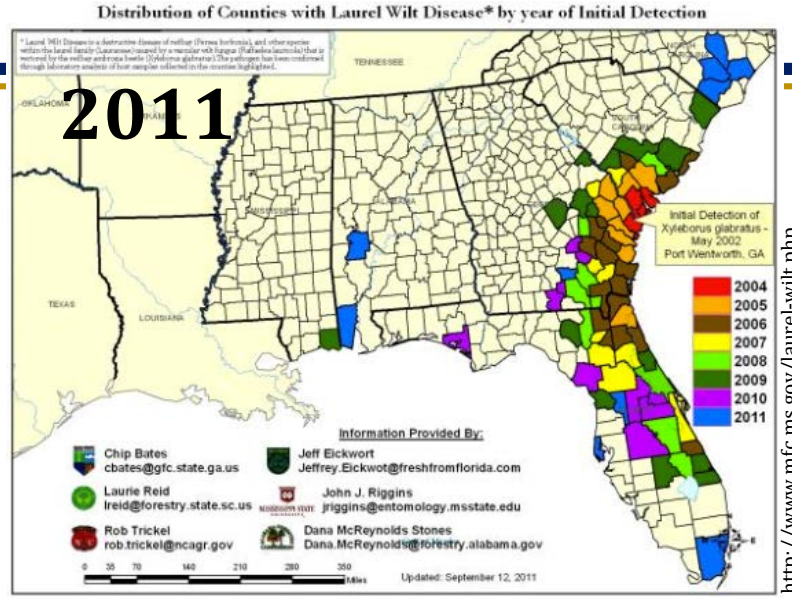
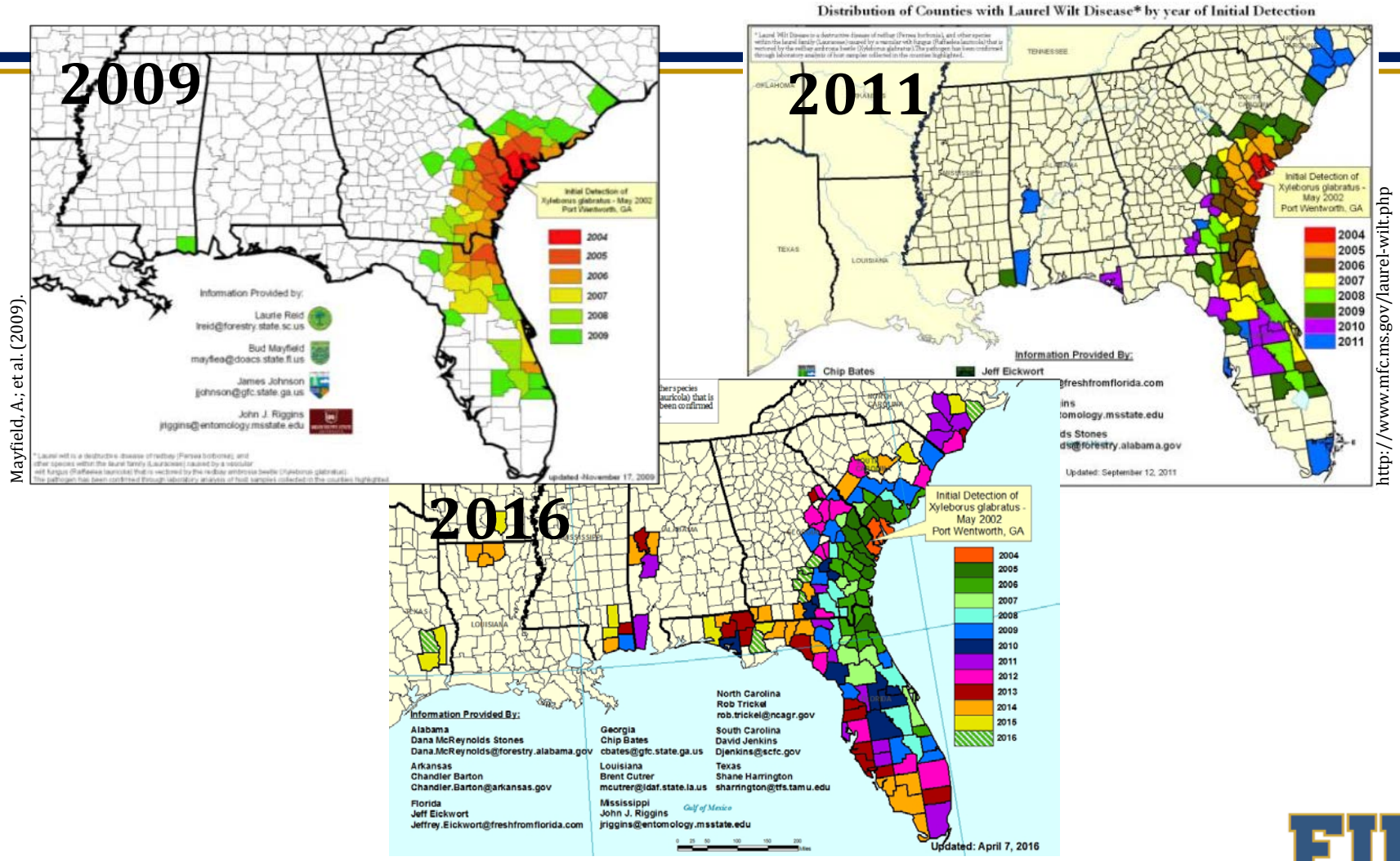


