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Host Volatiles Attractive to the Bronze Birch Borer *Agrilus anxius* Gory (Coleoptera: Buprestidae)

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OECD & DEFRA CONFERENCE ON EAB & BBB, VIENNA, AUSTRIA, OCT 1-4, 2018.

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Team Workers!!

- Peter Silk, Lucas Roscoe & Krista Ryall, CFS, Canada
- Peter Mayo, Gaetan LeClair, Matt Brophy, CFS, Canada
- Martin Williams, CFS, Canada.
- Claire Rutledge, Ct, USA; David Williams UK
- Gary Grant, CFS (deceased)

Background

- The buprestid beetles (Coleoptera: Buprestidae) - a diverse and widespread taxa,
- ~ 15,000 species worldwide.
- ~ 3000 Agrilus species known.
- The most important *Agrilus* species endemic to North America is the bronze birch borer, BBB (*Agrilus anxius* Gory).
- native to North America - attacks weakened and stressed birch trees (*Betula* sp.) and does not cause high levels of mortality in healthy trees in its native range.
- Concern has been raised about its possible introduction into Europe, the UK and Asia (Muilenburg and Herms 2013)-there may be a high risk of establishment and birch mortality on a continental scale.
- A detection tool such as a pheromone or kairomone-baited trap would be useful.
- THIS IS A WORK IN PROGRESS…started this year! Preliminary results.

COLEOPTERA

Major Families

The following nine families include about 70% of the North American beetle fauna:

- **Staphylinidae**
- **Curculionidae**
- **Carabidae**
- **Scarabaeidae**
- **Tenebrionidae**
- **Cerambycidae**
- **Elateridae**
- **Buprestidae**
- **Coccinellidae**

HOST ATTRACTION THE KEY???

Cerambycidae > 100 pheromones now known

Buprestidae ~ 1 !!!! EAB !

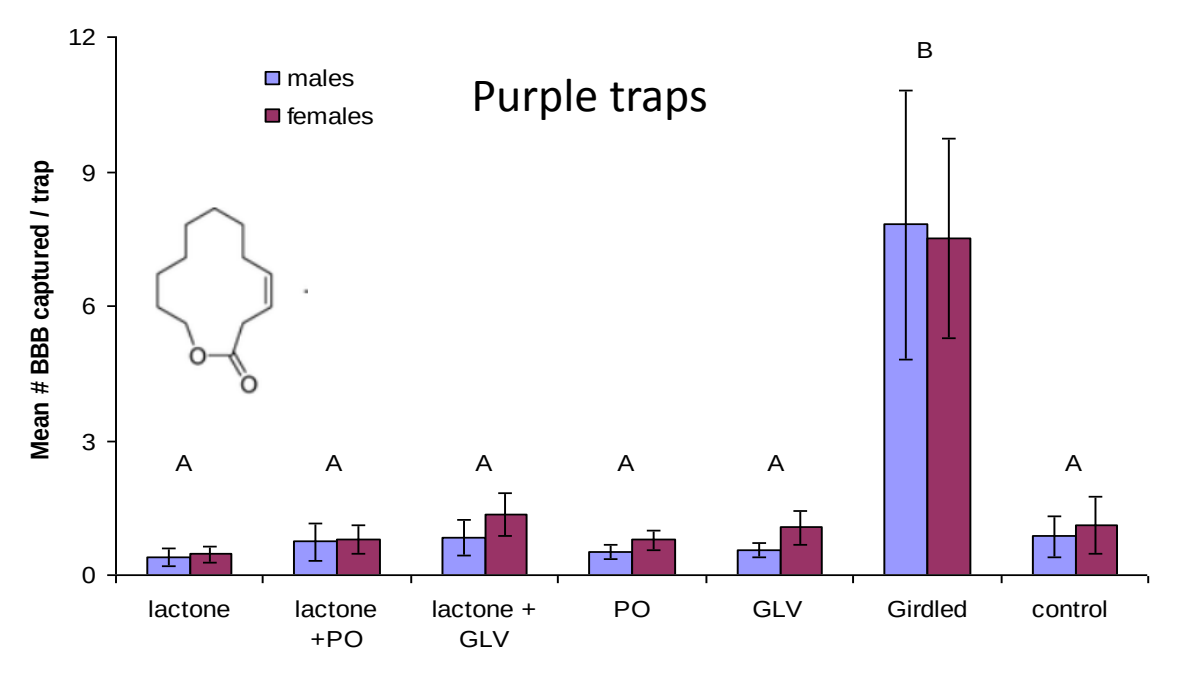
Damage to Birch

Thinning crown
Branch dieback
sap flow on trunk
Raised welts on bark
D-shaped exit holes

Larval galleries,
Feed in the
phloem

Initial Field Screening 2008:

Agrilus anxius



Needs to be repeated with green prism traps?

Silk & Ryall, unpublished

Effects of trap colour, trap position, and lure on detection of buprestids, esp. *Agrilus* spp.

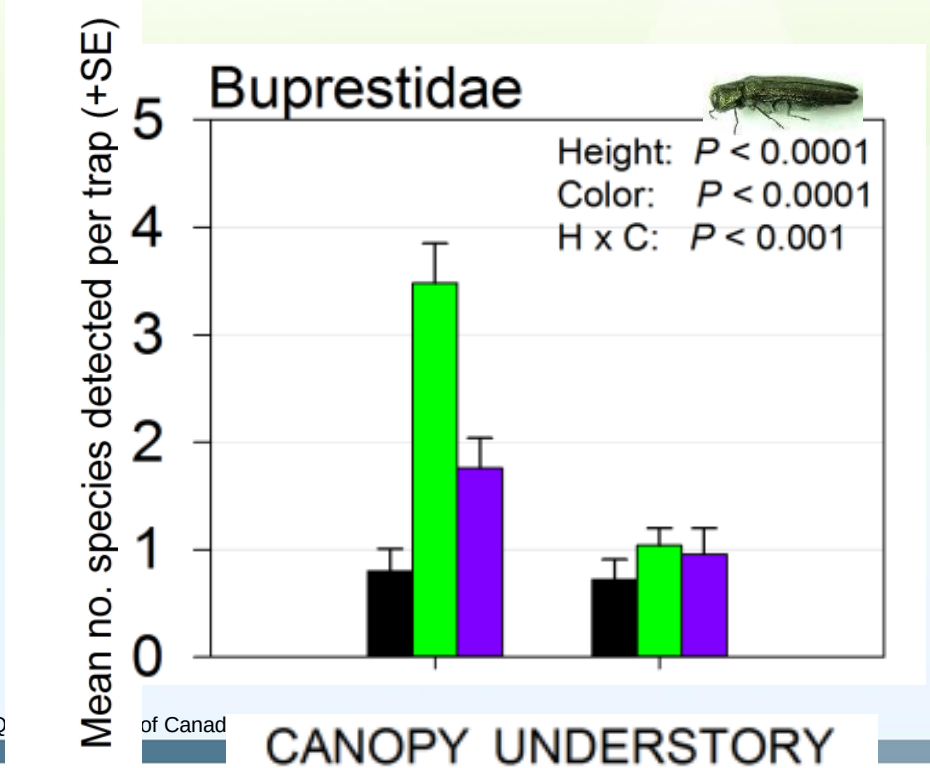
Sweeney, P. Silk, P. Mayo, D. Rassati, J. Gutowski, D. Miller, J. Francese, K. Ryall, C. MacQuarrie, Y. Li, Q. Meng, C. Hughes…unpublished

