# Antioxidants in Honey Bee Colonies

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

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## Objectives:

1. To determine if bees are tolerant of or repelled by antioxidants and nutrient (amino acid) supplements in sugar syrup; and if so, at what concentration.
2. To determine their lethality to the *Varroa* mites in a vial assay.

## Interpretive Summary:

Honey bee health is recently becoming the focus of research in an effort to determine some of the reasons for the decline in managed bee populations due to colony collapse disorder (CCD). This includes considering bee nutrition, since nutritional requirements pervade all aspects of bee biology and play a significant role in disease prevention by supporting immune responses. Our goal was to help bees combat the effects of poor diet, stress and pathogens by supplemental feeding of antioxidant and amino acid compounds. These were fed to free-flying bees in a choice test to determine which treatments were attractive or repellent compared to sugar syrup. Of the ten choices, L-cysteine, L-aspartic acid, and gallic acid were avoided or actually killed bees at a 5% solution. In a vial assay, the compounds were tested for *Varroa* mite mortality and while all killed mites at 500 µg concentrations, benzophenone and ferulic were the only two compounds that killed over 40% of the mites. The next step would be to assess the benefits of feeding antioxidants to bees to improve longevity, stress and pathogen defense.

## Materials and Methods:

*Feeding Trial:* The feeding responses to antioxidant- or amino acid-supplemented solutions were compared against sugar syrup controls. In each feeding trial, foragers were given a choice between a supplemented solution and a control (sugar syrup only), presented in Petri dishes. Each choice array consisted of three dishes of 24ml sugar syrup (50% w/w) solution and three of each treatment, randomly placed on an outside table near the Carl Hayden Bee Research Laboratory Apiary (Tucson, AZ). In each trial, the number of bees present at each dish at the end of two 10-minute intervals was counted by eye or camera.

Each of the compounds was tested at a 1%, 2%, and 5% solution. After each trial, new Petri dishes were used. The test compounds were: L-cysteine, L-aspartic acid, L-tryptophan, proline, L-histidine, ascorbic acid, uric acid, caffeic acid, gallic acid. These compounds were chosen because most are commonly found in nectar, honey, propolis, pollen (Gilliam et al 1980) and royal jelly (Nagai et al 2001) and would therefore naturally come in contact with bees. The percent preference data were compared via one-way ANOVA, both across antioxidant treatments for a given concentration, and then again within each antioxidant across three concentrations.

*Miticidal activity trial*: Mites were exposed to single compounds coated on the inside of a 20mL glass scintillation vial and 500µg of each test compound was applied in acetone to the inside of the vial and the solvent was allowed to evaporate, leaving only the test compound on the interior of the vial. By comparison, (before resistance), the miticides amitraz was lethal ($LD_{90}$) at 124 µg, coumaphos at 53 µg, and fluvalinate at 2.38µg (Elzen, et al. 2000). Compounds tested were: ascorbic acid, benzophenone, caffeic acid, citric acid, ferulic acid, L-aspartic acid, L-cystine, L-histidine, L-tryptophan, naringenin, proline and uric acid. The miticidal activity of these compounds was evaluated individually in vial bioassays. Control vials were prepared in the same manner using 0.5mL of acetone which does not affect mite survival (Elzen et al. 1988).

Live, adult *Varroa* mites were collected off removed infested brood and 5 mites were placed into each test vial without any food (Macedo et al. 2002). There were 5 replicate vials of each test compound and 5 control vials for each trial, which were placed into an incubator (Caron 6010, Marietta, OH) set at temperatures and humidity to simulate a hive environment (35C 50% RH). After 24 hours, vials were examined under a dissecting microscope. Mites were prodded with a probe to encourage movement and non-moving mites were recorded as dead. Mite mortality was recorded for each vial, (number of dead mites/the total number of mites in a vial) and expressed as % kill. We tested mortality from each concentration of each compound separately using the Chi-Square Test for Independence (data not shown). Mortality is compared to the expected mortality from the control vials (≤15% mortality) and summarized at percent mortality by treatment.

**Results and Discussion:**

**<u>Results of Feeding Trials</u>**

**1% Feeding Trial:** All of the supplemented solutions were eaten after 20 min. and there was no difference between treatments and sugar syrup visitation.

**2% Feeding Trial:** All of the supplemented solutions were equally visited and there was no difference; however, while gallic acid attracted the bees, the bees died before they could leave the dish. In the 2% L-tryptophan and L-histidine trial, the majority of bees ate the control syrup first, and then moved to the supplemented treatments after 20 minutes.

