Antioxidants in Honey Bee Colonies

Project No.: **09-POLL3-Sammataro**

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Objective:

Test antioxidants for miticidal activity against *Varroa* mite using the mite vial assay.

Interpretive Summary:

The goal of this project is to determine if feeding antioxidants to bees improves immune response, bee health and tolerance to *Varroa* mite. This will be done in a two step process. First, we will determine if any antioxidants have direct miticidal activity against *Varroa* mites. Second, we will investigate the effects of incorporating select antioxidants into supplemental bee food to determine if there is an increase in bee longevity.

For step one, we screened 7 antioxidants for direct miticidal activity against *Varroa* mite, including L-cystine, L-aspartic acid, L-tryptophan, proline, L-histidine, benzophene and naringenin. Of these, only benzophene killed more than 40% of the mites and requires further evaluation as a potential miticide (see **Figure 1**). We are continuing to test 8 to 10 additional antioxidants, such as quercetin, ascorbic acid (vitamin C), ascorbic acetate (Vitamin C ester), uric acid, caffeic acid, ferrulic acid, gallic acid, cinnamic acid, clove oil, pyruvate and α-tocopherol (vitamin E).

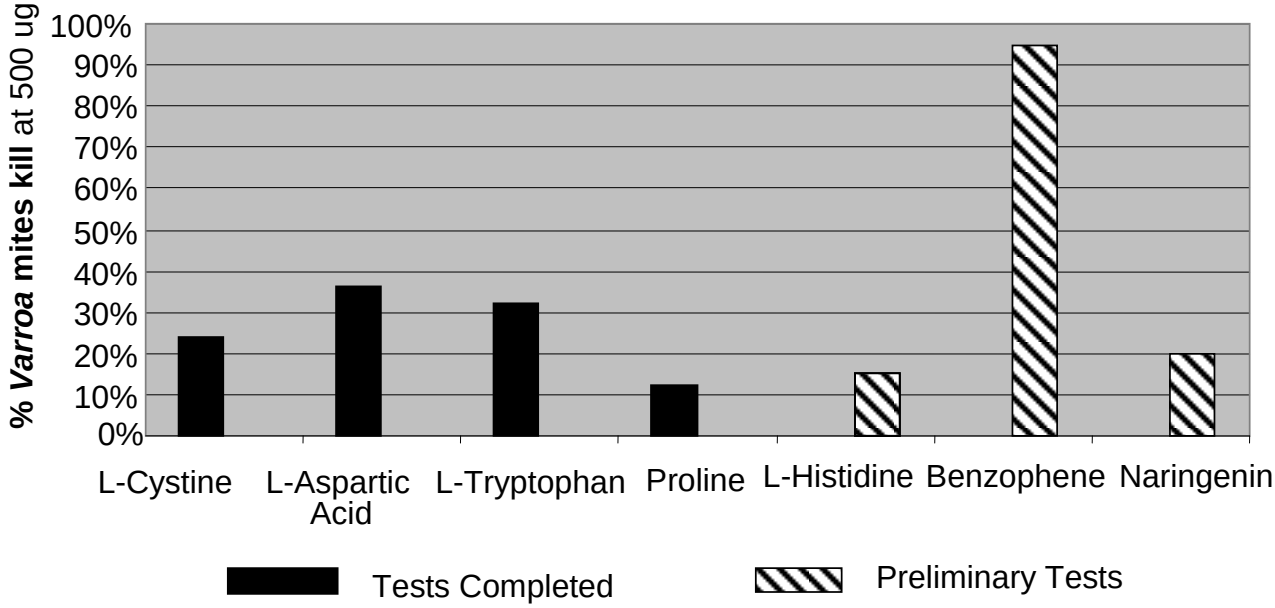
To conduct the mite vial assay, we diluted each compound to the desired concentration with a volatile, non-toxic solvent (typically acetone). Then a measured amount of one compound is coated onto the inside of a 20mL glass scintillation vial. After a few minutes, the solvent evaporates, leaving only the test compound on the interior of the vial. Control vials are prepared in the same manner using 0.5mL of plain solvent (acetone), which does not affect mite survival (Elzen et al. 1998).

Next, live, phoretic adult *Varroa* mites were collected by shaking adult honey bees with powdered sugar to separate the mites from the bees (Macedo and Ellis 2002). We

placed 5 mites into each test vial without any food (Elzen et al. 1998). Five replicate vials are prepared for each test compound at each concentration. In addition, at least there are 5 control vials for each trial. All test vials are placed into a warm incubator to simulate the conditions found inside honey bee colonies.

After 24 hours, test vials were examined under a dissecting microscope. Mites were prodded with a probe to encourage movement and non-moving mites were recorded as dead.

Figure 1. Results of 2008 Phase 1 Mite Testing for 7 Antioxidants



We will continue with initial screening to determine if we observe high mite mortality at a very high concentration of the test compound (500 ug; the active ranges of known *Varroa* miticides are below 250 ug). For each antioxidant, we will prepare 5 vials containing 5 mites each and 5 Control vials. If we observe promising mite mortality (>70%) in Phase 1, the compound will be passed to Phase 2.

Next promising compounds, including benzophene, will be re-tested over a lower range of concentrations until we determine the optimal dose (minimal contact concentration that results in maximum mortality) for that compound. Promising compounds with good mite mortality will be advanced into the second phase of this research: bee feeding trials assessing brood survival and bee longevity. This work will be done over the fall and winter, as mite numbers increase.

As a final note, the Antioxidants in Wax Cappings of Honey Bee Brood paper, supported by the Almond Board, is now in review with the Journal of Apiculture Research.