Mitigating Adverse Effects of Pesticides on Honey Bees Through Dietary Phytochemicals

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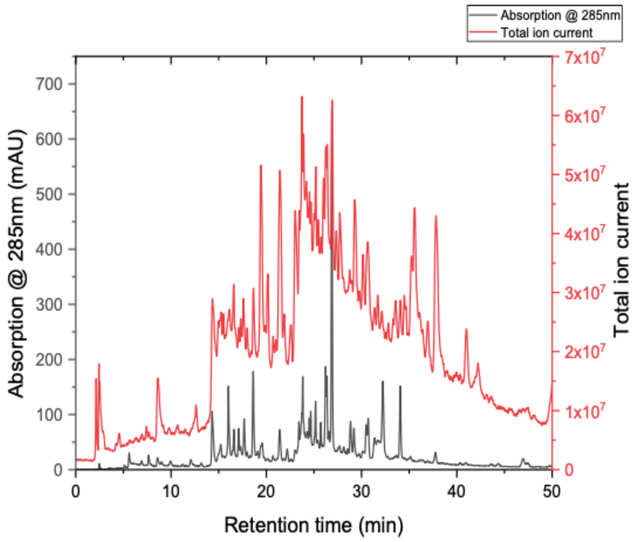
Introduction

- Many fungicides applied to almond orchards during bloom are considered bee-safe. However, beekeepers have reported sporadic occurrences of significant bee losses, particularly during larval and pupal stages, after fungicide applications (Mussen, 2008). Factors other than the fungicides might be involved in this intermittent bee mortality.
- Bees often ingest pesticides, including fungicides, along with phytochemical-rich food, which is detoxified predominantly by cytochrome P450 monooxygenases, potentially allowing toxicological interactions between pesticides and phytochemicals.
- According to our previous work, **consuming dietary phytochemicals ubiquitous in honey, including *p*-coumaric acid and quercetin, induces bee CYP450 gene expression** (Mao *et al*., 2011, 2013) and enhances detoxification of co-occurring tau-fluvalinate (Johnson *et al*., 2012), bifenthrin, β-cyfluthrin (Liao *et al*., 2017) and imidacloprid (Wong et al., 2018).
- For colony viability, healthy queen-rearing is essential. Nurses feed queen larvae in their queen cells and tend to them over the course of their development. **Nurse bees that consume pesticide-contaminated diets may experience changes in behavior and physiology that compromise queen care**. Pesticides in bee food might alter, quantitatively and qualitatively, the content of royal jelly, the queen larval food, by modifying mandibular and hypopharyngeal gland function, which in turn may compromise the quality of the next generation of queens.
- Our objectives were:
  - to determine the sublethal adverse effects of interactions between a fungicide and a tank-mixed insecticide **on bee flight performance**, **worker bee nursing behavior,** and **the quality of queens** raised by nurses that have ingested pesticides (in collaboration with R.M. Johnson et al., OSU), as well as **their interactions with phytochemicals.**
  - to **determine possible interactions between almond phytochemicals** and fungicides (in progress).
  - to evaluate the ability of phytochemical supplements to "rescue" impaired queen-rearing behavior and longevity and identify underlying **mechanisms** (via electroantennography).

Almond Honey Phytochemicals (preliminary data)



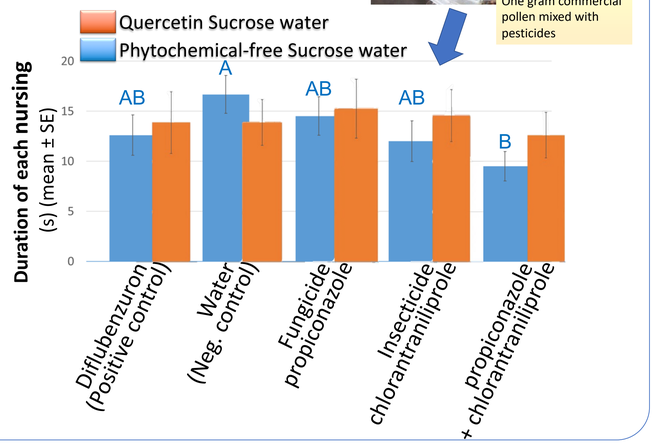
Two constituents, abscisic acid and the flavanone galangin, were identified from almond honey. The figure displays the UV absorption (285 nm) chromatogram (**black** line) and the total ion current chromatogram (**red** line) of an almond honey from HPLC-MS analysis.