Effect of Trap color, position, lure

-black, green, purple
-canopy, understory
-cerambycid lures ± ethanol

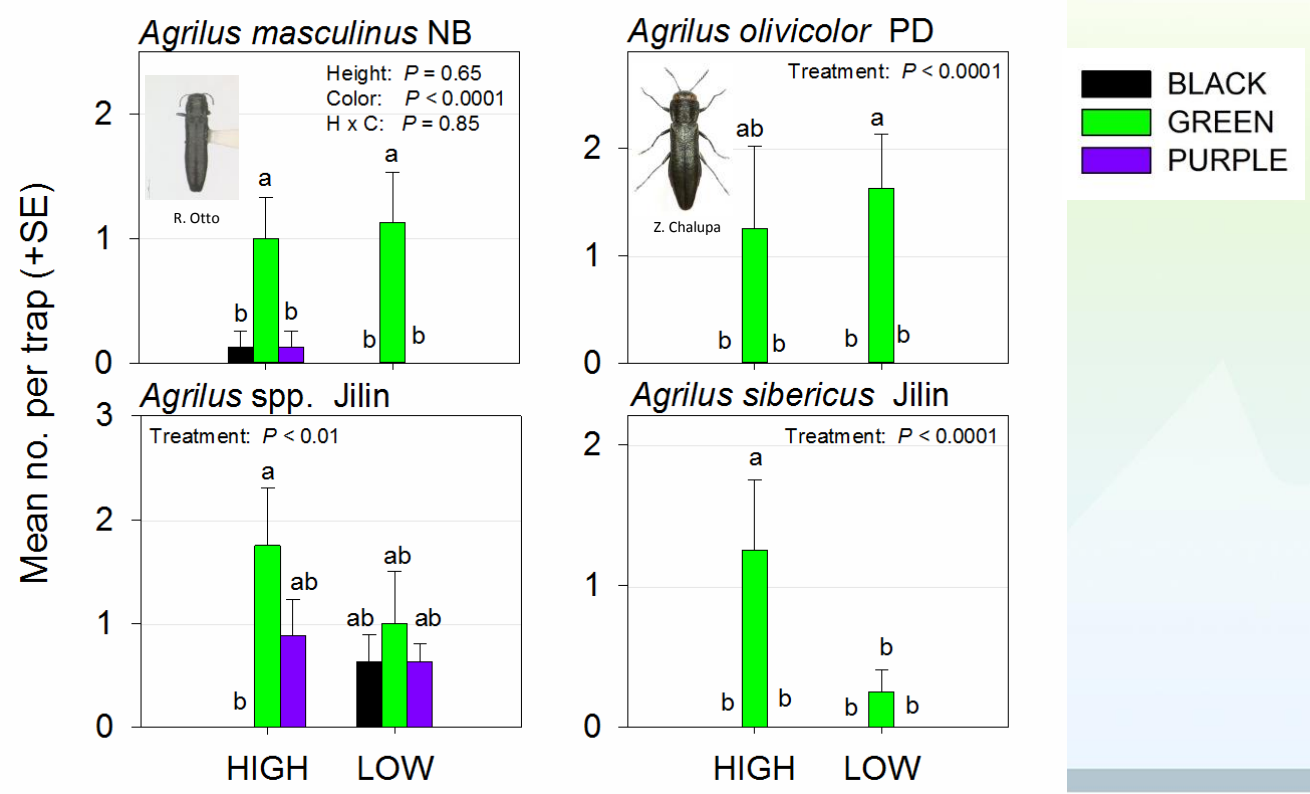
lure type ONLY important to cerambycids captured

Buprestid species richness per trap (Poland, Jilin, Georgia, Canada pooled, Sweeney et al. in prep.)

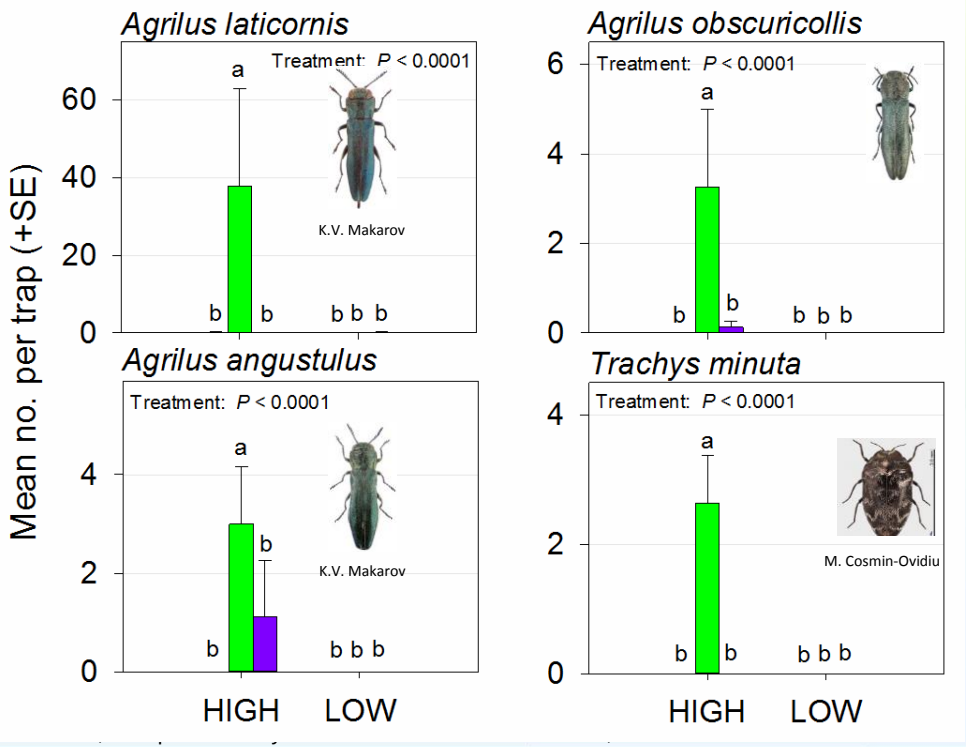


Green traps in the canopy detected the most buprestid species. Purple canopy traps detected more species than black traps

Agrilus detection was best in the upper canopy for most species but some species were also detected in the understory (Sweeney et al., in prep.)



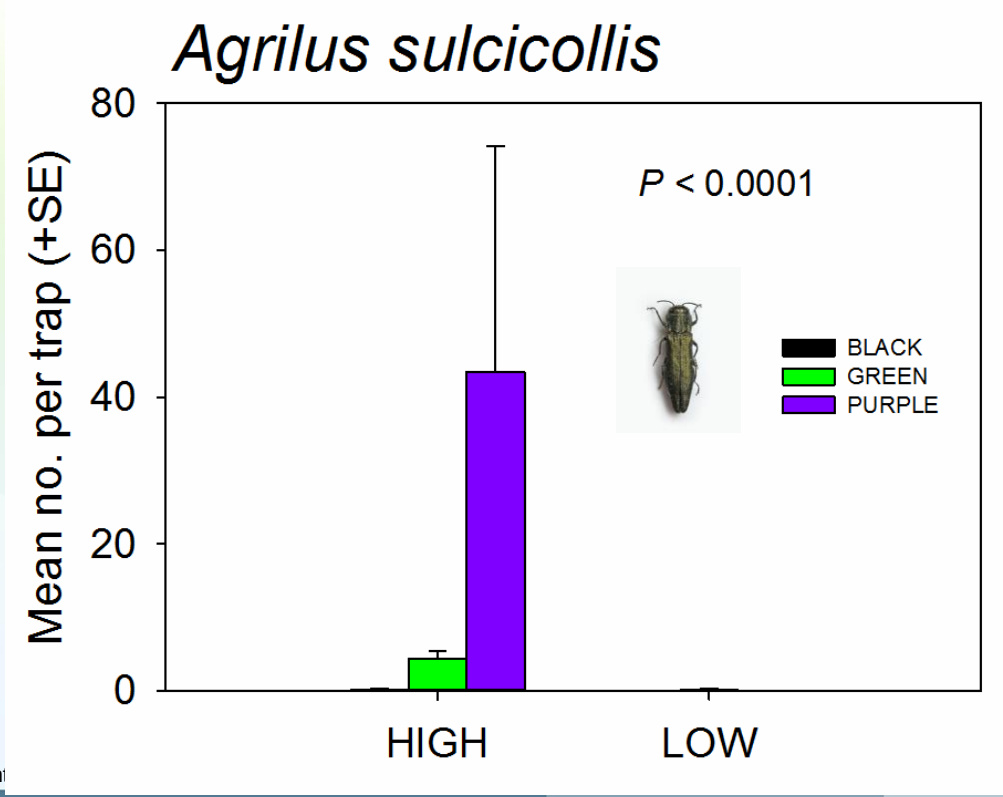
Green traps placed in the tree canopy worked best for detecting species in the Agrilinae subfamily (Sweeney et al., in prep.)



BLACK
GREEN
PURPLE

Poland 2015

But *Agrilus sulcicollis* was more attracted to purple traps, as were *Chrysobothris* spp.