**5% Feeding Trial:** In these trials, while there was no statistical difference in visitation vs. control, the L-cysteine and gallic acid solutions solidified and killed the bees that were feeding on it before the solution hardened. For all compounds, most of the bees were

attracted to the sugar syrup first and only moved to the trial solution once the sugar syrup was fully consumed. The 5% supplemented solutions that were the least visited were L-cysteine and aspartic acid ($F_{2,3}$=27.84, p=0.012, $F_{2,3}$=46.135, p=0.006,respectively); see **Figure 1**.

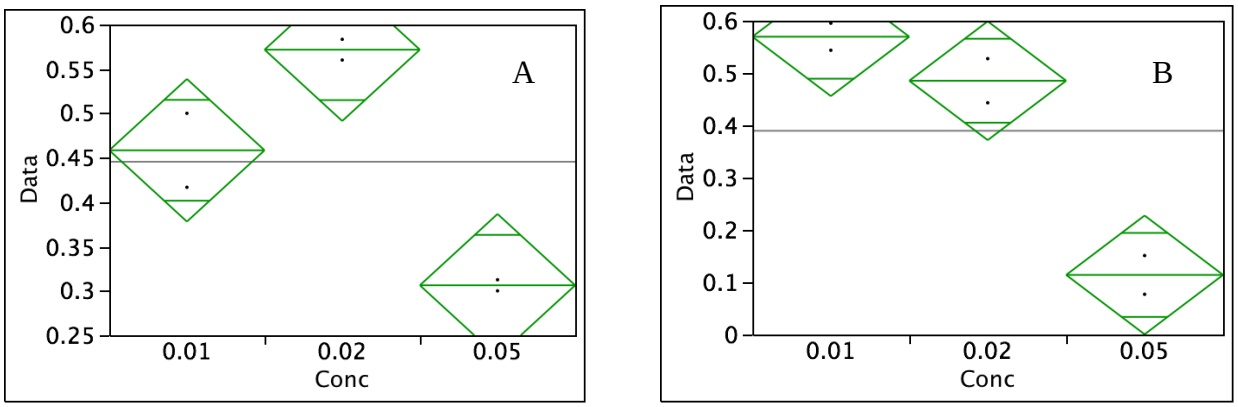


**Figure 1**. L-cysteine visitation (A) and aspartic acid (B) at 3 concentrations. The 5% solution was the least visited between the other concentrations.

## Results of Miticide Trials

Results are summarized in **Figure 2** and indicate that all of the compounds had miticide activity at 500µg, but that benzophenone and ferulic were the only two compounds that killed over 40% of the mites.