http://www.mfc.ms.gov/laurel-wilt.php

Laurel wilt distribution



Mayfield, A.; et al. (2009).

http://www.mfc.ms.gov/laurel-wilt.php

http://www.mfc.ms.gov/forest-health/disease/laurel-wilt-disease

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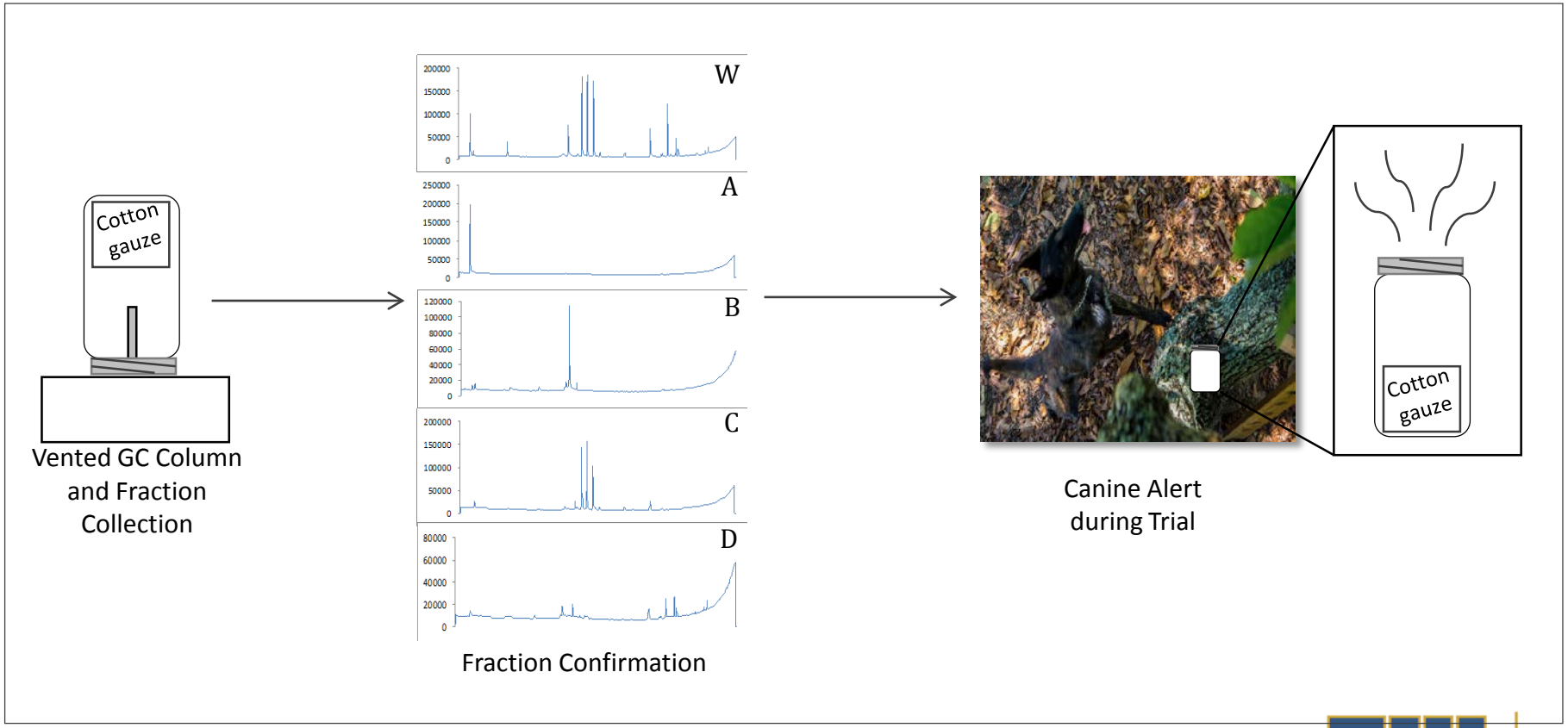
Raffaelea lauricola detection

