Antioxidants in Honey Bee Colonies: Amended Annual Report

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

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Objectives:

1) Test antioxidants for miticidal activity against Varroa mite using the mite vial assay.
2) Test whether antioxidants added to syrup provided to mite-infested honey bee colonies increases bee survival and longevity.

Interpretive Summary:

Work has continued on screening antioxidants and other compounds for direct miticidal activity against *Varroa* mite. At this time, we found that only benz killed more than 40% of the mites. We are repeating the tests and are now determining the effect of these compounds on bees. Once the laboratory assays are complete, we will proceed with tesing compounds in nucleus colonies. We had some setbacks early in 2011, due to poor weather, an extreme cold snap that killed many spring flowers and a continuing drought in the early summer; the result of this bad weather has produced few flowers for bees to forage, resulting in poor colony conditions and constant feeding of the colonies. Experiments have now resumed to determine which compounds kill bees and those which bees will tolerate. Nucleus colonies will be fed in a closed environment to assess bee longevity and bee tolerance as well as Varroa control.

Materials and Methods:

Objective 1) Test antioxidants for miticidal activity against Varroa mite using the mite vial assay: We tested live adult *Varroa* mites for each compound at nine concentration levels. To conduct the mite vial assay, we dilute 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 effect mite survival (Elzen et al. 1998). After 24 hours, test vials are examined under a dissecting microscope. Mites are prodded with a needle probe to encourage movement and non-moving mites are recorded as dead. We recorded mite mortality for each vial, (the number of dead mites divided by the total

number of mites in a vial) as % kill. Trials with 15% or more mortality in the controls were discarded and repeated.

Early results showed good varroicidal activity and when mite populations increase, we will repeat these trials in the fall of 2011 when bees recover and mites can be collected. Promising compounds with good mite mortality will be advanced into brood survival and bee longevity trials (**Objective 2**) and then into further mite suppression work.

Results and Discussion:

We have screened over 16 compounds for direct miticidal activity against Varroa mite, including Aspartic, Ascorbic, Ferulic, Gallic, Uric, Caffeic, Cinnamic and Citric Acids, Cysteine, Tryptophan, Proline, Quercetin, L-Histidine, Benz, Naringenin, and alpha-Tocopherol (vitamin E). Preliminary tests were repeated this year (2011) and some new compounds were selected; see **Figures 1a and b**. Because benz killed more than 40% of the mites, we selected it for further evaluation as a potential miticide. We tested benz at $LC_{90/95}$ and found good mite kill at low concentrations (25µg in the test vials); see **Figure 2.** We then tested different concentrations of benz on bees to see what concentration was detrimental to them. We found that any concentration over 500µg was harmful to bees; see **Figure 3**. Since it takes only about 25µg to kill mites, this makes it a potentially good compound to use in further testing. We are proceeding with the next phase of the experiment.

Research Effort Recent Publications:
Manuscripts are in preparation.

References Cited:
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.