Effects of Pesticides/Phytochemical Interactions on Queen Cell Nursing Behaviors

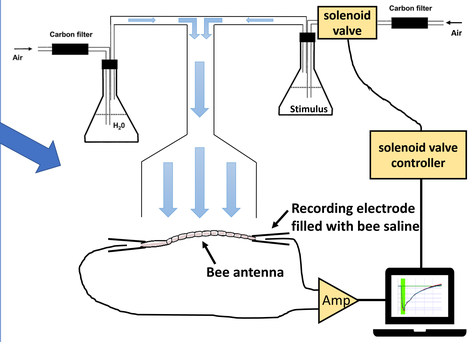
Mixed **propiconazole** and **chlorantraniliprole reduced the duration of visiting and nursing behaviors** of nurse bees consuming phytochemical-free sugar water.

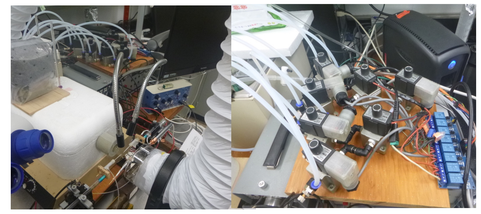
Consuming 250μM **quercetin-supplemented sugar water neutralized all impaired nursing behaviors** with all pesticide treatments. We detected no significant difference between any treatments.

One gram commercial pollen mixed with pesticides



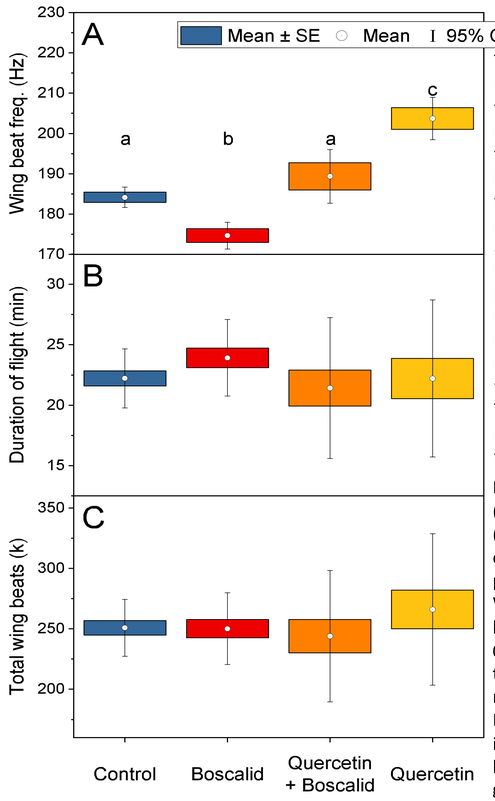
Effects of Pesticides on Olfactory Impairment via Electroantennography (EAG)





- We conducted assays to assess pesticide-induced altered olfactory responsiveness to brood pheromone as a possible mechanism underlying nursing behavior changes.
- We fed workers treatment pollens (water control, Dyne-Amic, Altacor plus Tilt, and Dyne-Amic+Altacor+Tilt) for one week and then measured their EAG responses to serial concentrations (5%, 10%, 20%, 40% and 100%) of synthetic non-volatile brood pheromone and a volatile brood pheromone, ocimene, to detect signs of olfactory impairment.
- In addition to the non-volatile brood pheromone, we also tested mineral oil as a negative control, and 2-heptanone, an alarm pheromone, as a positive control.
- Each antenna was tested with sequential exposure to the negative control, the serial concentrations of non-volatile brood pheromones, the volatile brood pheromone, and the positive control for each odor trial. We performed six odor trials per antenna.

