Testing a Novel Honey Bee Probiotic Formulation

Project No.: **10-POLL3-Sammataro/Carroll**

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Objectives:

1) Determine the effects of antibiotics on beneficial Lactic Acid Bacteria (LAB) in bees.
2) Determine if feeding LAB as a probiotic can increase or restore LAB in bees. (This work deferred, see Results and Discussion)
3) Determine the effects of antibiotic treatments against LAB and probiotic supplementation of LAB on honey bee nutrition and health.

Interpretive Summary:

The health of honey bees is threatened today by stresses that humans place on bees during commercial pollination and honey production. These stresses culminate in diseases and colony losses, and can lead to a loss of pollination and decreased honey yields. Honey bees rely on beneficial symbiotic microbes to digest food, to provide critical essential nutrients, and to crowd out pathogenic microbes. One group that appears to have particular importance to honey bees are thirteen different lactic acid bacteria (LAB) from the genera *Lactobacillus* and *Bifidobacterium*, which are located in the honey stomach and fresh food stores of honey bees worldwide (Ref. 1, 2, 3). LAB protect nectar and pollen from spoilage by other microbes during honey and bee bread formation. Also, LAB has been shown to suppress the growth of colony pathogens such as *Paenibacillus larvae*, the causative agent of American Foulbrood. We are assessing the microbes in bees to determine whether antibiotics, high fructose corn syrup (HFCS), and other supplements commonly used in bee management have a negative impact on these beneficial microfloras. If so, this could potentially change the recommendations for antibiotic and other treatments used in bee colonies. Preliminary tests of bees in cages showed that those fed antibiotics died more quickly (especially bees that were fed Fumagillin) compared to control bees fed untreated sugar syrup (**Figure 1**).

Materials and Methods:

Caged Bee Studies. Newly-emerged adult bees were collected from capped brood. Because these bees lack gut microbes, we exposed them to common sources of colony microbes in the first two days of their lives. They were given frames containing open nectar and bee bread (stored pollen) and were allowed to be fed by older adult bees by trophallaxis (food-sharing) across a screen divider. On the second day after emerging, approximately 250 bees (by weight) were placed into a Plexiglas and screen cage and then put in an incubator room maintained at 30°C and 40-45% relative humidity. Bees were provisioned with 1:1 sugar syrup, distilled water, and 10g of pollen patty. A small piece of beeswax comb was placed in each cage for cluster and storage space. Bee mortality and food and water consumption (sugar syrup, pollen, and water) were recorded every two days. Food supplies were changed and 10 bees were sampled for later nutritional and microbe analysis every 8 days (8 days being considered a "week"). On days 8 and 16, antimicrobial treatments were administered by the method and dose recommended on the label. Terramycin and tylosin were applied as a powder dusting (10mg in 1g of powdered sugar) and Fumagillin-b was introduced in the sugar syrup solution (87mg in 70mL sugar syrup). Bees were exposed to fumagillin in the feeder for 8 days after initial treatment and because they stored some of the food in the comb, they were exposed to fumagillin-treated sugar syrup for an extended time. Seven cage replicates were performed per treatment.

Results and Discussion:

Preliminary work showed that bees in cages fed antibiotics died more quickly than bees fed sugar syrup. Bees fed fumagillin displayed higher mortality than control bees fed the untreated syrup (**Figure 1**), but they may have been exposed to antimicrobials longer than the topical applications because treated sugar syrup was also stored in the comb cells. When sugar syrup consumption was examined, fumagillin-fed bees consumed significantly less syrup than control bees fed the untreated sugar syrup, a trend that appeared to increase as the treatment time continued. Fumagillin may act as an antifeedant to honey bees.

Because of the change in collaborators (work is now being done by USBARCO and the University of Arizona Proteomics Consortium) anticipated work on the LAB have been delayed. In addition, the drought and hard winter of 2010 has put all of our colonies in a stressed situation where young bees are not hatching and the queens are not laying. This has left us with little brood and few bees to hatch out for our cages. The first cage run gave us some good information on bee mortality, but the experiment will be repeated when bee populations increase.

The caged bee trial will be done again in the fall of 2011 to examine the effects of antibiotic treatments. Later, the effects of LAB supplementation on honey bee health and honey stomach (HS) microbial communities (Objective 2) will be explored, to determine whether the microbial populations (LAB) can be augmented or re-established through feeding microbially-acitve bee bread. Treatment times will follow this schedule:

Day 0 to 8	no treatment
Day 8 to 16	antimicrobial treatment
Day 16 to 24	antimicrobial treatment
Day 24 to 32	no treatment (first latent period)
Day 32 to 40	probiotic supplementation treatment
Day 40 to 48	no treatment (second latent period)

The first and second latent periods (noted second olumn in chart) allow us to gauge the effects of the antimicrobial and probiotic treatments after the treatment period.