Poland 2015

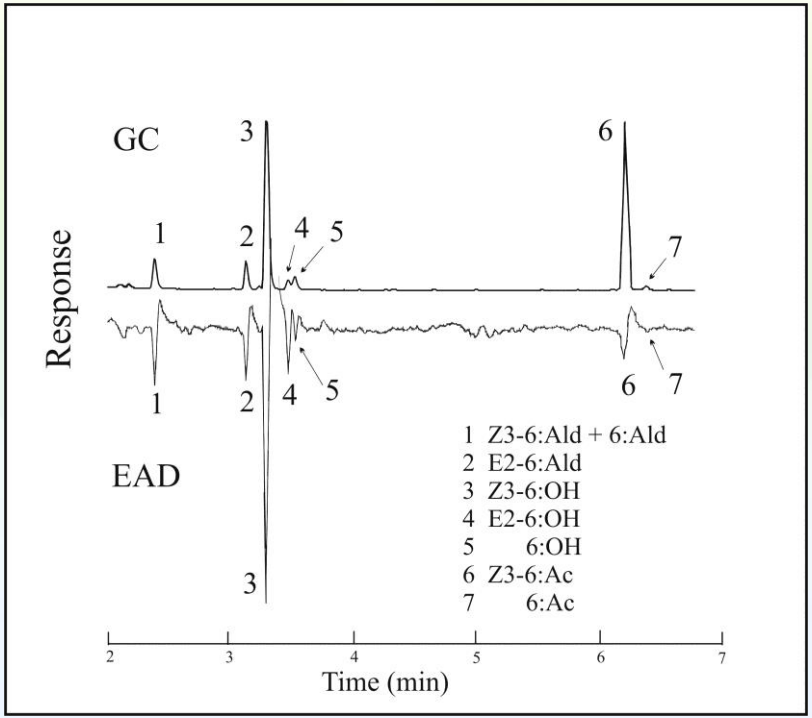
BBB: Chemical C(l)ues?

- Looking for chemical cues??
- Host volatiles…leaves , bark?

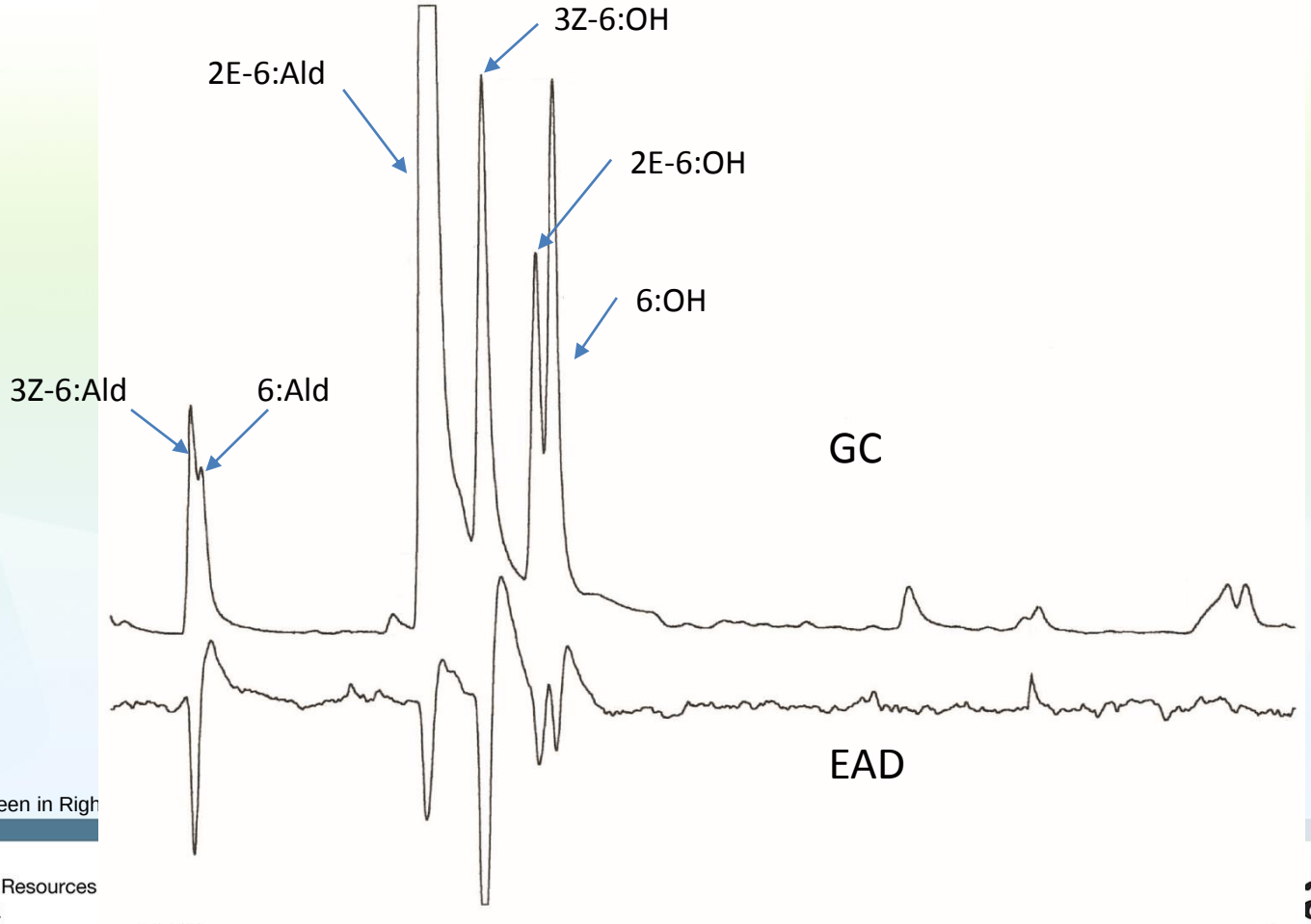
Possibility of a pheromone in *A. anxius*…similar to EAB?

Synergism of pheromone/kairomone???

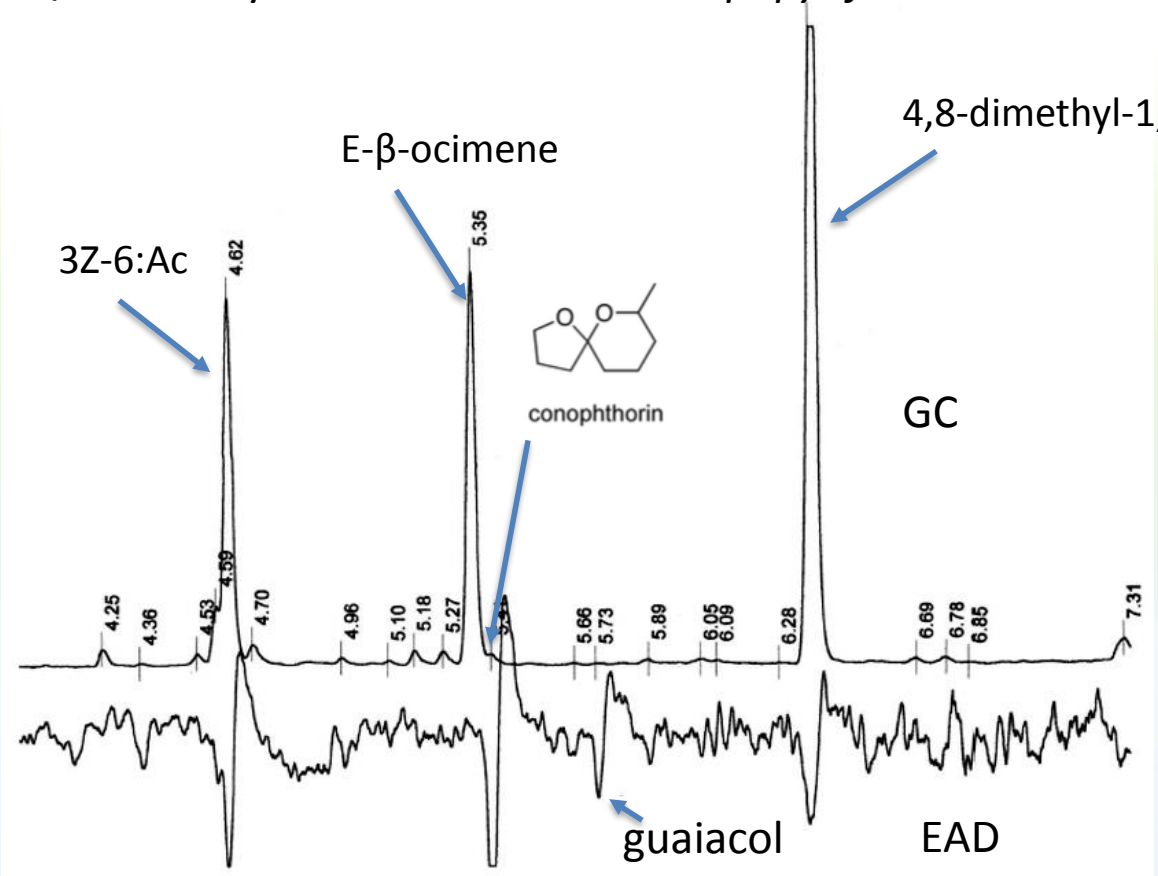
GC-EAD Response from Male EAB Antenna to Aeration Extract of White Ash Foliage