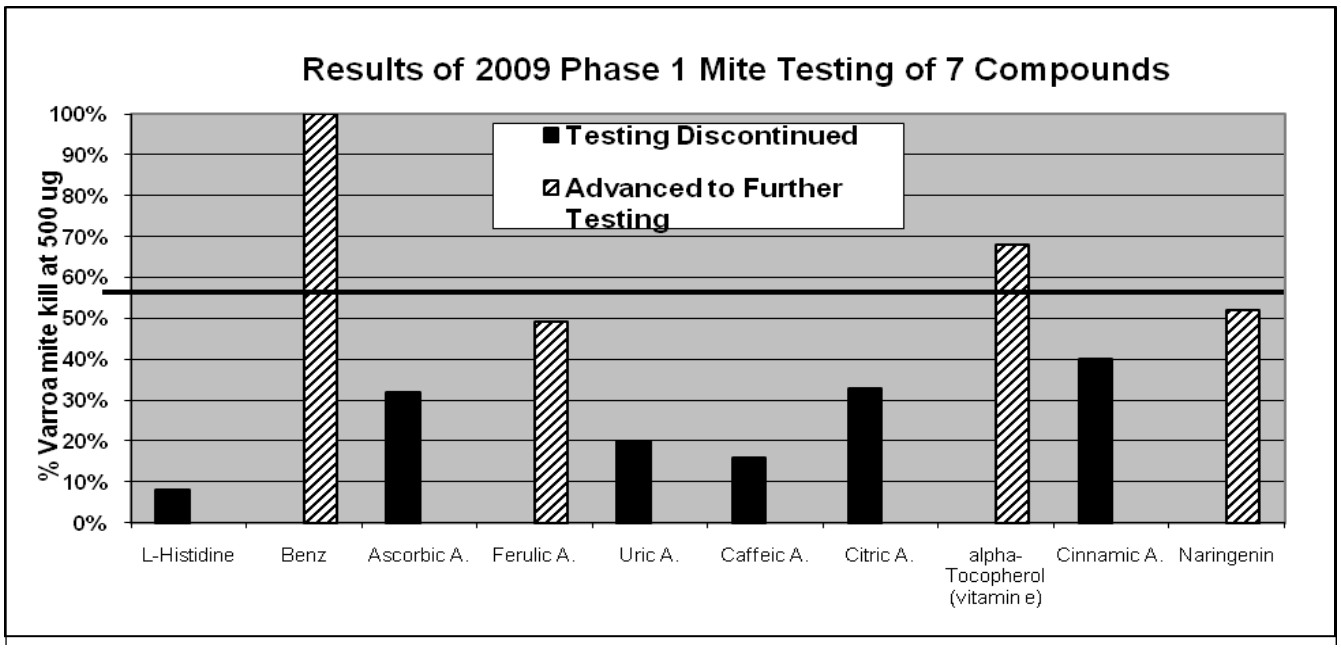

Figure 1a. Percent Varroa mite kill (500µg compound) in 2009 on seven compounds.

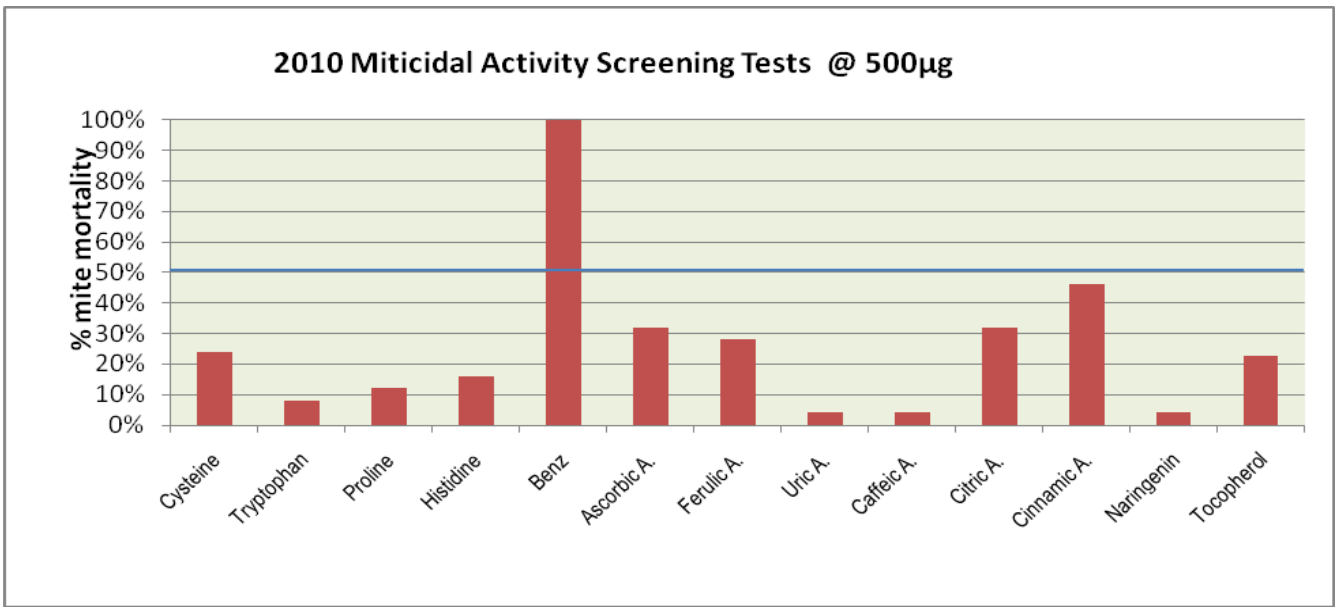

Figure 1b. Percent Varroa mite mortality on 13 compounds tested in 2010. Some of the same compounds had different kill results which could be due to seasonal or nutritional changes.