- Only current method of early identification is canine detection
  - Canines were trained using LDPE bags holding infected wood
- Canines are run in suspected avocado groves and identify infection prior to visual detection is possible
- Infected wood is not an ideal training aid!

Method



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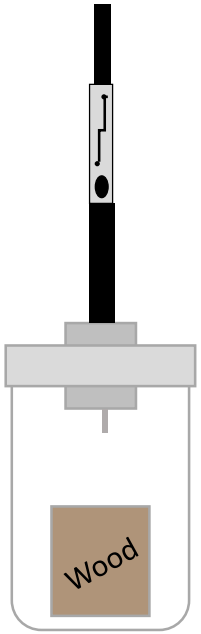
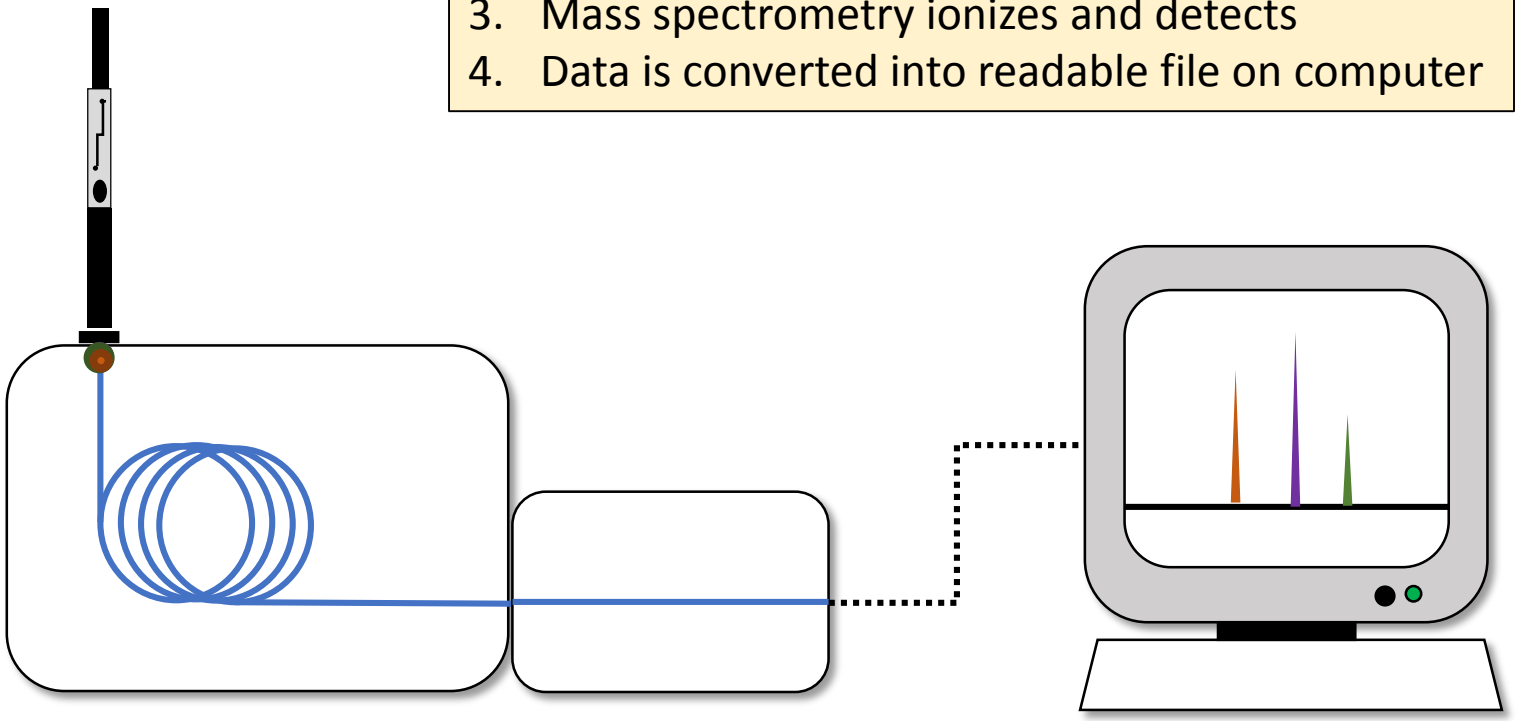
HS-SPME-GC-MS