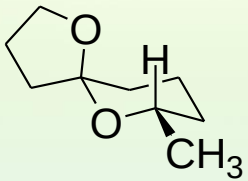
GC/EAD responses BBB male SPME; GLV's from Birch foliage



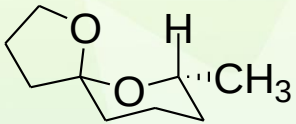
GC/EAD analysis: birch leaves *Betula papyrifera*



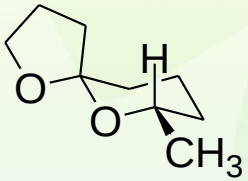
Conophthorin stereoisomers



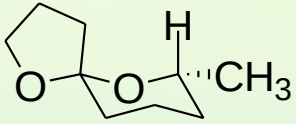
E-(7*S*)-conophthorin
(5*S*,7*S*)



E-(7*R*)-conophthorin
(5*R*,7*R*)

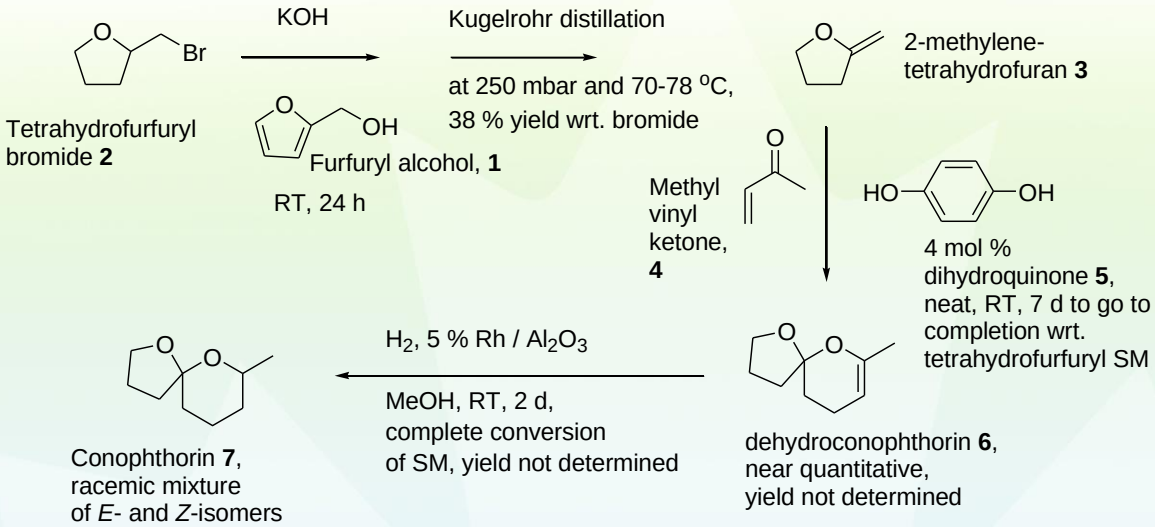


Z-(7*S*)-conophthorin
(5*R*,7*S*)



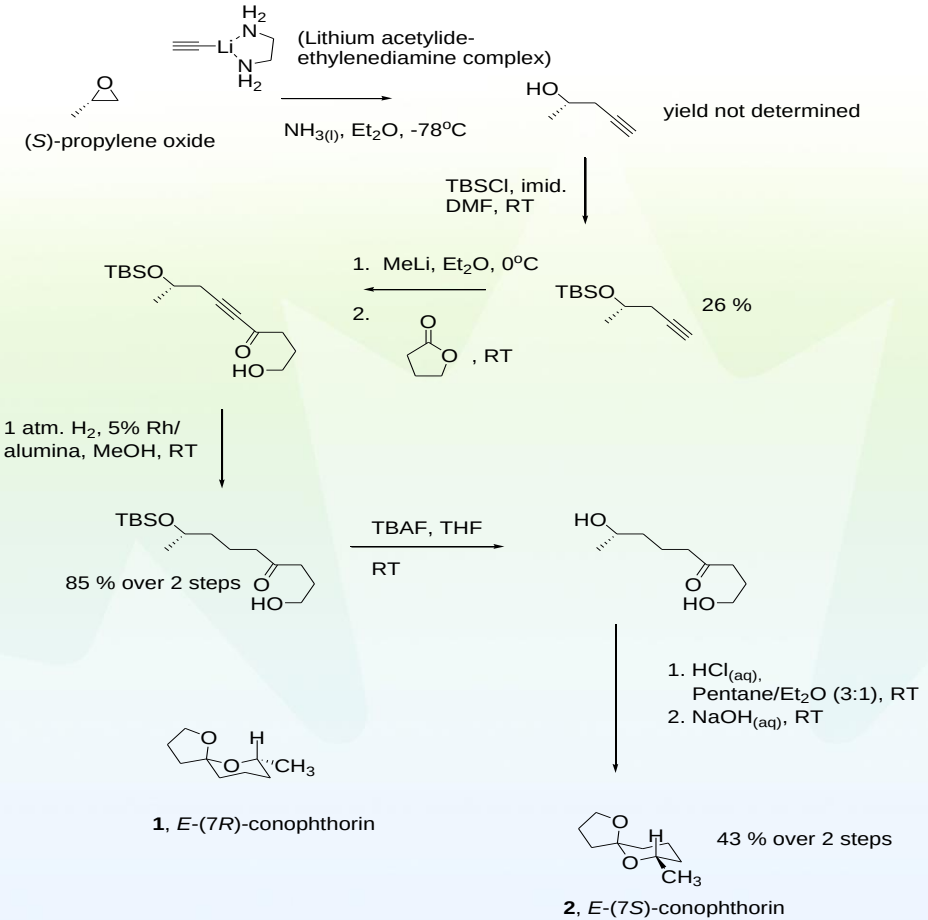
Z-(7*R*)-conophthorin
(5*S*,7*R*)