We will further assess honey bee health by mortality and growth performance, nutrient reserves, and biochemical markers of nutritional stress in adult bees. Bees will be sampled from day 0 at seven day intervals for analysis of stomach flora contents, hypopharyngeal glands (used to produce brood food), and key biochemical indicators of bee health and stress. All bees sampled will be stored in the -80C freezer. Later, we will analyze the protein content of the hypopharyngeal glands and honey stomachs, using protocols developed by USBARCO and by the proteomics facilities at the University of Arizona. We expect that all bees will start with the same levels of LAB. For bees fed antibiotics followed by supplementary LAB, the bacteria should initially decrease with antibiotic treatment and then increase after we add supplementary LAB-rich sources. We expect that bees from treatment groups with LAB will perform better than bees from treatment groups without LAB, in terms of decreased mortality, higher body weights, and greater nutritional reserves. The next step will be to conduct a similar set of controlled feeding studies on nucleus colonies ("nucs") isolated in flight arenas. If time permits, we may examine the effects that different dietary carbohydrates have on LAB in a separate second series of studies comparing sucrose and high fructose corn syrup (HFCS) as carbohydrate sources. Ultimately, we hope to reduce the negative impact of antimicrobials on honey bee colonies at vulnerable times of the year, such as emergence from late winter dearth during the almond pollination season.

Research Effort Recent Publications:
Manuscripts will be forthcoming.

References Cited:
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2. Vásquez A, Olofsson TC, Sammataro D. Apidologie. 2009; DOI: 10.1051/ apido:2008063
3. Vásquez, A, Olofsson, TC. J. Apicult. Res. 2009; DOI:10.9836/IBRA.1.48.3.07

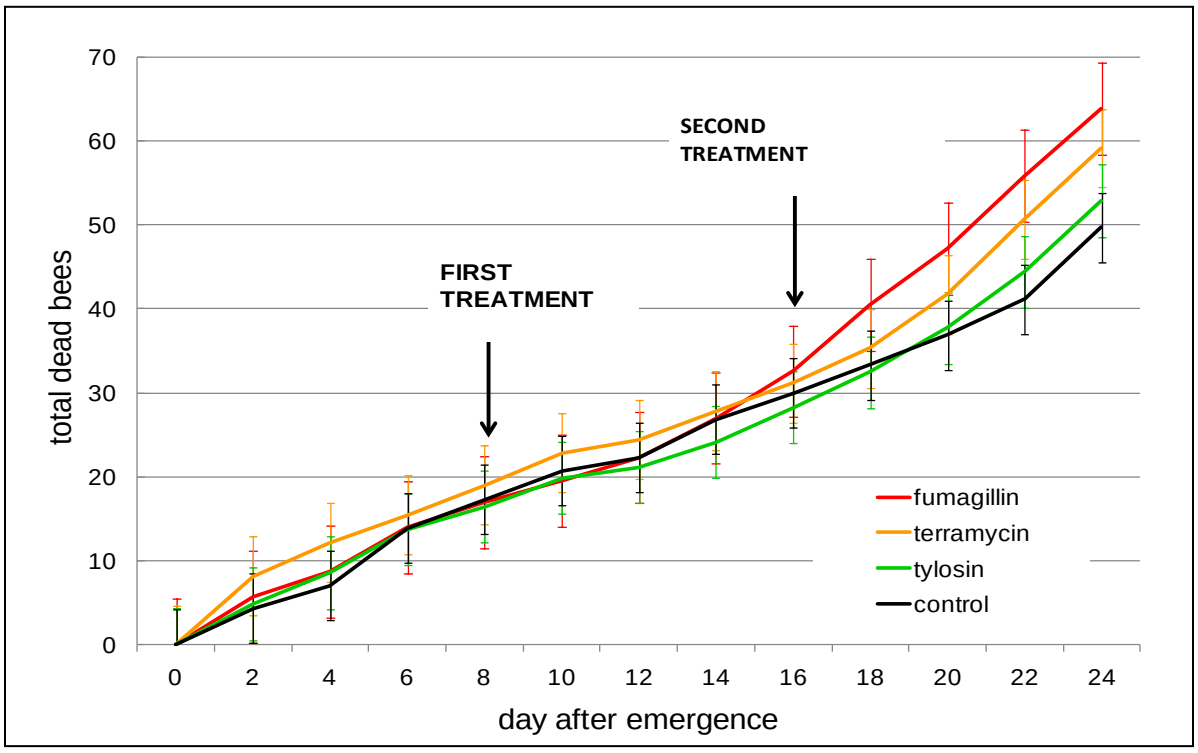

Figure 1. Average cumulative bee mortality in caged bee experiment. Antimicrobial treatments were applied twice at day 8 and day 16 (arrows) either in a powdered sugar dusting (terramycin and tylosin) or in sugar syrup (fumagillin). Error bars are standard error (SE).