1. Analytes collected on SPME fiber
2. Gas chromatography separates compounds
3. Mass spectrometry ionizes and detects
4. Data is converted into readable file on computer





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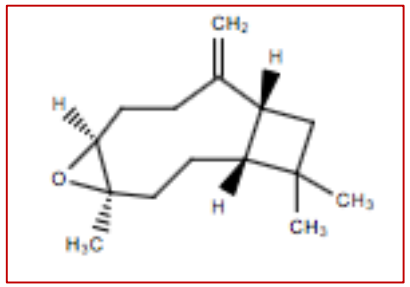
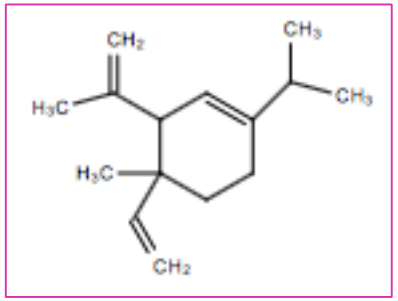
VOC identification

Infected

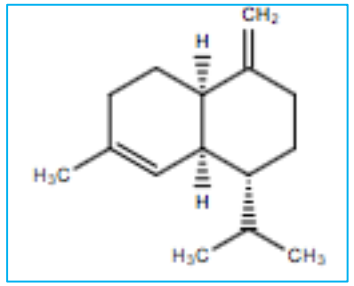


Caryophyllene oxide

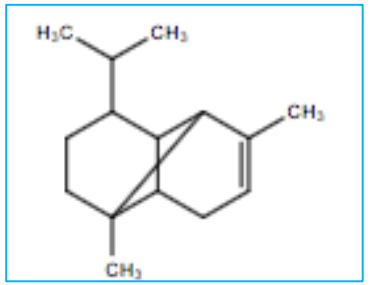
Both