Synthesis of racemic conophthorin



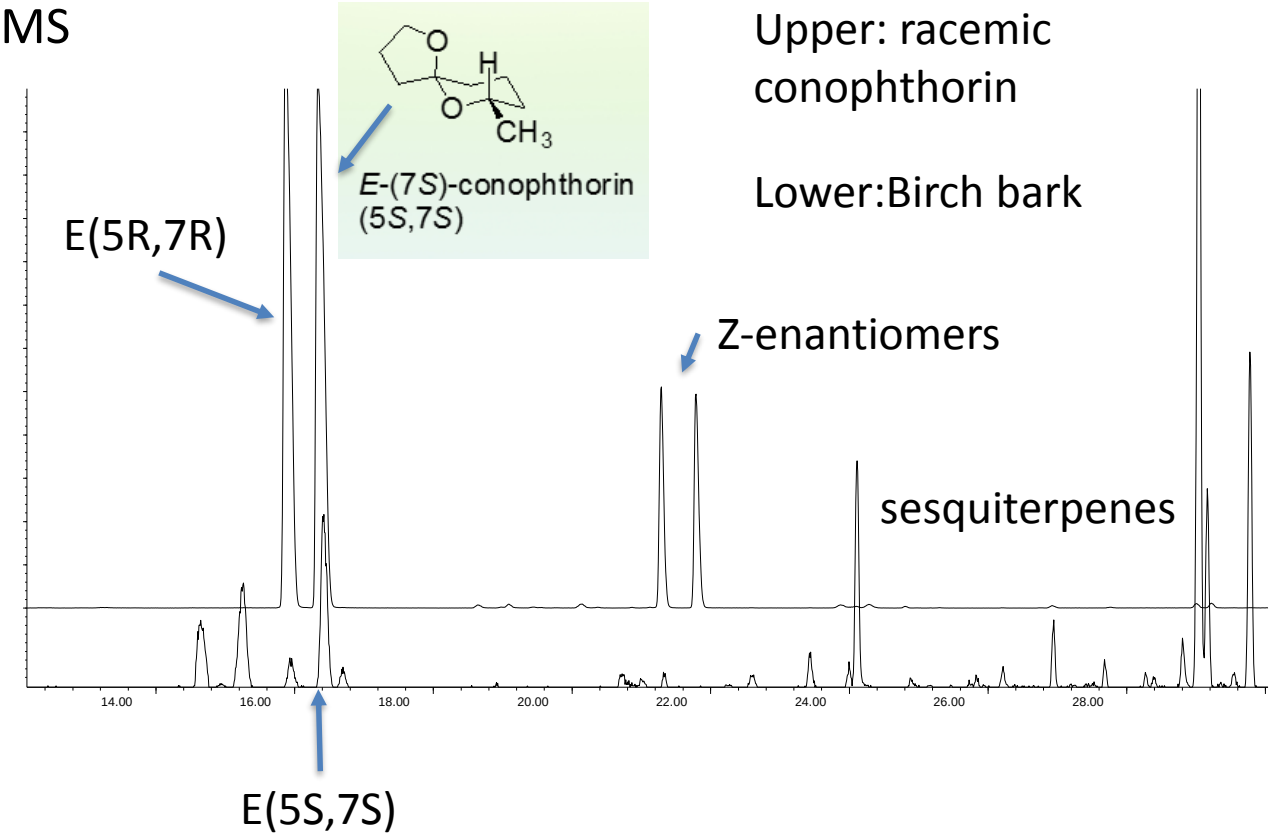
Kessler, S. N.; Neuburger, M.; Wegner, H. A. *Eur. J. Org. Chem.* **2011**, pp. 3238–3245, DOI 10.1002/ejoc.201100335.
Ireland, R. E.; Häbich, D. *Tet. Lett.* **1980**, Vol. 21, pp. 1389-1892.
Jacobson, R.; Taylor, R. J.; Williams, H. J.; Smith, L. R. *J. Org. Chem.* **1982**, *47*, 3140-3142.

SYNTHESIS of chiral enantiomers



Jacobson, R.; Taylor, R. J.; Williams, H. J.; Smith, L. R. *J. Org. Chem.* **1982**, *47*, 3140-3142.

CHIRALITY OF WHITE BIRCH CONOPHTHORIN: GC/MS

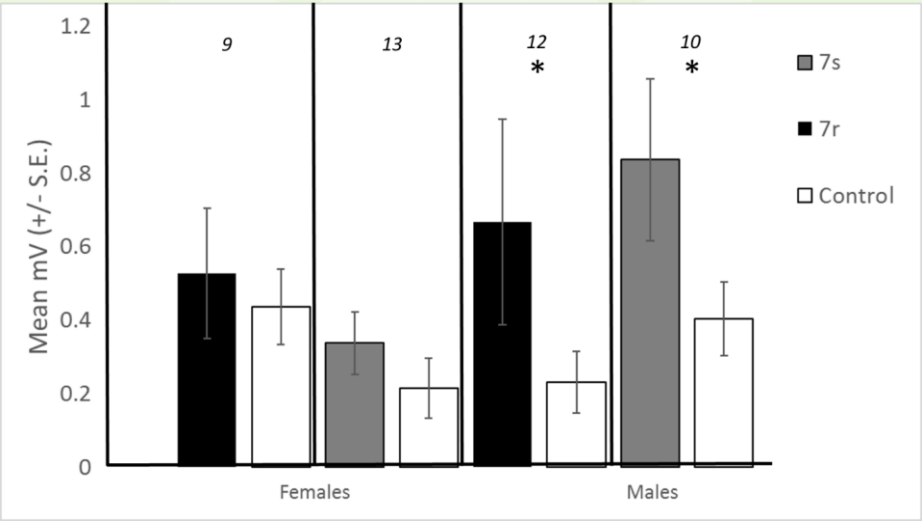


Upper: racemic conophthorin

Lower:Birch bark

GC/MS: β-cyclodextrin column chromatography

EAG Responses

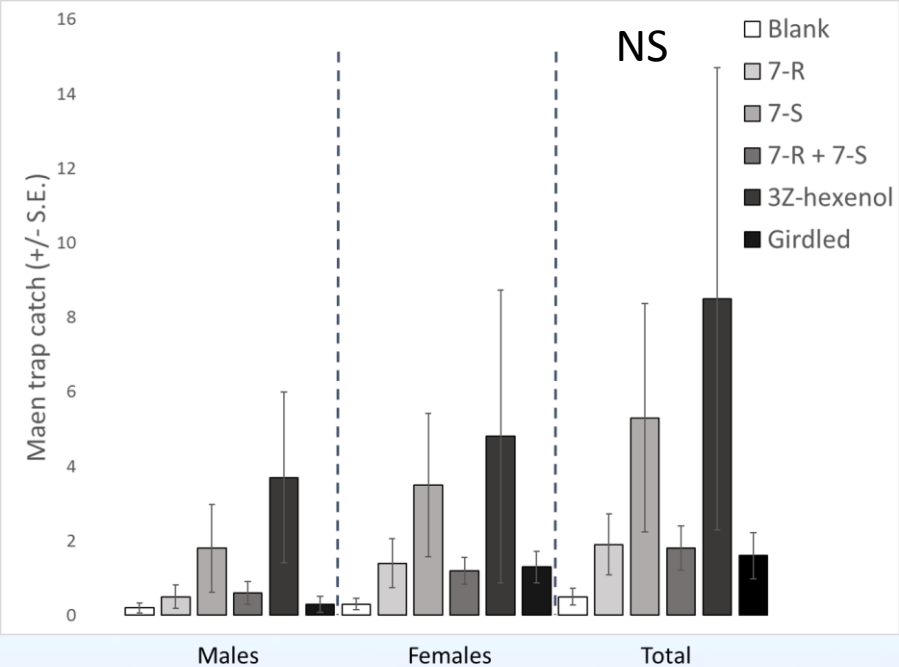


EAG analysis of standards

Standard Compound*	**Response** (+- < 0.5mV; ++++ ~3-4 mV)
6:Ald	+
E2-6:Ald	+
3Z-6:OH	**++++**
benzaldehyde	++
1-octen-3-ol	+
3Z-6:Ac	+
Benzyl alcohol	+
Salicylaldehyde	++
Limonene	+
Z-β-ocimene	++
Conophthorin	**++++**
Octanol	+
Guaiacol	++
β -caryophyllene	+

