

## Report on “Increasing Colony Survival through Innovative Queen Nutrition”

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The original objective of this experiment was to improve queen quality by supplementing grafted larvae with a naturally occurring pheromone given off by young larvae called e-beta ocimene ( $e\beta$ ). Commercial beekeepers regularly cite poor queen quality as a major factor in colony losses. While queens in the past lasted more than one year, few queens currently survive the beekeeping season, adding considerable cost in replacement queens and lost pollination units to beekeeping operations. My PhD research had indicated that  $e\beta$  was a “feed me protein” signal that we believed would stimulate greater feeding, resulting in better quality queens that produce a stronger pheromone signal and thus are less likely to be replaced.

### **Experimental Results:**

This grant was for innovative research and as is often the case, theory and practice don't always align. The first year of this grant we faced both terrible weather conditions with frequent rains that complicated queen rearing experiments. The experiment required larvae of a specific age, less than 12 hours post hatching. This required caging queens and then grafting larvae 4 days later. The frequent rains made this extremely difficult to conduct, as we couldn't pull the appropriately aged larvae and nurse bees on rainy days.

Despite poor weather conditions, we did our best to prevail. However, we also ran into some technical issues that required numerous revised protocols. Early attempts at supplementing the larvae with  $e\beta$  directly into the grafted queen cups resulted in very high mortality rates of 80% or more. We needed mortality rates below 20%, otherwise the technique would never be feasible for commercial operations. The oily substance of the pheromone appears to interfere with larval respiration and they are cannibalized by the attending worker larvae.



Figure 1. Well stocked queenless swarm box, filled with young nurse bees collected from healthy colonies. These swarm boxes were stocked with approximately 7 lbs of bees. They were provided with two frames of grafted cells (QC), two frames of open nectar (N) and one full pollen frame (P) in the following configuration N-QC-P-QC-N. Each frame of queen cells had 30 grafted cells, and 60 were inserted per swarm box.

We thus attempted numerous other means of supplementing the grafted larvae, including impregnating the queen cups with wax that had been infused with  $e\beta$ . However,  $e\beta$  is a volatile substance. We tried infusing  $e\beta$  into the melted wax and then coating the queen cups in that infused wax versus a control wax. We saw no difference in larval weight between queens grafted into treated vs control waxed cup.

So we went back to the drawing board to figure out another means of trying to supplement larvae without killing the grafted larvae. After trial and error, we found that double grafting cells worked well. We would graft larvae, place the grafted cells into a well-stocked queenless swarm box filled with 7 lbs of nurse bees (see Fig. 1), then remove the cells 24 hours later (see Fig. 2). The bees would have started to elongate the queen cups and filled the entire plastic base with royal jelly.

At this stage the initially grafted larvae were discarded, different doses of  $e\beta$  injected into the bed of royal jelly, and another set of larvae less than 12 hours post-hatching were grafted onto the bed of royal jelly. These cells were then returned to the swarm box for 36 hours, then moved into a strong queenright finishing colony with the cells placed above a queen excluder.

One day prior to emergence, the cells were inserted into hair roller cages and the virgins allowed to emerge into these protective cages (see Fig. 3). Unfortunately, by the time we had figured out the successful protocol for rearing the supplemented queens, and installed virgins into mating nucs, it was too late in the season for good mating results.

Initial results were too variable across replicates and not significant, though when pooled across all replicates there was a trend toward increased weight in supplemented queens (see Fig 4). We plan to conduct a follow-up experiment this spring using the double-grafting protocol we have now



successfully established. We have all the necessary equipment, bees and supplies available to conduct this follow-up.



Figure 2. Queen cells after 24 hours in the swarm box. At this stage, the initially grafted larvae can be discarded, the  $e\beta$  pheromone supplement injected into the brood food jelly at varying doses, and another larva grafted on top.



Figure 3. Virgins emerging into hair roller cages.

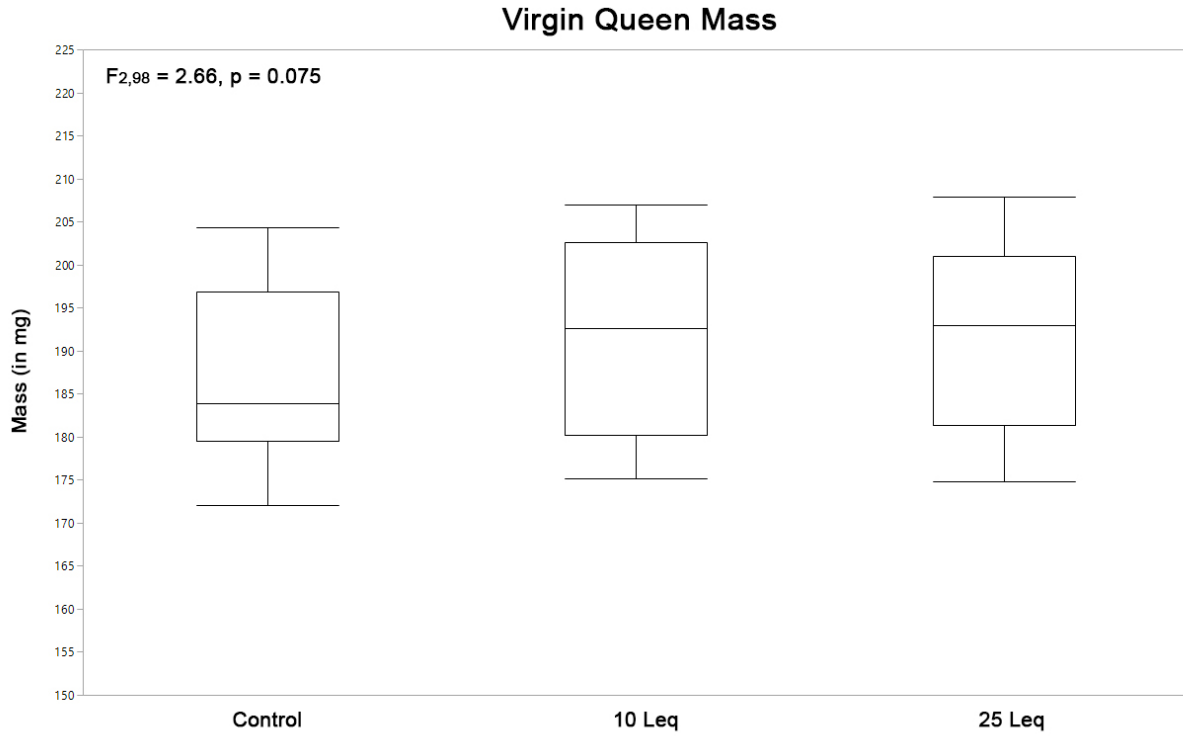


Figure 4. Mass of virgin queens at time of emergence, pooled across all replicates. There was a trend toward increased weight in the queen larvae supplemented with 10 and 25 Leq compared to controls, though this was not significant ( $p = 0.75$ ).