Developing a choice bioassay to quantify chemically mediated behavior for the walnut twig beetle

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Introduction

- Walnut twig beetle (*Pityophthorus juglandis* Blackman) is an invasive species from the west coast.
- Forms symbiotic relationships with fungi, *Geosmithia morbida,* to cause thousand cankers disease (TCD).
- Black walnut (*Juglans nigra*) is susceptible to TCD.
- Black walnut is valued over $500 billion in standing trees.
- Semiochemicals are used by WTB to find a host. Once a host is found, male WTB release aggregation pheromones to attract more beetles.

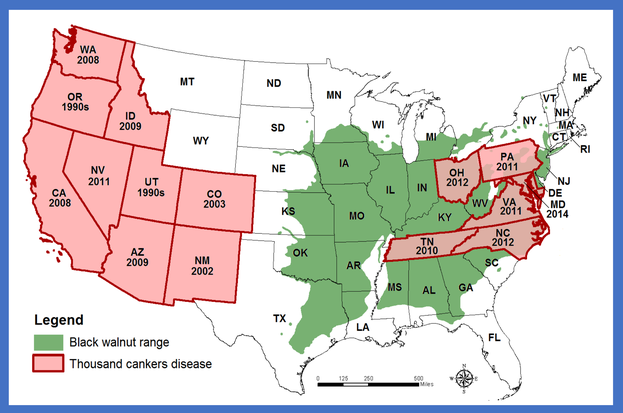

Figure 1: A map showing susceptible black walnut range (green) and states that already have TCD reporting's (red)

Rationale

- WTB causes serious harm to black walnut.
- Finding the best compounds to attract/repel WTB is important.
- In order to identify these compounds, a reliable method to test beetle response is necessary.
- This project tested two types of olfactometers to measure response of WTB to different attractants.

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Results

- Olfactometer box
  - Females showed no significant difference in choices between either the walnut twig or prenol and the blank.
  - Males spent longer time over prenol, however; the difference was not significantly different. Males showed no preference between the walnut twig and blank.
- Olfactometer Y-tube
  - Regardless of time interval, WTB showed no significant difference in choice between ethanol and walnut extract.

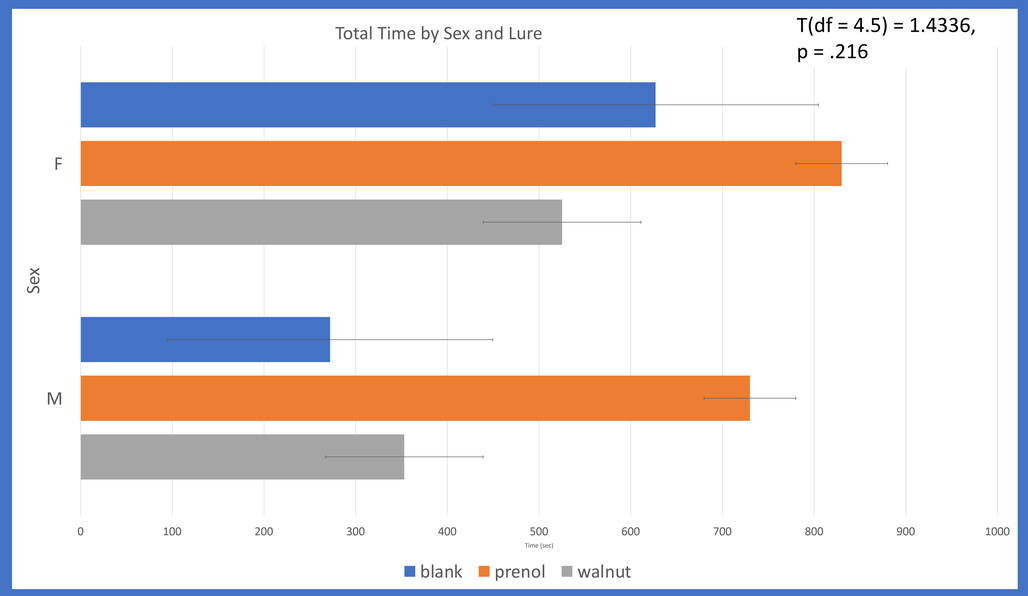


Figure 2: Beetle retention time over stimulus blank, prenol, and walnut twig between males and females using the box olfactometer