*10 ng

Field Trapping NB: green prism traps 2017



Conophthorin
2 mg/septum

3Z-hexenol: pouches
~40 mg/day

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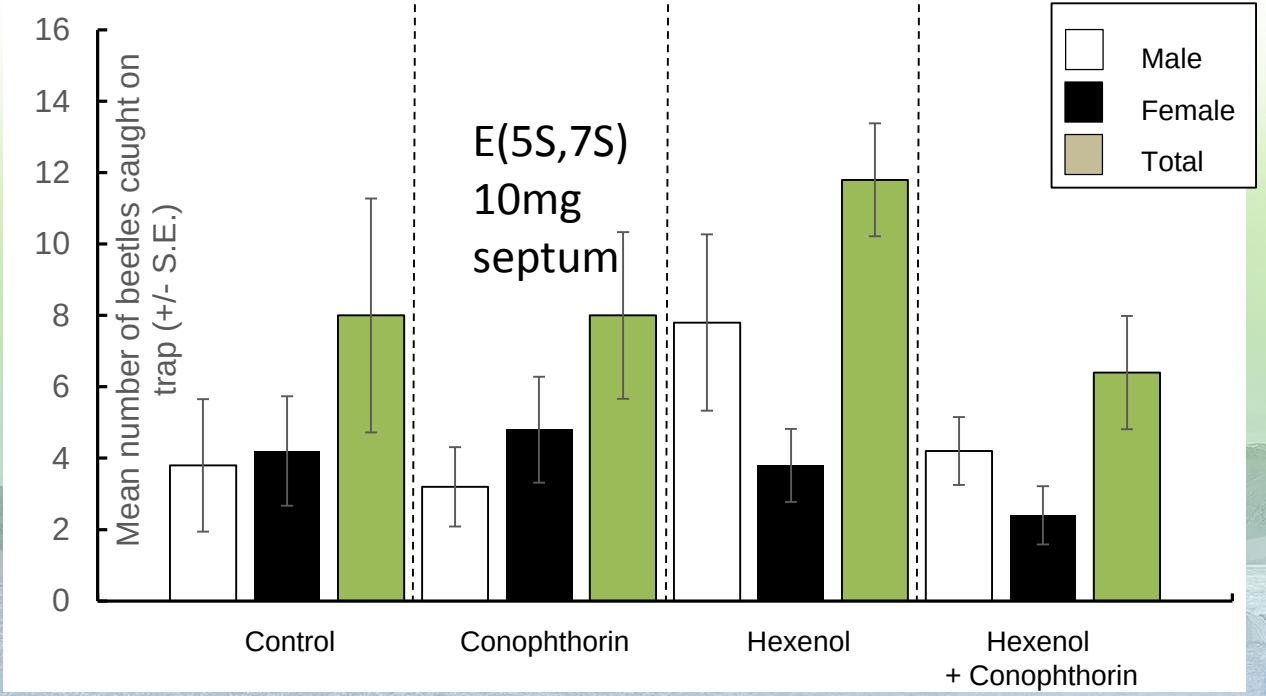
2018 NS



Mean trap catches of *Agrilus anxius* Gory on baited green prism traps outside of Sudbury, ONT., July 10-25, 2018. 10 mg.con./septum
Connecticut trapping…similar results (Rutledge/Williams)

REPEAT WITH PURPLE TRAPS!!

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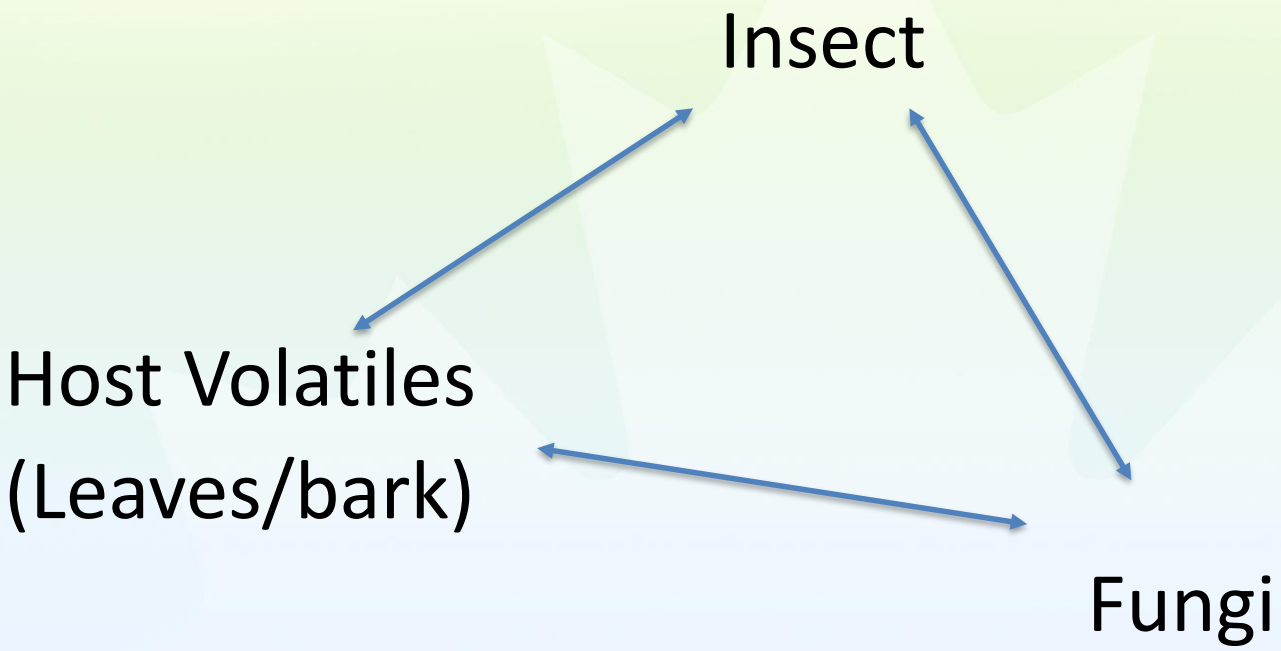
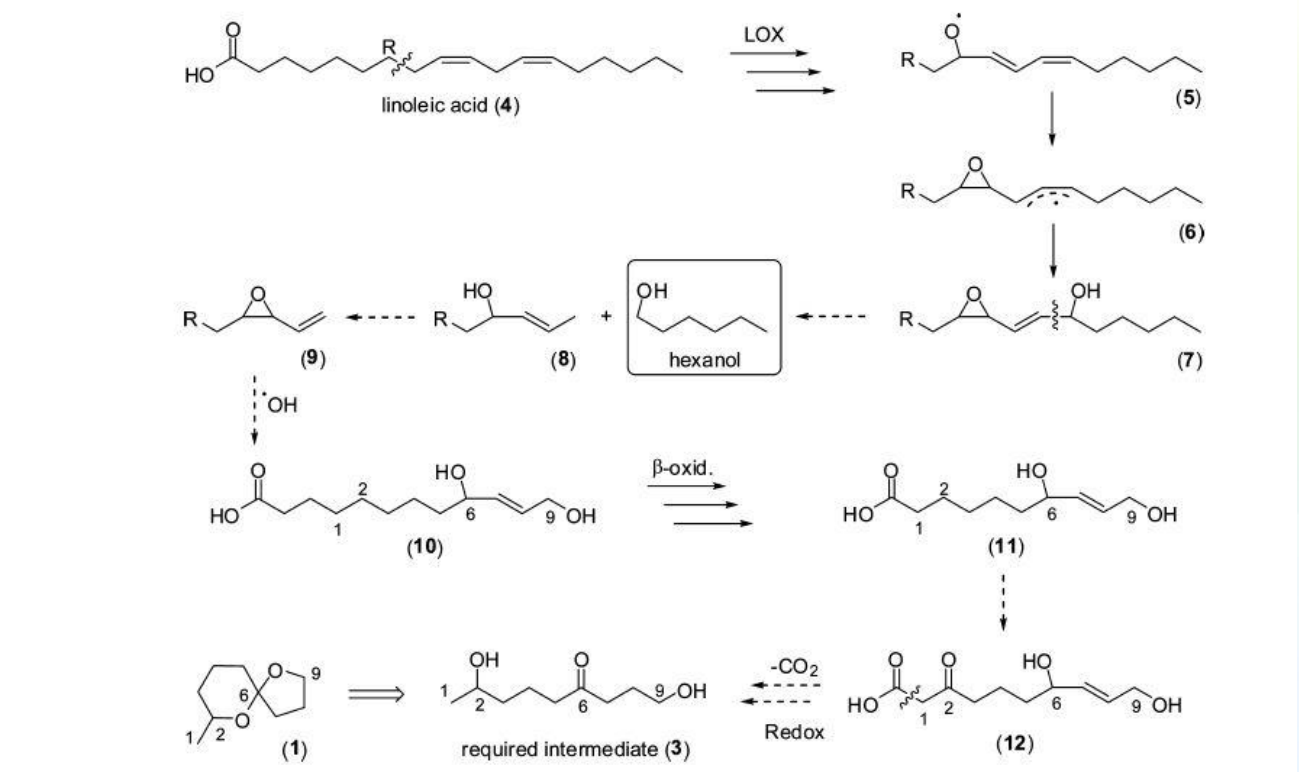
Host/Fungal signature??

- May offer some clues??
- Beck et al. (2012) have shown the fungal production of the spiroketals conophthorin and chalcogran from polyunsaturated fatty acids (linoleic, linolenic) in almonds.
- The birch volatilome may be more complex??