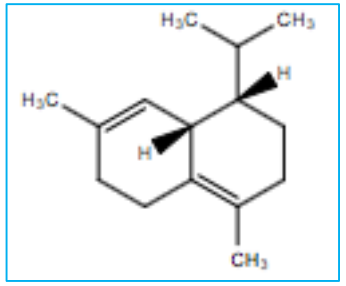
δ-Elemene



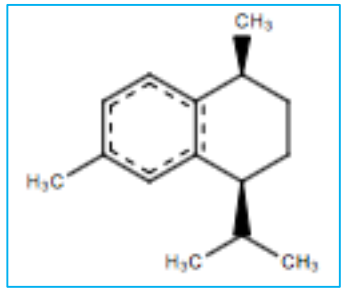
γ-Muurolene



Copaene

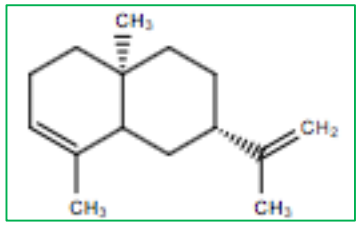


δ-Cadinene

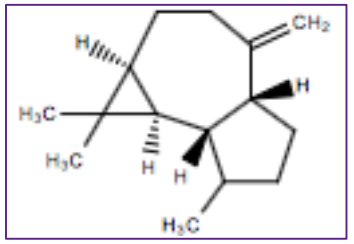


Calamenene

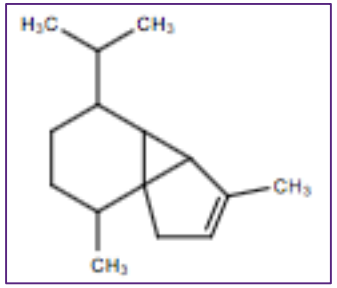
Healthy



α-Selinene



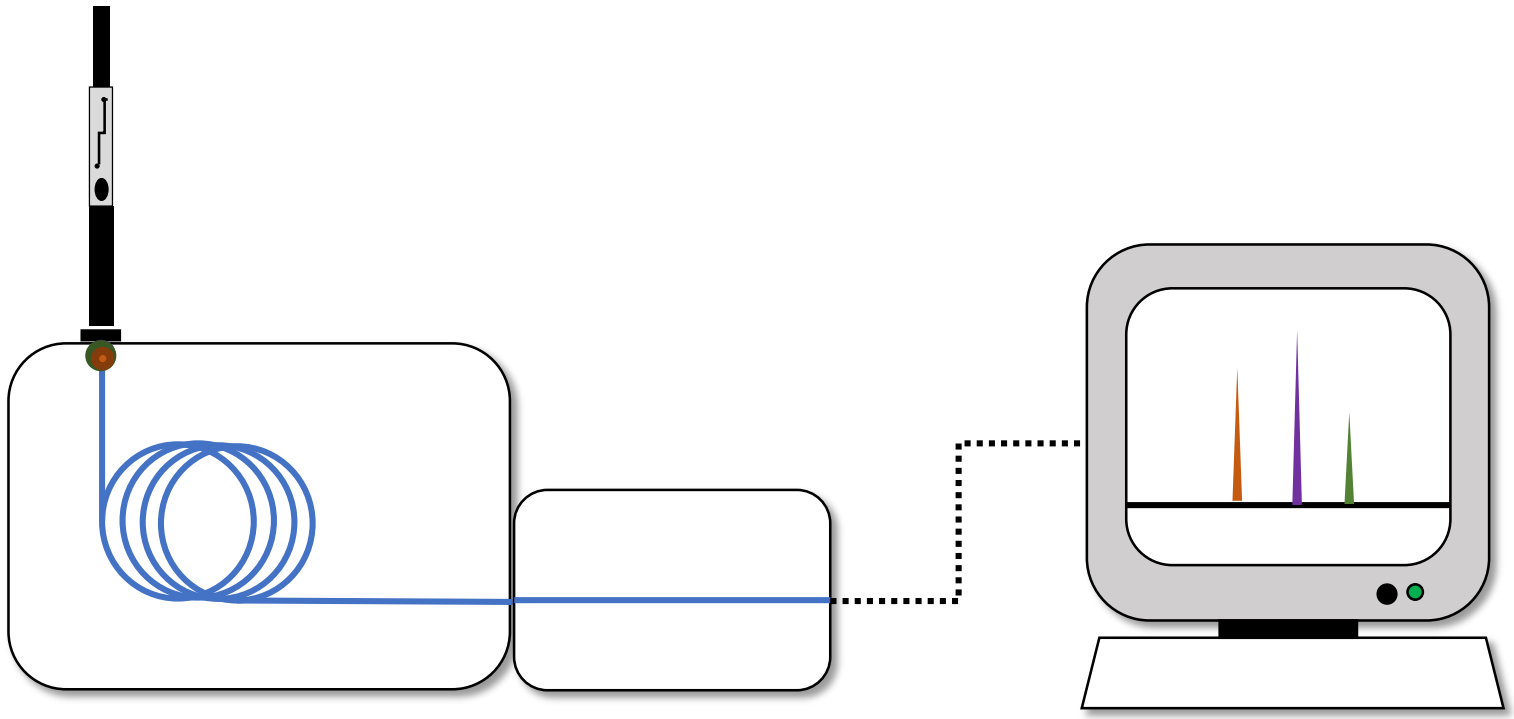
(-)-Alloaromadendrene



α-Cubenene

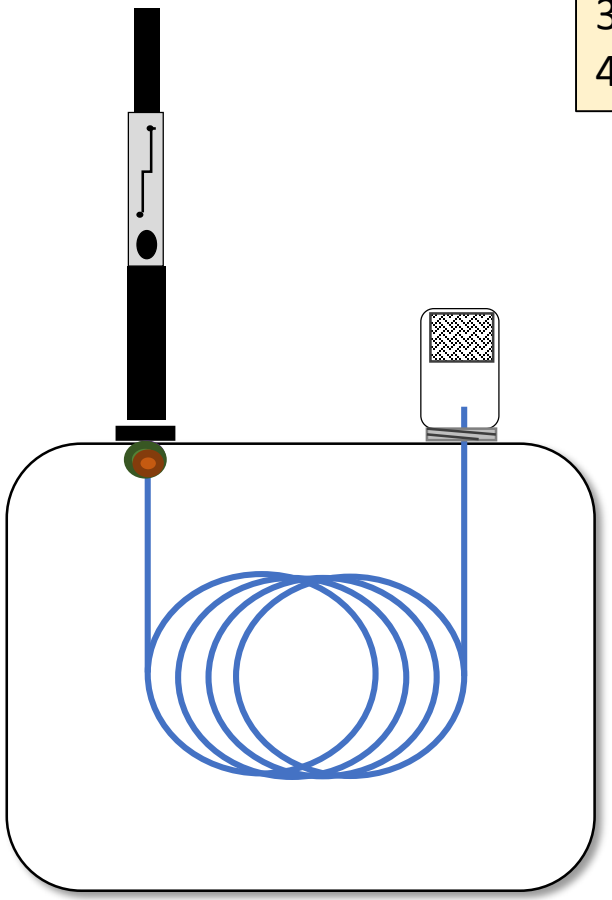
Humulene | Germacrene | Cadinene, Muurolene, & Calamenene | Eudesmene | Guaiene