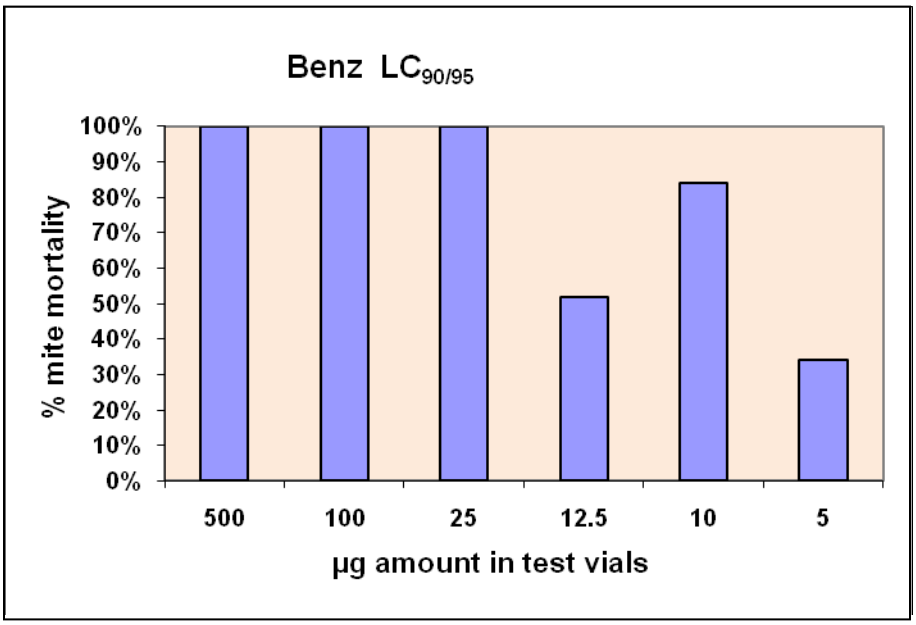

Figure 2. Amount of benz (in µg) that killed Varroa mites in vials. Concentrations over 25ug killed mites at a rate of 100%.

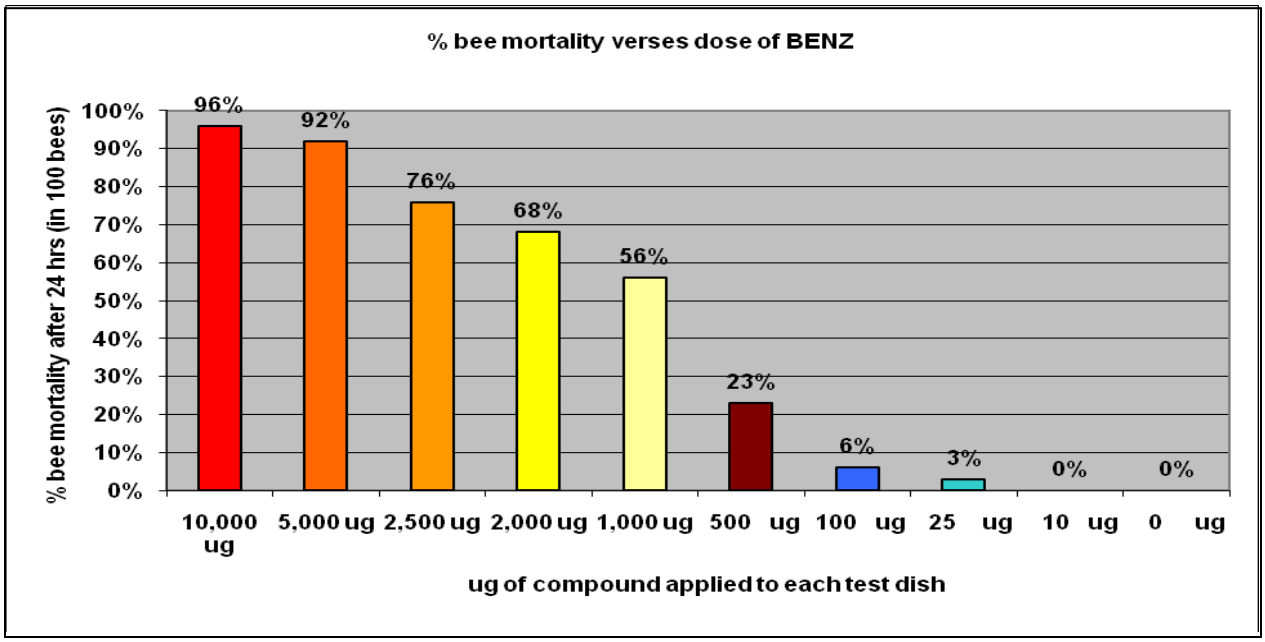

Figure 3. Amount of benz (in µg) that we tested for bee mortality. At 25µg (the amount it takes to kill mites) bee mortality in Petri dishes was 3%.