Hypothesize a 3 way interaction?



Hypothesized biosynthesis for 1-hexanol and conophthorin from fungal spores on linoleic acid. Beck et al. 2012



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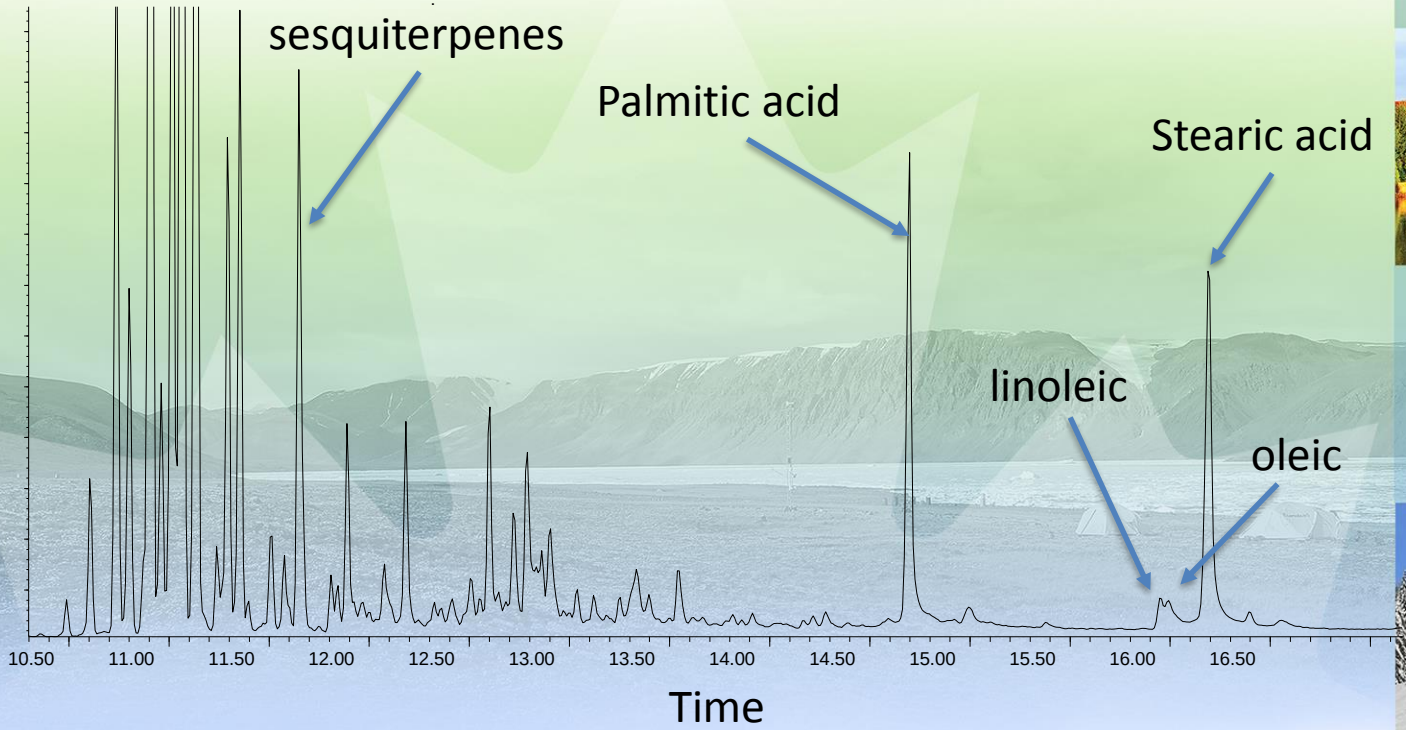
GC/MS of Birch Phloem crude extract



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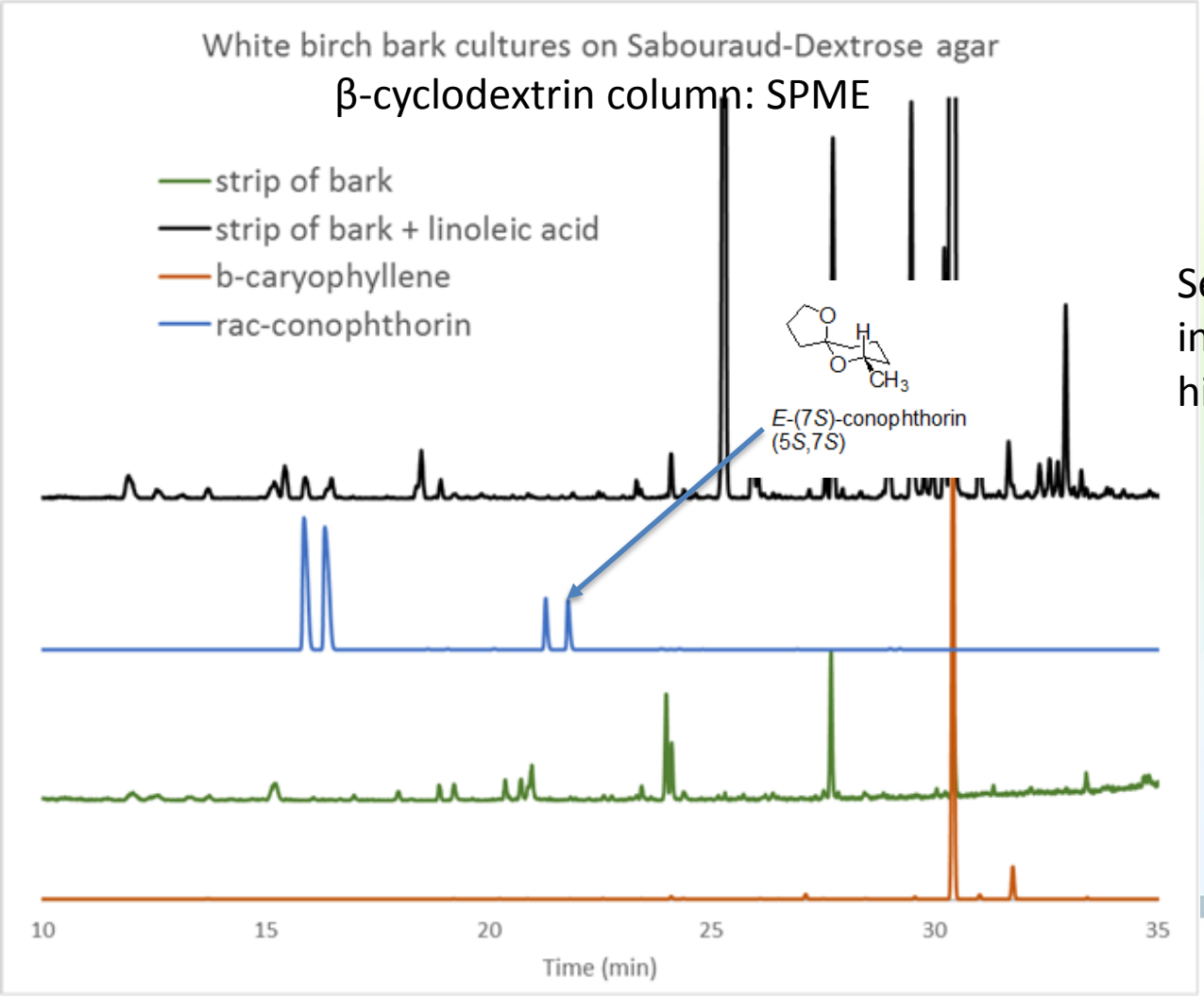

Sesquiterpenes and fatty acids including linoleic acid are found in high levels in birch phloem

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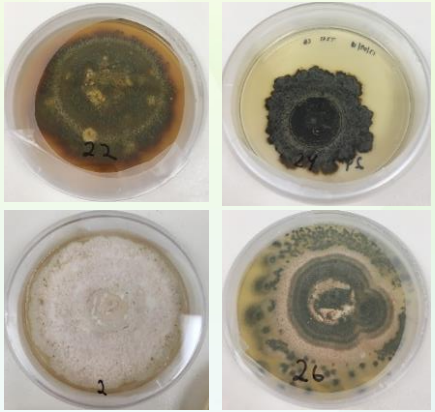
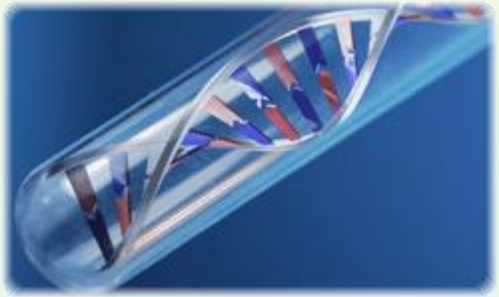
Volatilome analysis of birch associated fungi

