Imidacloprid Methods

Used Koppert Bees! (Not BioBest)

DWV infection: How much DWV inoculum necessary for DWV infection?

To accomplish objective 1, purified deformed wing virus isolate was prepared at the University of Maryland by collaborator Dr. Humberto Bonchristiani. Five commercial bumble bee colonies were obtained and tested for 3 RNA viruses (black queen cell virus, Israeli acute paralysis virus, and deformed wing virus) upon arrival. Of the five colonies, all 5 were infected with black queen cell virus and 4 were infected with deformed wing virus. The results of this preliminary testing provide evidence that commercial bumble bee colonies may be contributing to RNA virus spread. These results also presented many challenges, as I need virus free colonies for upcoming experiments. Despite these challenges, I conducted two pilot experiments to determine a. the effectiveness of the virus inoculum and b. effect of imidacloprid on bumble bee survivorship.

To test the virus inoculum on bumble bees, one hundred bumble bee (Bombus impatiens) workers were transferred to individual containers and assigned to one of 5 treatments: 4 different concentrations of DWV and a control. After a 5-hour period without food, each bee was fed 10 ul of an inoculum containing DWV and 50% sucrose. The control bees only received 10 ul of 50% sucrose. All bees were given pollen and 30% sucrose ad libitum for 14 days. Mortality and morbidity were recorded. After 14 days, all surviving bees were transferred to -80**°**C. Using RT-qPCR I analyzed two of the groups and found that bees fed the inoculum had higher DWV levels than the control group. Results will provide data on the amount of DWV necessary to cause an infection in bumble bees, the variation of viral infection I can expect among individuals and methodological information on inoculation protocols.

Imidacloprid Experiment results- food intake & virus results

In a second pilot experiment, I tested the effect of different concentrations of imidacloprid on bumble bee survivorship and also tested whether exposure influenced the viral loads already present. This pilot experiment was necessary to ensure bees would experience only sublethal effects of imidacloprid in the larger future experiment while still surviving during the length of the experiment. Twenty bees were assigned to each of 4 treatments and a control. Treatment groups were fed pollen and 30% sucrose ad libituminoculated with different concentrations of imidacloprid: 0.1, 1, 10, and 20 parts per billion (ppb) for 8 days. The control received 30% sucrose only. Sucrose consumption was measured for five days. Bees in the 20 ppb and 10 ppb group consumed significantly less sucrose ([figure 1](http://mysare.sare.org/wp-content/uploads/SARE_Report_Figures-1.pdf)). In light of these important preliminary results, bees will be fed sucrose inoculated with less than 10 ppb imidacloprid in future experiments to ensure the bees eat and receive the pesticide exposure treatment. Since the bees arrived already infected with DWV and BQCV, I will use RT-qPCR to test bees to see if virus levels were affected by the pesticide exposure.