Column vent method



Column vent method

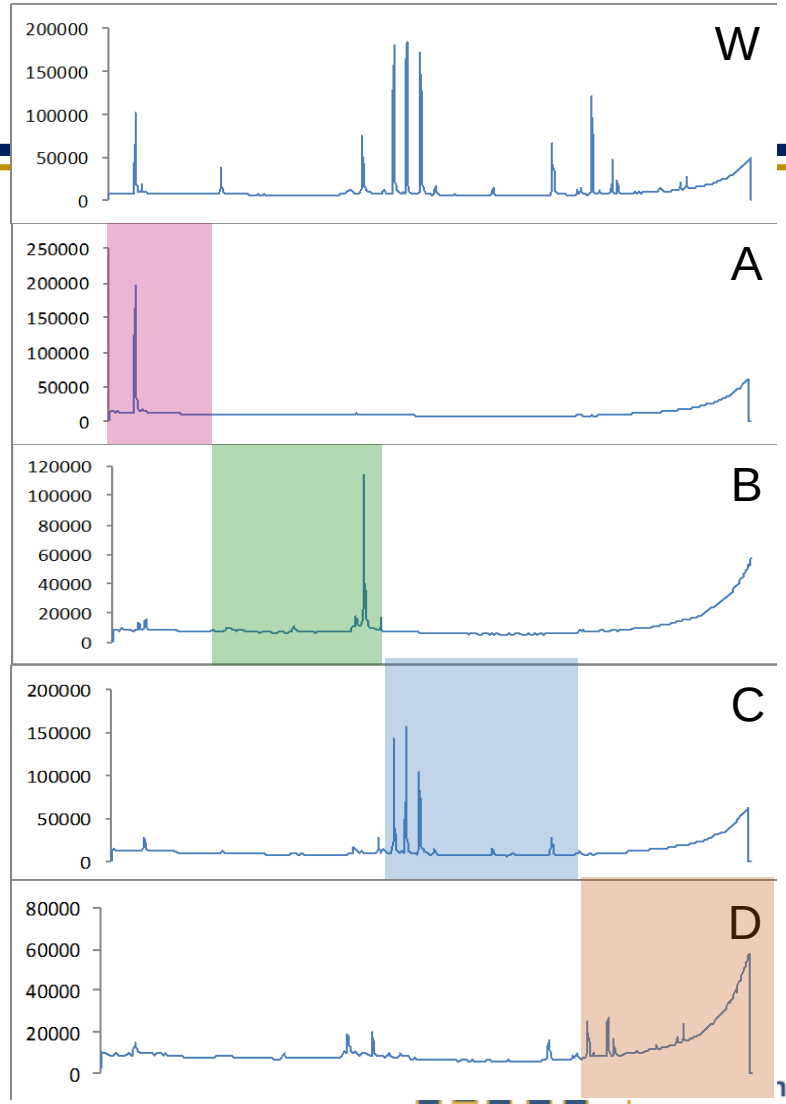
1. Analytes collected on SPME fiber
2. Gas chromatography separates compounds
3. Compounds are collected on cotton gauze
4. Contents are verified using HS-SPME-GC-MS