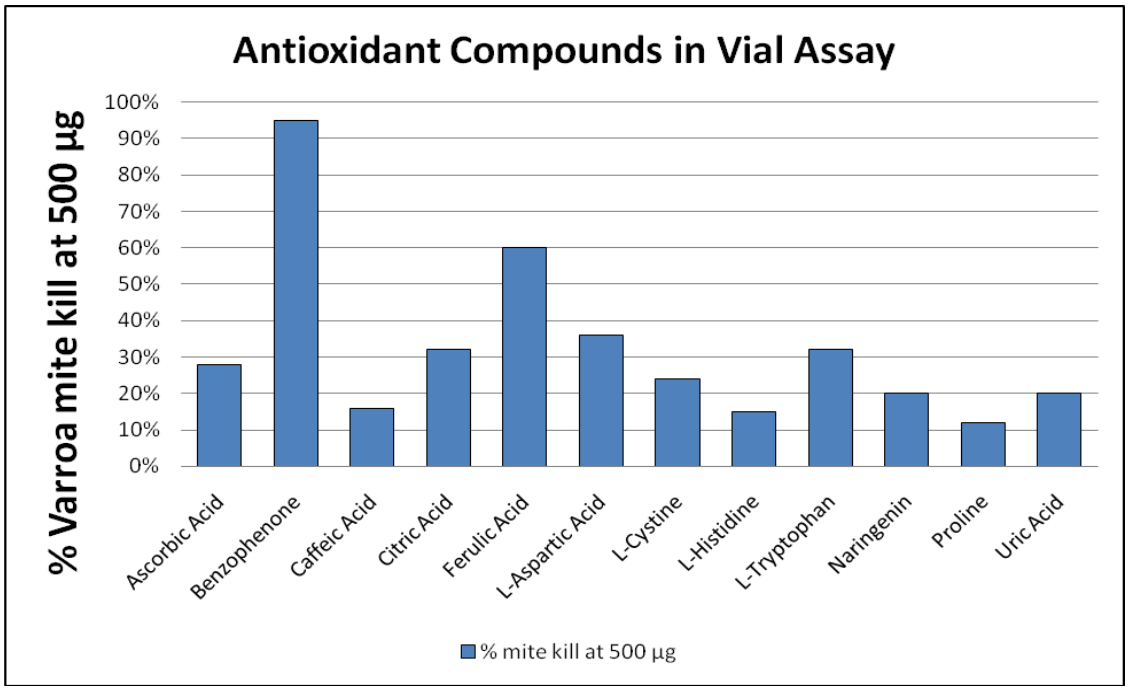


**Figure 2**. Treatment compounds used in the vial assay to kill *Varroa* mites.

## Discussion:

In this preliminary feeding study, we found that bees do have a preference to some of the antioxidant compounds while others (gallic acid, L-cysteine, aspartic acid) had to be eliminated due to their toxic effects on or avoidance by bees. For the next phase of the feeding study, we will add the compounds into syrup for longevity study of caged bees. One of the compounds, naringenin, has been found in large amounts in Sonoran desert pollen (LaBlanc et al. 2009) and will be the subject of future feeding and *Varroa* experiments. Proline was found to be attractive to bees in a feeding experiment (Bertazzini et al.2010); this and other compounds may play an important role in honey bee health as well as defense against parasites and pathogens. Johnson et al. (2009) observed that bees either do not have a complex immune system, or the genes involved in immune response were compromised by viral infections. Because bees can have multiple viruses (Chen et al. 2004), our goal is to help bees combat the effects of poor diet, stress and pathogens by supplemental feeding.

The next step would be to assess the benefits of feeding antioxidants to bees to improve longevity, stress and pathogen defense. Additionally, the future work for the *Varroa* control is to determine the LC90-95 (concentration which kills 90-95% of mites) for promising compounds. Next, we will take that concentration (or higher) to caged bees and look for detrimental bee effects and mode of action, which will help to determine our delivery route (contact, systemic, etc.). As a final step, and assess how to distribute the compounds to achieve effective doses in the hive environment.

## Recent Publications:


Sammataro, D., B. LeBlanc, J. Finley and M.C. Carroll. 2010. Antioxidants in wax cappings of honey bee brood. Apidologie, in press.

Ruiz-Matute, M. Weiss, D. Sammataro, J. Finley and M. Luz Sanz. 2010. Carbohydrate composition of High-Fructose Corn Syrups (HFCS) used for bee feeding: effect on honey composition. J. Agric. Food Chem. 58: 7317–7322.

Cicero, J. M. and D. Sammataro. 2010. The salivary glands of adult female *Varroa destructor* (Acari: Varroidae), an ectoparasite of the Honeybee, *Apis mellifera* (Hymenoptera: Apidae). *Internat. J. Acarology*. Vol. X, in press.

Sammataro, D., J. Cicero. 2010. Functional morphology of the honey stomach wall of European honey bees (*Apis mellifera* L. ) as viewed by SEM. *Annals Entomol. Soc. Am*.. Section A, in press.

Sammataro, D. and M. Weiss. Comparison of Productivity between Honey Bee Colonies Supplemented with Sucrose or High Fructose Corn Syrup (HFCS). In prep.

Sammataro, D. and A. Avitabile. 2010. Beekeeper's Handbook. 4th ed. Cornell Un. Press. In press

**References Related to this Reserch:**


Ball, B. 1997. *Varroa* and viruses. *In* Munn, P.; Jones, R. (eds) *Varroa! Fight the mite*. International Bee Research Association; Cardiff, UK. pp. 11–15.

Bertazzini, M., P. Medrzycki, L. Bortolotti, L. Maistrello. 2010. Amino acid content and nectar choice by forager honeybees(*Apis mellifera* L.). *Amino Acids*. 39: 315–318.

Brodschneider, R. And K. Carilsheim. 2010. Nutrition and health in honey bees. *Apidololgie* 41: 278-294.

Chen, Y, Y. Zhao, J. Hammond, H. Hsu, J. Evans, M. Feldlaufer. 2004. Multiple virus infections in the honey bee and genome divergence of honey bee viruses. J. Invertebrate Pathology 87: 84–93

Dimri, U., Ranjan, R.; Kumar, N.; Sharma, M. C.; Swarup, D.; Sharma, B.; Kataria, M. 2008. Changes in oxidative stress indicies, zinc and copper concentrations in blood in canine demodicosis. *Veterinary Parasitology*. 154: 98-102.

Elzen, P. J., Eischen, F. A., Baxter, J. R., Pettis, J., Elzen, G. W., Wilson, W. T. 1988. Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations. *Am. Bee J.* 138: 674-676.

