Antioxidants in Wax Cappings of Honey Bee Brood

Project No.: **08-POLL3-Sammataro**

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Objectives:

1) Determine antioxidant levels in the wax cappings of honey bee brood and compare cappings from Varroa-infested and uninfested brood.
2) Test antioxidants found in wax cappings for miticidal activity against Varroa mite using the mite vial assay.

Interpretive Summary:

As research continues on the causes of the colony collapse disorder (CCD) in honey bees and the myriad of challenges they face, including diseases, parasitic mites and pesticides, the role that nutrition in the bees' diet may be included in this list. Nutritional requirements pervade all aspects of bee biology and play a significant role in disease prevention by supporting immune responses. In Varroa mite-infested colonies, a healthy immune system is crucial. Mites pierce the cuticle of bees to feed on bee hemolymph, and may decrease the amount of hemolymph available to the bee. Antioxidants and antioxidant enzymes make up a large portion of the honey bee immune system. We investigated if there was a difference in the antioxidant level of wax cappings from infested vs. non-infested brood in workers and drones from several bee lines. The results confirm that there is a difference in antioxidant activity in cappings of bee brood between Varroa- infested vs. non-infested cells. This indicates that the bees are responding to mite invasion and activity while in the pupal cell. These results demonstrate that antioxidant activity is dynamic in a bee colony and may shed some light on the nutritional requirements and even supplements that could benefit bees.

The next phase of this work will be to feed bees supplementary antioxidants in their food to help boost their immune system and perhaps mitigate the harmful effects of mite predation of bee larvae. We will also look at if particular antioxidants are lethal to mites in laboratory assays.

Materials and Methods:

Two types of samples were collected; cappings from Varroa-infested vs. uninfested brood and cappings from certain queen lines. Colonies headed by specific lines of queen honey bees included Russian, African, Hygienic, California Italians and SMR and all were located at the Carl Hayden Bee Research Center (Tucson AZ). Five colonies per line were all installed on new beehive equipment and foundation and maintained for several years in the same hive furniture. Cappings were collected from known-aged brood, obtained by caging the queen for 24 hours (Finely and Sammataro 2008). Cappings that were from infested cells were stored separately from uninfested cells. All cappings were put in Eppendorf tubes and stored in the freezer (0F -19.4C) until needed. Cappings from drones were collected from a California Italian colony and separated

Five cappings from infested vs. uninfested brood were weighed; these were the small samples. In the second experiment, we pooled all the cappings from all four African bee colonies (A), all days (738.45mg) and all three Russian colonies (B), all days (834.4mg). The rest of the mite cappings (C), all from the Russian colonies were also pooled (59.4mg).

To dissolve the wax in the small samples, we added 100uL of methanol and put each tube in the ultrasound for 10 min. after which they were placed in the -70 overnight. The next day, after they defrosted, 200uL more of methanol was added and again placed in the ultrasound for 10 min.

Determination of Antioxidant Activity Using the DPPH Radical Scavenging Method.

The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay is a robust, facile antioxidant assay. We used a modification of the assay conditions reported by Hatano et al. (1988). Ethyl alcohol (280 uL) is mixed with the solvent extract (72 uL) in a scintillation vial and DPPH solution (242.5 uL of a 51 mg DPPH/100 mL methanol) added; the vial is capped and vortexed. The more active samples turn color, from purple to light yellow. Immediately following, 160 uL is transferred to each of the 96 wells of the microplate and the samples are run in triplicate. The micro-plate is sealed (Thermal Seal, Excel Scientific, Wrightwood, CA) and incubated in the dark for 1 hour at room temperature. After a programmed 30 second shake cycle, the absorbance is recorded at 517 nm. Extraction solvents (72 uL) volumes is used as controls for each solvent extract. The percent activity is calculated by the following equation by substituting the mean of the absorbance values:

$$\% \text{ scavenging activity} = 100 \times \frac{[\text{control-sample}]}{\text{control}}$$

Determination of Antioxidant Activity Using the FRAP Assay.

The ferric reducing – antioxidant power (FRAP) assay was performed according to conditions reported by Benzie & Strain (18). Water (350 uL; 18 M-Ohm; Barnstead) was mixed with 100 uL of FRAP solution. The FRAP solution was freshly prepared by

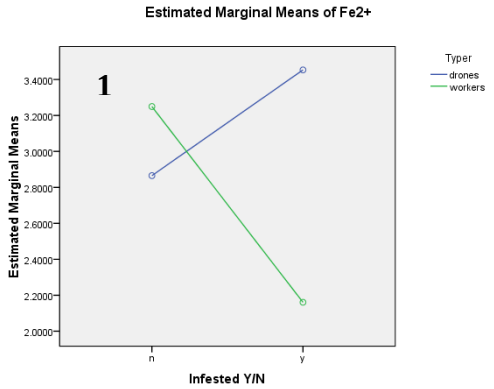
mixing: 10 mM 2,4,6-tripyridyl-S-triazine (TPZ) in 40 mM HCl, 20 mM $FeCl_3$ solution and 0.3 M NaOAc/HOAc buffer at pH=3.6 at (1:1: 10) parts per volume respectively.

