

# The effect of imidacloprid on honey bee queen fecundity

Jamilyn A. Martin,<sup>1</sup> Julia D. Fine,<sup>2</sup> Amy Cash-Ahmed,<sup>2</sup> and Gene E. Robinson<sup>2,3,4</sup>

Kaskaskia College, Centralia, Illinois<sup>1</sup>, Carl R. Woese Institute for Genomic Biology<sup>2</sup>, Neuroscience Program<sup>3</sup>, Department of Entomology, University of Illinois at Urbana-Champaign<sup>4</sup>

## Introduction and Background

- Imidacloprid is a neonicotinoid insecticide commonly used in agricultural settings to control insect pests by acting as an agonist of acetylcholine receptors and inducing paralysis and mortality<sup>1</sup>.
- In small doses, imidacloprid can cause loss of memory and foraging ability along with impaired learning and a lowered immune response in western honey bees (*Apis mellifera*)<sup>1</sup>.
- **Effects of neonicotinoid insecticides on colony reproduction have been documented including decreased colony expansion<sup>2</sup>, queen failure and replacement<sup>2,3</sup>, and decreased queen egg laying<sup>4</sup>.**
- For this study, we examined the effects of imidacloprid on the fecundity of queen bees when their worker attendants (Figure 1) were exposed to low doses of imidacloprid through their food source using a novel, lab-based, Queen Monitoring Cage (QMC) system.
- Our results will help elucidate the effect of imidacloprid on the egg laying behaviors of honey bee queens.
- By comparing the results generated using QMCs to previous studies using full-sized colonies, we will attempt to validate the use of QMCs as a risk assessment tool.



Figure 1: Queen bee and her worker attendants



Figure 2: Queen Monitoring Cage with bees

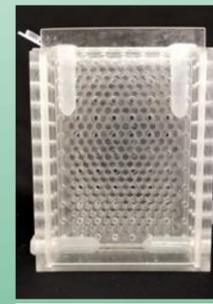


Figure 3: Queen Monitoring Cage



Figure 4: Honey bee egg

## Project Description

### Materials

- One-day-old adult worker bees were obtained from an apiary located at the University of Illinois at Urbana-Champaign. Mated queens were purchased from Olivarez Honey Bees, Inc. Orland, CA.
- QMCs were composed of plexiglass with small holes in the walls for ventilation (Figures 2 and 3). Each QMC contained 2 egg laying plates positioned vertically and serving as the inner walls of the cages. These custom-made injection-molded, polystyrene plates were patterned with 64 hexagonal wells measuring 5.1 mm across and 11 mm deep, mimicking the dimensions of the cells in natural honey bee brood comb.

### Method Summary

- Sixty cages were assembled, each containing approximately sixty day-old bees and one mated queen.
- **The cages were divided into 4 treatment groups consisting of different part per billion (ppb) concentrations of imidacloprid in sucrose solution or pollen diet (Table 1) and queen egg laying (Figure 4) and diet consumption were monitored the next 14 days.** Worker mortality was recorded on the final day of the trial.

Table 1: Dietary treatment names and descriptions.

Treatment	Sucrose Feeder	MegaBee Feeder	Water Feeder
Control	Two feeders of 50% (w/w) sucrose	One feeder of MegaBee® (easily regulated pollen supplement)	One feeder of water
IP	Two feeders of 50% (w/w) sucrose	One feeder of MegaBee® with 10ppb imidacloprid	One feeder of water
10 ppb	Two feeders of 50% (w/w) sucrose with 10ppb imidacloprid	One feeder of MegaBee®	One feeder of water
50 ppb	Two feeders of 50% (w/w) sucrose with 50ppb imidacloprid	One feeder of MegaBee®	One feeder of water

- Differences between the number of eggs laid and diet consumed by treatment were evaluated by ANOVA, Student's t-tests, and post hoc Tukey HSD tests. Significance was evaluated at the  $\alpha=0.05$  level.

## Results

- **Treatment significantly affected egg laying (ANOVA,  $p=0.0001$ , Figure 5).** The most eggs laid were laid by queens in the IP and Control treatment groups. Relative to IP queens, queens in the 10 ppb treatment laid fewer eggs. Queens in 50 ppb group laid significantly fewer eggs relative to all treatment groups (Tukey HSD).
- Total water consumption was highest in the IP treatment group relative to all treatments (ANOVA,  $p=0.003$ , Tukey HSD, Figure 6)
- Treatment significantly affected sucrose consumption, with a significant difference detected between IP and 50 ppb (ANOVA,  $p=0.005$ , Tukey HSD, Figure 7).
- A trend was observed between treatment and MegaBee® consumption (ANOVA,  $p=0.10$ ). Total pollen consumption was significantly greater in IP treatment group relative to 50 ppb (Student's t-test,  $p=0.02$ , Figure 8).
- Mortality was not significantly different across treatments (ANOVA,  $p=0.22$ ).