Elzen, P. J., J. R. Baxter, M. Spivak, W. T. Wilson. 2000. Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos. *Apidologie* 31: 437-441.

Evans J. D. and J. S. Pettis. 2005. Colony-level impacts of immune responsiveness in honey bees, *Apis mellifera*. *Evolution* 59:10, 2270-2274.

Felton, G.W. 1995. Antioxidant defenses of invertebrates and vertebrates, in: Ahmad S. (Ed.), *Oxidative Stress and Antioxidant Defenses in Biology*, Chapman & Hall, New York, pp. 356-434.

Gilliam, M. W.F. McCaughey, B. Wintermute. 1980. Amino acids in pollens and nectars of citrus cultivars and in stored pollen and honey from honeybee colonies in citrus groves. *J. Apic. Res.* 19: 64-72.

Gregory, P.G., Evans, J.D., Rinderer, T. and de Guzman, L. 2005. Conditional immune-gene suppression of honeybees parasitized by *Varroa* mites. *J. Insect Sci.* 5:7, online: insectscience.org/5.7

Herbert, E.W. Jr., J.T. Vanderslice, D.J. Higgs. 1985. Effect of Dietary Vitamin C Levels on the Rate of Brood Production of Free-Flying and Confined Colonies of Honey Bees. *Apidologie* 16: 385-394.

Joanisse, D.R. and K. B. Storey. 1996. Oxidative stress and antioxidants in overwintering larvae of cold-hardy goldenrod gall insects. *J. Exper. Biol.* 199: 1483-1491.

Johnson, R. M., J. D. Evans, G. E. Robinson, and M. R. Berenbaum. 2009. Changes in transcript abundance relating to colony collapse disorder in honey bees (*Apis mellifera*). *PNAS* 106: 14790–14795. www.pnas.org/cgi/doi10.1073/pnas.0906970106

Kanbar, G. and Engles, W. 2003. Ultrastructure and bacterial infection of wounds in honey bee (*Apis mellifera*) pupae punctured by *Varroa* mites. *Parasitol. Res.* 90: 349-354.

Kanbur, M.; Atalay, O.; Ica, A.; Eraslan, G.; Cam, Y. 2008. The curative and antioxidative efficiency of doramectin and doramectin + vitamin AD3E treatment on *Psoroptes cuniculi* infestation in rabbits. *Research in Veterinary Science*. 85: 291-293.

LeBlanc, B. W., O.K. Davis, S. Boue, A. DeLucca and T. Deeby. 2009. Antioxidant activity of Sonoran Desert bee pollen. *Food Chemistry*. 115: 1299-1305.

Lipinski, Z. and K. Zoltowska. 2005. Preliminary evidence associating oxidative stress in honey bee drone brood with *Varroa destructor*. *J. Apic. Res*. 44: 126-128.

Macedo, P. A., Wu, J. and Ellis, M. D. 2002. Using inert dusts to detect and assess *Varroa* infestations in honey bee colonies. *J. Apic. Res.* 40 (1-2): 3-7.

Nagai, T., S. Mizuho, R. Inoue, H. Inoue, and N. Suzuki. 2001. Antioxidative activities of some commercial honey, royal jelly and propolis. *Food Chemistry*. 75: 237-240.

Naug, D. 2009. Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biol. Conserv*. 142:2369-2372.

Pirk, C. W.W., C. Boodhoo, H. Human, S.W. Nicolson. 2010. The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). *Apidologie* 41: 62–72.

Sahinler, N., A. Gul and A. Sahin. 2005. Vitamin E supplement in honey bee colonies to increase cell acceptance rate and royal jelly production. *J. Apic. Res*. 44: 58 - 60 DOI 10.3896/

Sauer, J.R., Essenberg, R.C. and Bowman, A.S. 2000. Salivary glands in ixodid ticks: control and mechanism of secretion. *J. Insect Physiol.* 46 (7): 1069-1078.

vanEngelsdorp, D., J. Hayes, Jr., R. M. Underwood and J. Pettis. 2008. A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. *PLoS ONE*. 3(12): e4071. doi: 10.1371/journal.pone.0004071.

Williams, J.B., S.P. Roberts, M.M. Elekonich. 2008. Age and natural metabolically-intensive behavior affect oxidative stress and antioxidant mechanisms. *Exp. Gerontology*. 43: 538-549.

Yang, X. and Cox-Foster, D. L. 2005. Impact of an ectoparasite on the immunity and pathology of an invertebrate: Evidence for host immunosuppression and viral amplification. *PNAS* 102 (21): 7470-7475.