

The Effect of Increasing Ozone Concentration on Pheromone Communication Between Mountain Pine Beetles (*Dendroctonus ponderosae*)

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Introduction

- **Mountain pine beetles (MPB)** play a role in forest health by eliminating infected trees and encouraging forest regeneration.
- Outbreaks of MPBs can lead to an epidemic when healthy trees are attacked.
- MPBs communicate through the use of **pheromones**, which are often affected by the shift in conditions surrounding host trees.
- A rise in the emission of **air pollutants** results in an increased concentration of **ozone**. This can interfere with pheromone communication by affecting the microclimate and breaking down the pheromones carbon-carbon bonds. (Jiang, N, 2023)
- This study is to analyze the effect of increasing **ozone concentration** on MPBs pheromone communication.

Methods

- Three sealed acrylic boxes were assembled. Each with 5 Porapak Q adsorbent tubes attached to the top, connected to a motor.

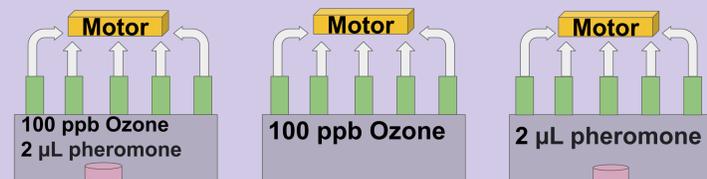


Figure 1 - Boxes 1, 2, and 3

- 4 different pheromones (**trans-Verbenol, Verbenone, exo-Brevicomin and Frontalin**) were used in the experiment.
- Each Pheromone would react for 2 and 6 hours, respectively in each box.
- The collection occurred for 2 hours: motor flow rates were set to 450 ml/min. Each tube was removed and stored in -80°C.
- For the extraction a **0.001%** solvent was made of **heptyl acetate** in **dichloromethane**.
- The beads from each adsorbent tube were removed and poured in 2 ml centrifuge tubes with 1 ml of the solvent.
- Each sample was **vortexed for 30 seconds, sonicated for 10 minutes and centrifuged for 15 minutes**.
- After pipetting and filtering the samples into a vial, the vials were stored in -40°C until results were analyzed in the GC/MS.

Results

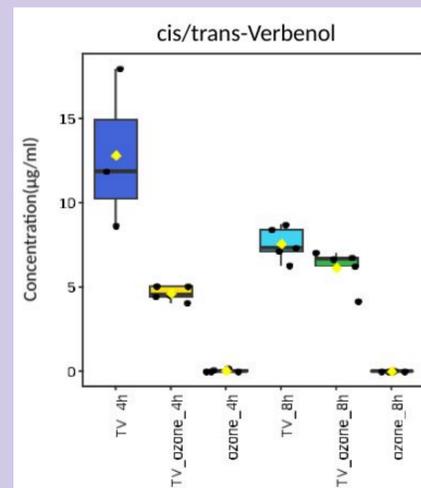


Figure 2 - trans-Verbenol

- There was a **decrease** in Pheromone concentration of 4h samples from box 1 to box 3
- The 8h samples had an insignificant difference in concentration comparing box 1 to 3.

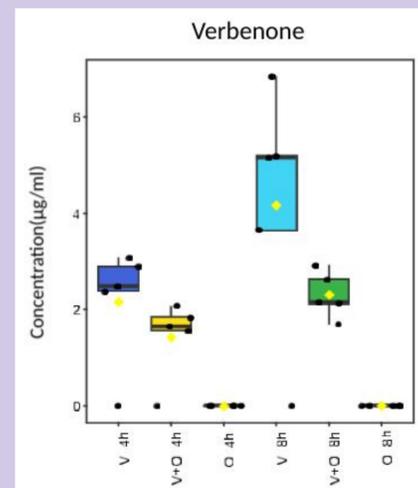


Figure 3 - Verbenone

- Concentration of pheromone **decreased** in box 1 with Ozone, compared to box 3 with air. This occurred in both the 2 and 6 hour reactions.

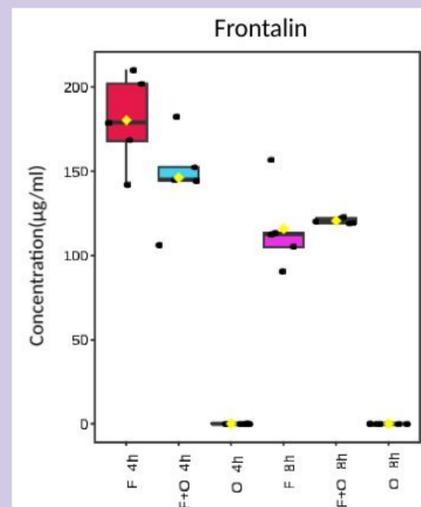


Figure 4 - Frontalin

- Pheromone concentration degraded in the 2 hour reaction.
- The 6h reaction had an insignificant difference in concentration in box 1 vs box 3.

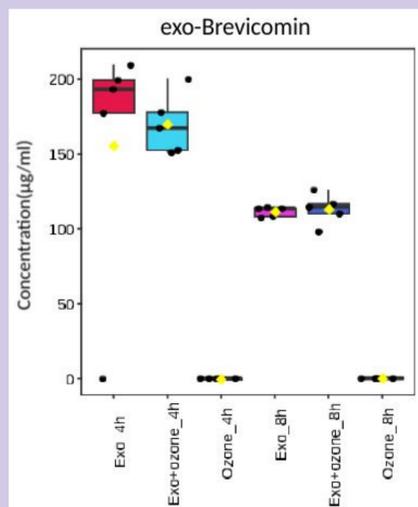


Figure 5 - Exo-Brevicomin

- Exo-Brevicomin did not show decreased amounts of pheromone concentration in the presence of ozone in the 2 or 6 hour reactions.

Conclusions

- Our study concluded that in the presence of 100 ppb of ozone, the pheromones **trans-Verbenol, Verbenone, and Frontalin** will begin to degrade.
- **exo-Brevicomin** had no significant changes in pheromone concentration when exposed to ozone for the 2 and 6 hour reactions.
- In the 8h Pheromone+O₃ **Frontalin** samples the ozone had no significant effect on the pheromone concentration. Indicating, that the ozone was likely unable to remain as O₃ in the box for the full 6 hours: potentially converting to oxygen.
- **Verbenone** degraded more in the 6 hour reactions than the 2 hour. Meaning that Verbenone has a different diffusion rate and breaks down more in longer reaction periods.
- **trans-Verbenol** had a decrease in concentration during the 2 hour reaction with ozone proving that ozone does reduce pheromone concentration. The 8 hour results were insignificant due to the lack of variability between samples with ozone and those with air.
- Ozone has a varied effect on different pheromones. Increased ozone can cause degradation, loss of function or little to no result depending on the pheromone.

References

- Knaden, Markus, et al. "Human impacts on insect chemical communication in the Anthropocene." *Frontiers in Ecology and Evolution* 10 (2022): 214.
- Jiang, N. J., Chang, H., Weißflog, J., Eberl, F., Veit, D., Weniger, K., ... & Knaden, M. (2023). Ozone exposure disrupts insect sexual communication. *Nature Communications*, 14(1), 1186.

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