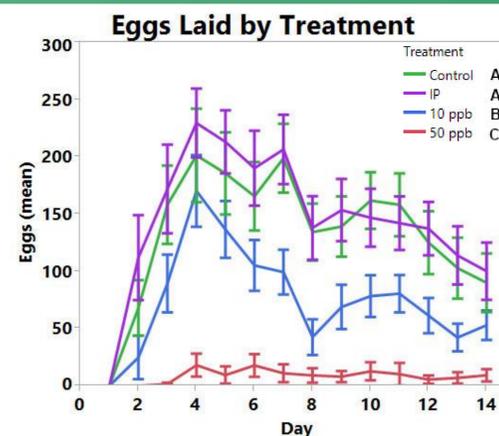


Figure 5: Average daily egg laying  $\pm$  SEM by treatment with significant differences between groups identified by letters.

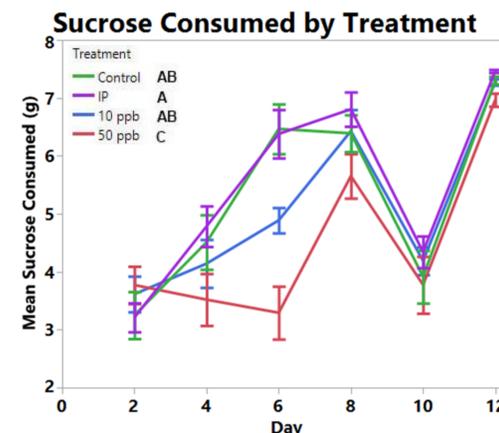


Figure 7: Average daily sucrose consumption  $\pm$  SEM by treatment with significant differences between groups identified by letters.

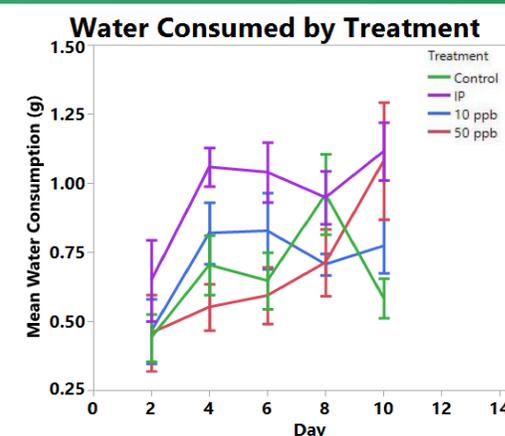


Figure 6: Average daily water consumption  $\pm$  SEM by treatment with significant differences between groups identified by letters.

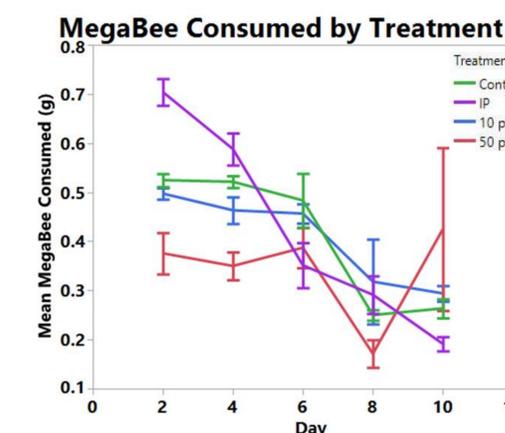


Figure 8: Average daily MegaBee® consumption  $\pm$  SEM by treatment.

## Conclusions

- **The effects of imidacloprid on egg laying in QMCs mirror the effects reported for full colonies<sup>4</sup>,** suggesting that the system can be used as a robust risk assessment tool.
- The relationship between sucrose consumption and treatment is suggestive of a dose dependent anti-feedant effect when imidacloprid is administered in sucrose solution.
- The trend for higher MegaBee® consumption by workers in the IP treatment group relative to 50 ppb suggests that pollen consumption may be stimulated when imidacloprid is administered in a pollen diet.
- The increased initial water consumption for the IP group suggests that imidacloprid administered in pollen may be detoxified via a different pathway than when it is administered in sucrose solution. More work is needed to separate this effect from dose dependent differences in metabolism.

## Future Work

Future work should include replication and possibly an exploration of the results of the 10ppb imidacloprid in pollen. This experimental group laid a high number of eggs despite the agrochemical treatment, which may be related to higher pollen consumption, which is suggested by the MegaBee® consumption trend. Additionally, more work is needed to determine the effects of the imidacloprid treatment on workers and queens that resulted in varying egg laying performance across treatments.

## References

1. Goulson D (2013) REVIEW: An overview of the environmental risks posed by neonicotinoid insecticides. *J Appl Ecol* 50(4):977–987.
2. Dively GP, Embrey MS, Kamel A, Hawthorne DJ, Pettis JS (2015) Assessment of Chronic Sublethal Effects of Imidacloprid on Honey Bee Colony Health. *PLoS ONE* 10(3):e0118748.
3. Sandrock C, et al. (2014) Impact of Chronic Neonicotinoid Exposure on Honeybee Colony Performance and Queen Supersedure. *PLoS ONE* 9(8):e103592.
4. Wu-Smart J, Spivak M (2016) Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. *Scientific Reports* 6:32108.

## Acknowledgments

Financial support was provided by the National Science Foundation under grant #NSF REU 1559908/1559929, as part of the Phenotypic Plasticity Research Experience for Community College Students, through the University of Illinois at Urbana-Champaign Institute for Carl R. Woese Genomic Biology and Parkland College. <http://precs.igb.illinois.edu/>

Special thank you to the project's PIs, Dr. Nathan Schroeder and Dr. C. Britt Carlson. Thank you to the entire Robinson lab for the mentoring and patience.