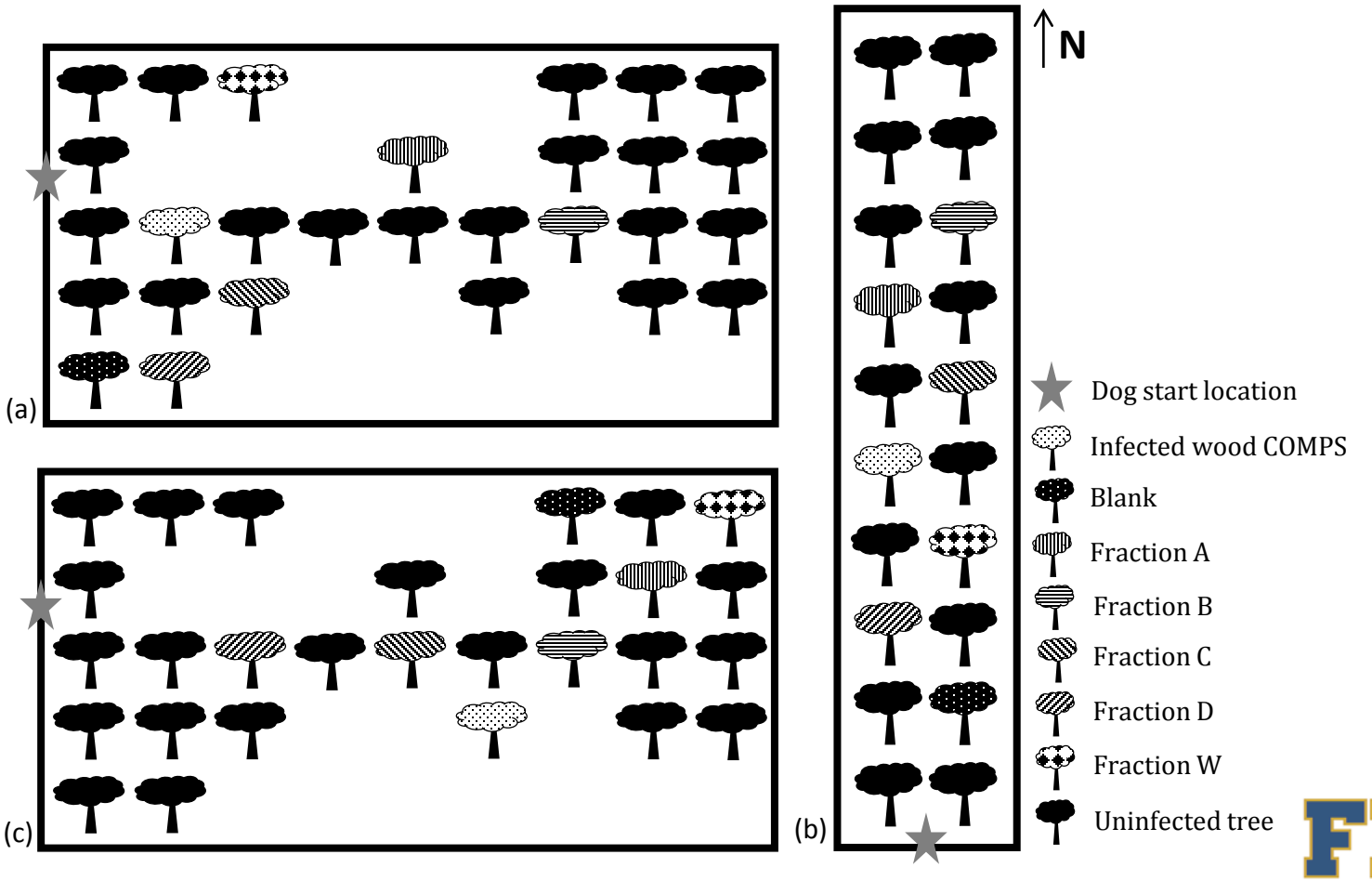
Label	Fraction begin (min)	Fraction end (min)
A	0.00	10.00
B	10.01	20.00
C	20.01	30.00
D	30.01	39.98
W	0.00	39.98

Verification

1. SPME of infected tree VOCs
2. Desorbed into vented column (0.53mm ID)
3. Fractions collected on cotton gauze in glass vials
4. 1 hour equilibration
5. SPME of glass vials
6. Desorbed into GC-MS (0.25mm ID)