1. Sampling

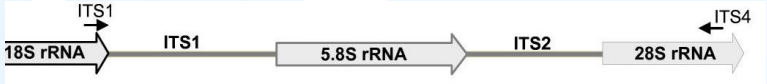
2. Pure culture isolation (32)



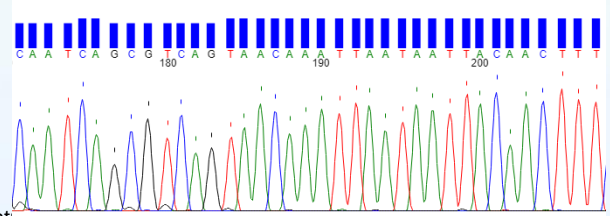
3. DNA extraction



4. Fungal barcode PCR



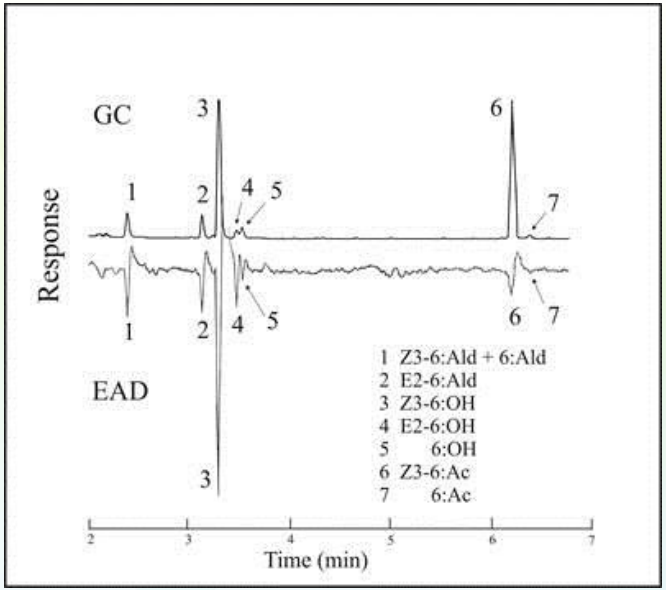
5. Barcode sequencing

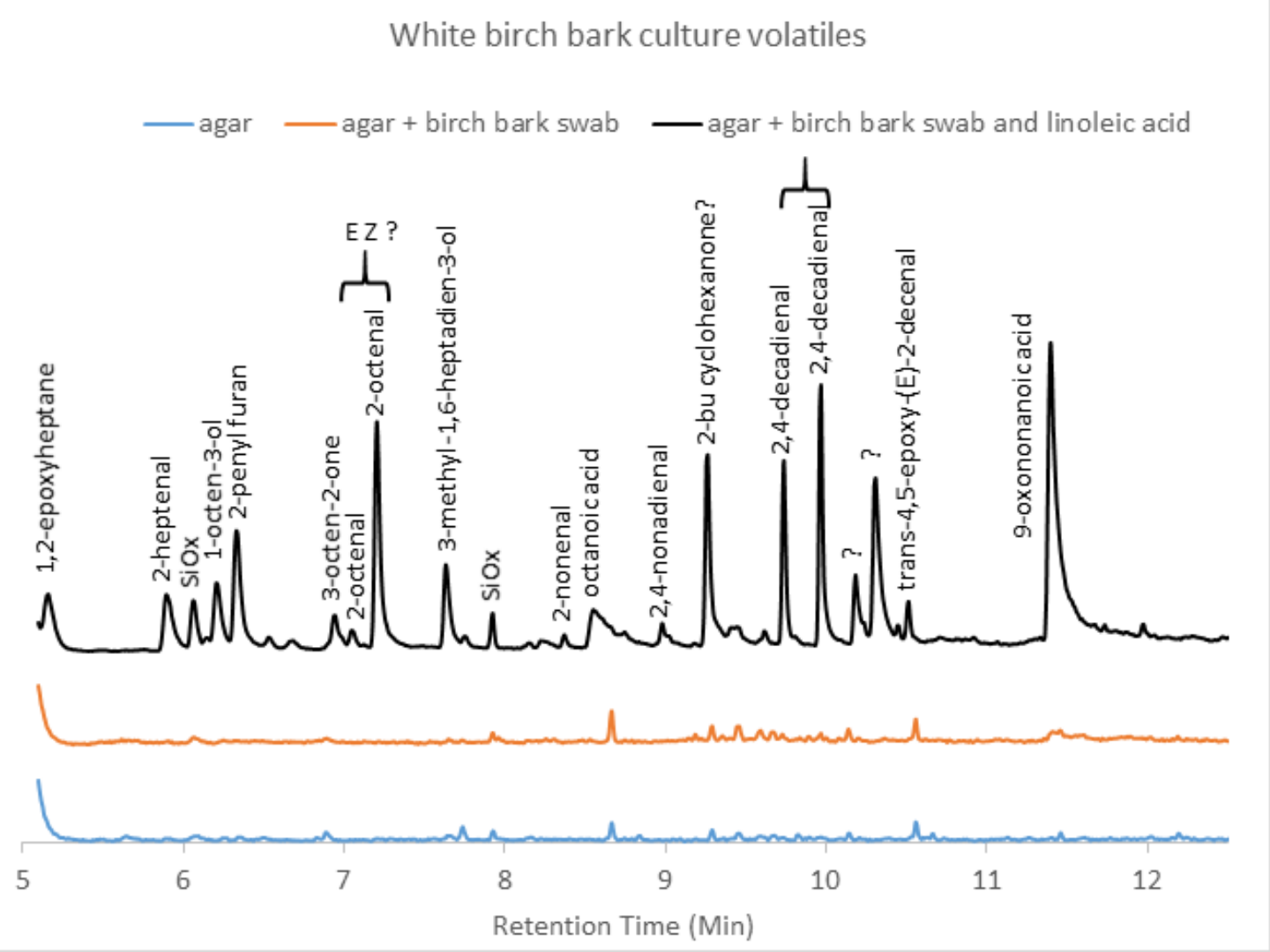


6. Compare barcode sequence to fungal libraries for Identification

Volatilome analysis

- Birch trees sampled (3): 2 live, 1 cut down
- Sample types: 2 swab and 2 wood bark pieces/tree
- Results: 32 pure cultures
  - Tree 1 (dead): 11 pure cultures (7S, 4P)
  - Tree2 (live): 12 pure cultures (8S, 4P)
  - Tree 3 (live): 9 pure cultures (3S, 6P)
- 28 Ascomycetes, 3 Basidiomycetes and 1 unknown
- 20 different genus, 3 confirmed species, need more marker data for species Id
- Next steps:
  - Volatile analysis of pure cultures (SPME GC/MS)
  - GC/EAD analysis





AUTOOXIDATION and /or FUNGAL DEGRADATION???

Silk, Ryall, Roscoe et al. unpublished

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FURTHER WORK

- *Agrilus anxius* Rosetta Stone yet to be discovered?
- Screen host volatiles (leaves, bark)…EAG, olfactometry, trap color?
- Screen fungal volatiles…EAG, olfactometry, trap color?
- Screen for pheromonal activity; females?

  (e.g. GC/EAD techniques)…BBB is not trapped by EAB pheromone!
- Trapping experiments… STAY TUNED!!!

  Recommend for now:
- green trap baited with 3Z-hexenol in the canopy?: very weak data!
- *Or* purple trap on girdled birch tree

Acknowledgements

- OECD & DEFRA
- Hugh Evans, Mariella Marzano and Gernot Hoch Organisers of the conference
- Thanks to VIENNA!
- The Silk Group: Lucas Roscoe, Peter Mayo, Gaetan LeClair, Dave MaGee (UNB), Deepa Abeysekera, Glen Forbes, Matt Brophy, Rosanna Lamb