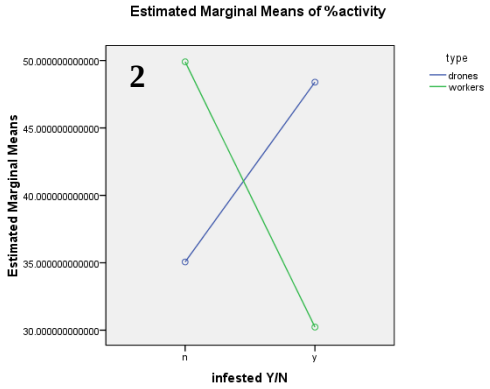
$[Fe^{2+}]_{mM} = 0.235 \times (Abs@593\ nm) + 0.0054;\ R^2=0.9992$

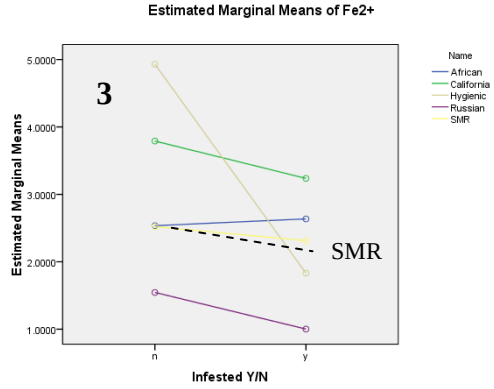
Quercetin, a flavanol, was selected because it is a polyphenolic that is found in bee collected pollen, fruits and legumes. By performing the FRAP and DPPH assay under identical conditions as the extracts and by varying the concentrations of quercetin, we could determine the antioxidant activity of the extracts in terms of quercetin equivalents.

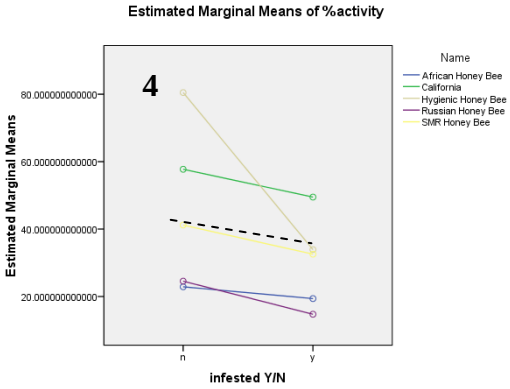
Results & Discussion:

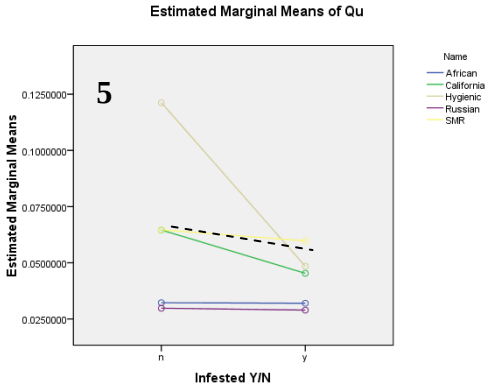
In our preliminary investigations, we did find some significant differences in the activity of the cappings between infested and non-infested workers and drones. The results are summarized in the graphs.











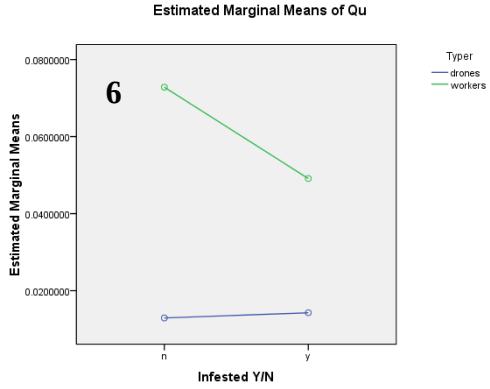


Fig 1. The results of the FRAP assay indicates that activity in drone capping increases if the drone brood is infested; in worker cappings, the activity decreases if infested.

Fig 2. The percent activity from the DPPH assay shows the same changes from infested drones and workers as in the FRAP assay.

Fig. 3. There were some interesting differences between lines of bees. Russians differed from all other lines in the FRAP assay and had the least difference between infested and non-infested brood cappings.

Fig 4. The results of the DPPH assay indicates that the Russian bees were different from all the other lines except the African bees and the SMR (dashed line) were different from all except the Californa Italians.

Fig. 5. Quercitin equivalents assay demonstrated that the African, Russian and California Italians bees differed from the Hygienic and SMR lines, and the SMR was different from all other lines. The Russian and African lines had the least activity between the infested vs. non-infested cappings and the hygienic ahd the most difference with a decrease in activity from non-infested to infested.

Fig. 6. The Quercitin activity dropped significantly in workers that were infested, but rose in infested drones.

Conclusion:

The results confirm that there is a difference in antioxidant activity in cappings of bee brood between Varroa- infested vs. non-infested cells. This would indicate that the bees are responding to mite invasion and activity while in the pupal cell. These results demonstrate that antioxidant activity is dynamic in a bee colony and may shed some light on the nutritional requirements and even supplements that could benefit bees. The next phase of this work would be to feed bees supplementary antioxidants in their food to help boost their immune system and perhaps mitigate the harmful effects of mite predation of bee larvae. We will also look at if particular antioxidants are lethal to mites in laboratory assays.