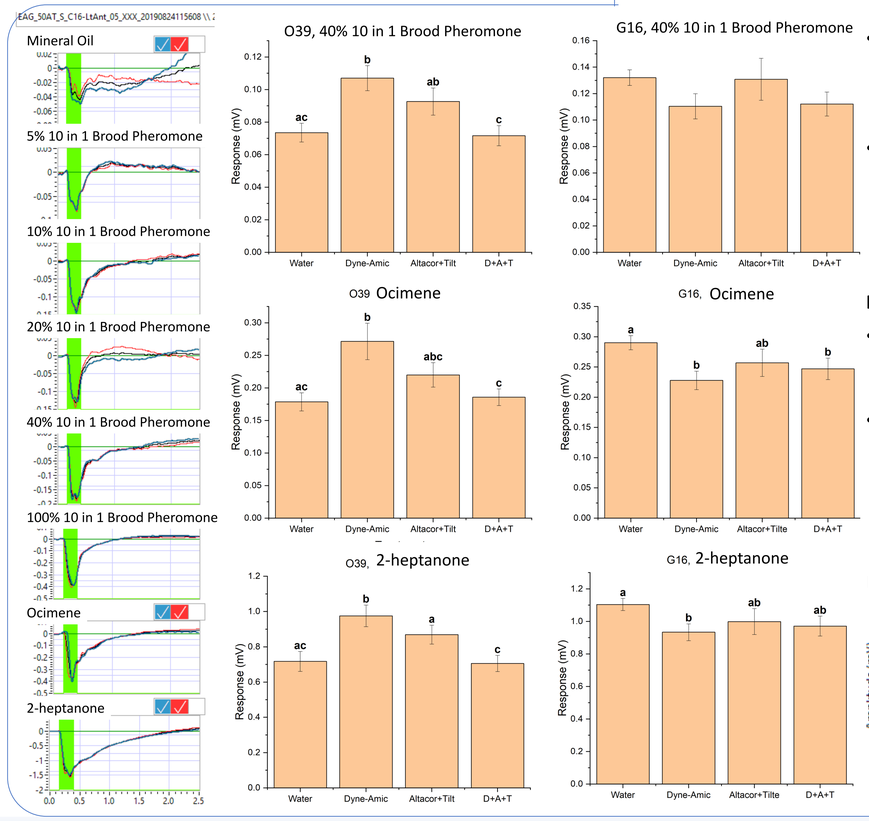
Flight Performance on Workers



The fungicide boscalid inhibits mitochondrial complex II, part of the ATP-generating system that provides energy to cells and tissues. When bees consume boscalid with sugar water, their wingbeat frequency declines, possibly impairing their foraging ability. However, pollen and honey contain quercetin, a phytochemical that boosts ATP levels in flight muscles. When bees consumed quercetin with boscalid, wingbeat frequencies were restored to normal levels. Thus, their natural diet may protect bees against fungicide toxicity.

Effect of a phytochemical (quercetin) and fungicide (boscalid) individually and combined on the flight performance of foragers. A) Wingbeat frequency was affected by treatments ($df = 3$, $\chi = 88.59$, $p < 0.001$). B) duration of flight and C) total wingbeats per flight were not affected by treatments. Different lower-case letters indicate significant differences between treatments ($p < 0.05$, generalized estimating equation).

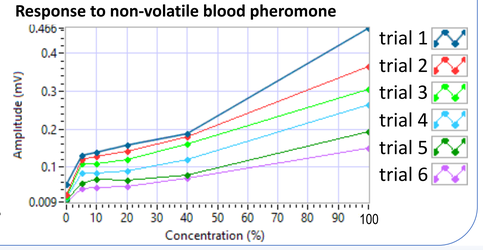
Liao L-H, Wu W-Y, Dad A, Berenbaum MR. 2019 Fungicide suppression of flight performance in the honeybee (*Apis mellifera*) and its amelioration by quercetin. Proc. R. Soc. B 20192041. http://dx.doi.org/10.1098/rspb.2019.2041 (*in press*)



Results:

- The mixture of the fungicide Tilt and the insecticide Altacor did not alter the olfactory responses of nurses.
- However, the adjuvant Dyne-Amic may alter the olfactory response of nurse bees to brood pheromones and the alarm pheromone 2-heptanone. Nonetheless, the responses were different between hives. Further analysis is ongoing.

Response to non-volatile blood pheromone



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