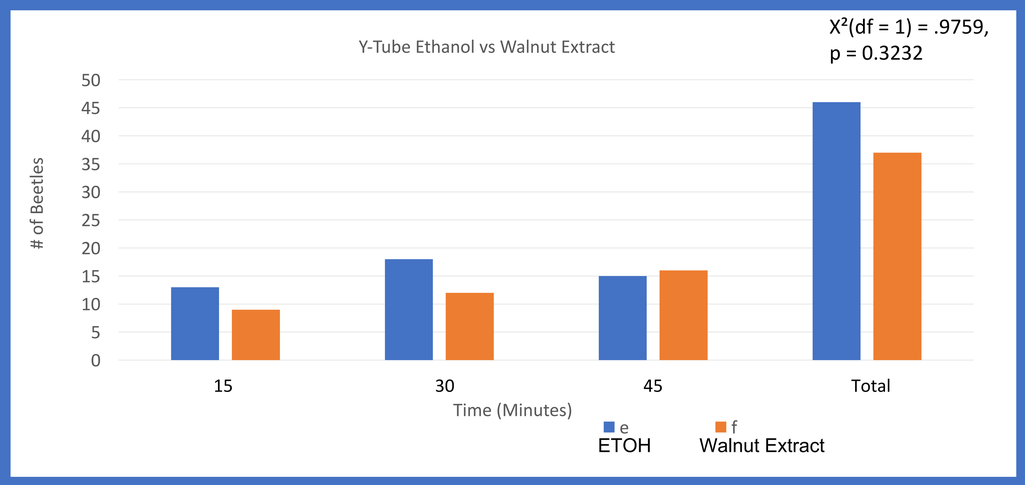


Figure 3: The number of beetles choosing ethanol vs walnut extract at each time interval (15, 30, 45) and the total time.

Methods

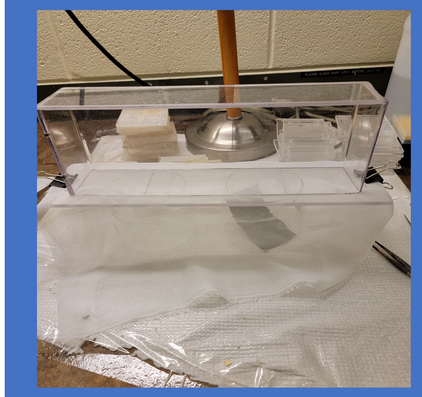
- Beetles from Walla Walla, WA were reared inside buckets in the lab.
- Two olfactometers were used for the project

Box

- Beetles presented with two stimuli
  - A walnut twig or prenol vs blank
- Time = 5 minutes per beetle
- Retention time of beetles above stimuli analyzed with Welch's two sample t-test

Y-tube

- Beetles presented with two stimuli
  - Ethanol extracted walnut vs pure ethanol
- Time = 45 minutes total (15, 30, 45 min intervals)
- Numbers of beetles at each stimulus per time interval analyzed with chi-square test



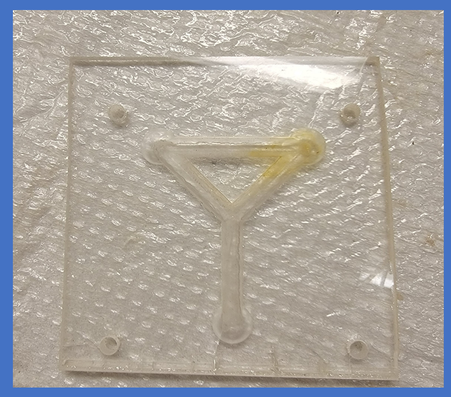


Figure 4-5: Olfactometer box (left) and olfactometer Y-tube (right)

Discussion

- Female WTB did not have a preference for either choice.
  - Other factors could be involved in host recognition.
- Males seem to show slight, but statistically insignificant preference for prenol over blank and walnut.
  - Could mean males are more sensitive to WTB aggregation pheromones.
- Beetles did not show a difference in retention on ethanol and walnut twig.
- Future work includes:
  - Conducting additional trials for more statistical power.
  - Conducting similar experiments using other attractants or repellants.

References

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