Canine trial design



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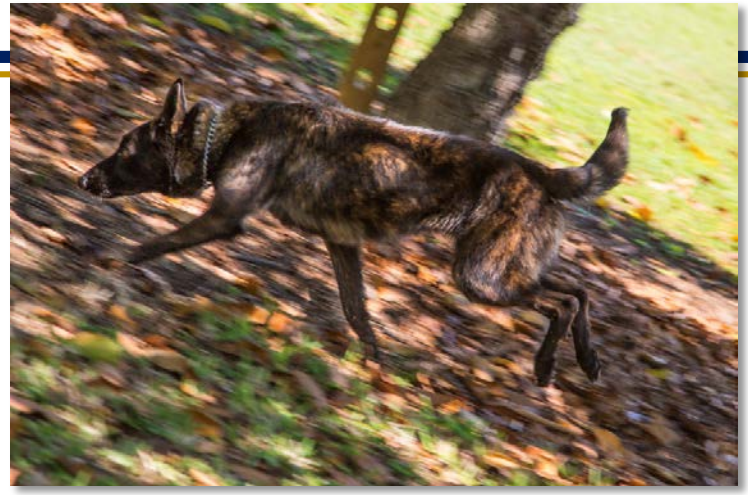
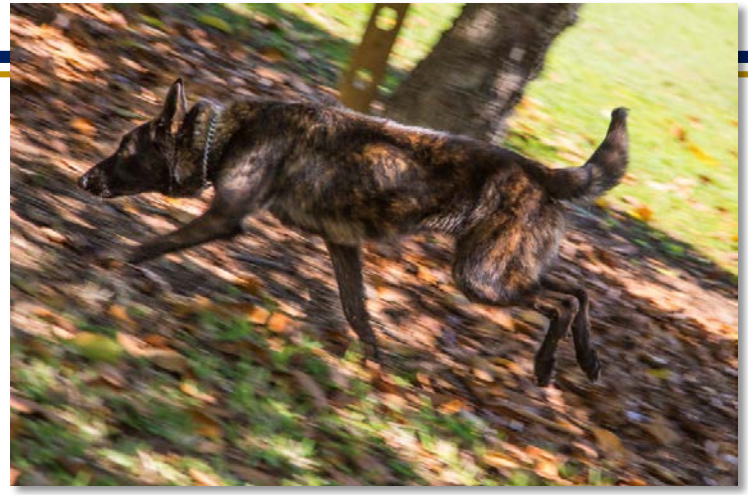
Canine trial results

Column Venting Results (n=2)		
Training Aid	Alert Rate (%)	No Alert Rate (%)
Blank	0.0	100.0
COMPS	100.0	0.0
W	100.0	0.0
A	50.0	50.0
B	33.3	66.7
C	0.0	100.0
D	0.0	100.0



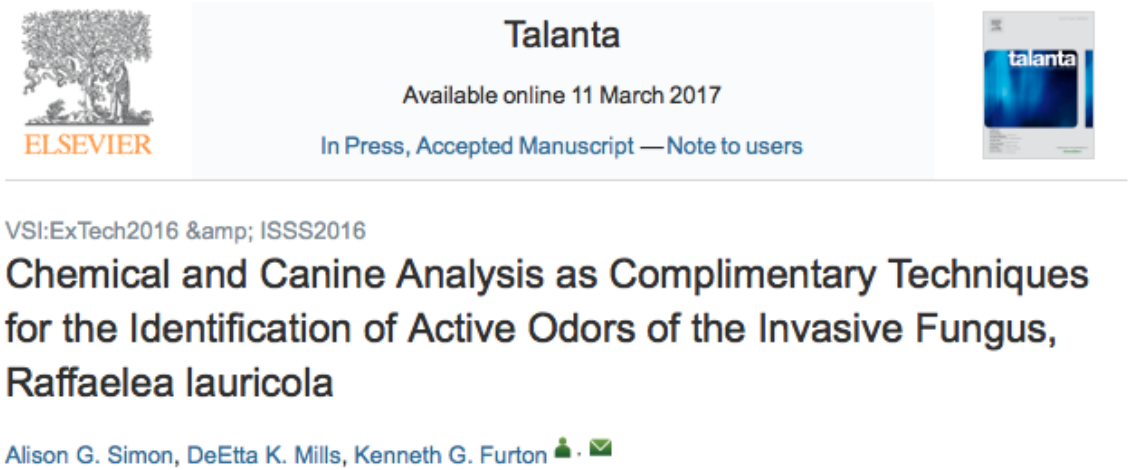
Canine trial results

Column Venting Results (n=2)		
Training Aid	Alert Rate (%)	No Alert Rate (%)
Blank	0.0	100.0
COMPS	100.0	0.0
W	100.0	0.0
A	50.0	50.0
B	33.3	66.7
C	0.0	100.0
D	0.0	100.0



Conclusions

- Method allows canines to detect active odorants based on chromatographic areas of interest
- More streamlined active odor identification
- Training aids do not need live cultures or infected samples
- Reduces dependence on live training aids



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Questions?

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