Notes from the Lab:
The Latest Bee Science Distilled

by Scott McArt

Almond growers should avoid using insecticides and adjuvants during bloom

As I write this article, approximately 2.1 million honey bee colonies are pollinating ~1.3 million acres of nut-producing almond trees in California. Since the estimated number of colonies in the United States is 2.92 million according to the USDA National Agricultural Statistics Service, that means ~70% of the country's managed honey bee colonies are currently in almond orchards in the central valley of California. If we assume each colony is comprised of ~10 frames of bees, that's around 20 billion bees.

How big of a number is 20 billion bees? Well, last year ~30 million eastern monarch butterflies successfully migrated to their overwintering grounds in the Sierra Madre mountains of Mexico. The eastern monarch migration is widely regarded as the largest insect migration event in the world. But if the numbers above are correct, over the past month there were ~600 times more honey bees that made an *assisted* migration to California almond orchards, mostly on the backs of 18-wheel trucks!

Dylan Ricke

Photo 1. *Co-author Chia-Hua Lin adds nurse bees to a swarm box used in the experiment.*

All this is to say if we care about honey bee health in the United States, almond pollination is perhaps the single most important time to ensure that bees aren't exposed to harmful levels of pesticides. But is that the case? Are the pesticides that almond growers apply during bloom safe for bees? What about the "inert" adjuvants in pesticide sprays, are they safe for bees? These are the topics for the fifty-second Notes from the Lab, where I summarize ***"Pollen treated with a combination of agrochemicals commonly applied during almond bloom reduces the emergence rate and longevity of honey bee (Hymenoptera: Apidae) queens,"*** written by Dylan Ricke and colleagues and published in the ***Journal of Insect Science*** **[2021].**

Bees are always exposed to a cocktail of pesticides during crop pollination. For example, honey bees are simultaneously exposed to 17 pesticides on average during apple pollination in New York (McArt et al. 2017). During Michigan blueberry pollination, honey bee-collected pollen contains 35 pesticides on average (Graham et al. 2021). My lab has analyzed several samples from beekeepers conducting California almond pollination and we typically find ~20 pesticides in bee bread, trapped pollen, or foraging bees.*

Chia-Hua Lin

Photo 2. *Research assistant Will Passifiume grafts worker larvae.*

Chia-Hua Lin

Photo 3. *Research assistant Will Passifiume holds a frame of queens from the experiment.*

What does this mean for pesticide risk to bees during pollination? It means we need to understand the impact of co-exposures, not just the impact of exposure to a single pesticide. With this goal in mind, Ricke and colleagues set out to understand how co-exposure to several pesticides that are commonly applied during bloom impact development and survival of new queens.

For their study, the authors chose to focus on individual and combined effects of chlorantraniliprole (active ingredient in Altacor), propiconazole (Tilt), an organosilicone and alkyphenol ethoxylate spray adjuvant (Dyn-Amic), and diflubenzuron (Dimilin 2L) as a positive control since the harmful effects of diflubenzuron to honey bee larvae have already been documented (see Wade et al. 2019). Chlorantraniliprole and diflubenzuron are both insecticides, propiconazole is a fungicide, and organosilicone and alkyphenol ethoxylate spray adjuvants are used to improve pesticide performance (wetting, particle size, etc.) during application. All are labeled for use during almond bloom, and tank mixes of the pesticides and adjuvants (i.e., combining multiple products into one spray) are common.

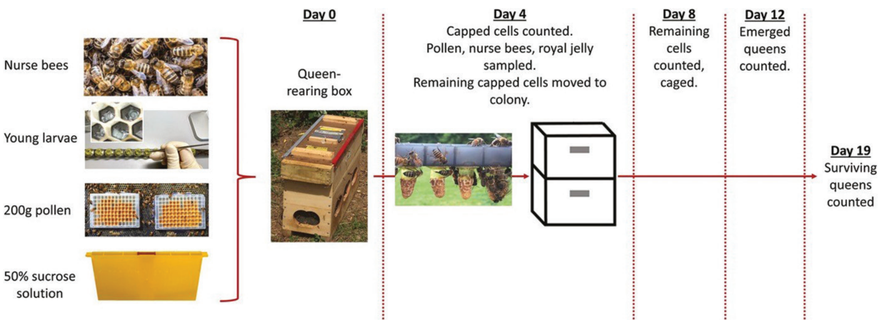

Figure 1. *The queen-rearing approach used for this study. Queen-rearing boxes were prepared on day 0. On day 4 (96 h later), samples of pollen, nurse bees, and the royal jelly from a subset of capped queen cells were taken for chemical analysis. Capped cells were counted and moved to a strong incubating colony. On day 8, the remaining cells were counted and caged. On day 12 through day 19, living and dead emerged queens were counted every 2-3 days. Any queens not emerging by day 19 were counted as dead.*

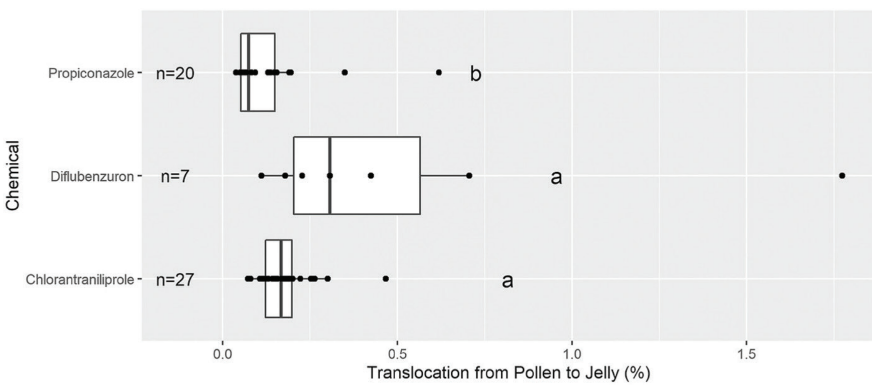

Figure 2. *Translocation rates of each chemical from treated pollen into royal jelly. Each point represents the translocation rate of the given chemical measured from a single queen-rearing box trial. Rates were calculated as the proportion of the concentration of each chemical in jelly over the concentration measured from treated pollen.*

To test the impact of the pesticides and adjuvants on queen development and survival, the authors created swarm boxes that were provisioned with contaminated pollen (Photo 1). Concentrations of the pesticides were chosen to mimic high but field-realistic exposures based on maximum application rates: 40 parts per million (ppm) chlorantraniliprole, 90 ppm propiconazole, 100 ppm diflubenzuron, and 0.8% by weight of the adjuvants.

Each swarm box received contaminated pollen, sucrose, nurse bees, and 30 young larvae grafted into queen cups on a cell bar frame (Photos 1 & 2). After 4 days (96 hrs), samples of pollen, nurse bees, and jelly were taken for pesticide residue analysis, and capped cells were counted and transferred to a strong incubating colony. On day 8, the remaining cells were counted and caged (Photo 3). On day 12 through day 19, living and dead emerged queens were counted every 2-3 days. A full schematic of the experimental methods is shown in Figure 1.

So, what did they find? Do pesticides fed to nurse bees in pollen make it into royal jelly that's fed to developing queens? Yes, but much less than what's present in contaminated pollen. As seen in Figure 2, nurse bees are quite efficient at filtering pesticides as they consume contaminated pollen and make jelly. In doing so, nurse bees essentially function as the colony's liver, absorbing and filtering toxins. On average, less than 0.5% of each pesticide made it through nurse bees and into royal jelly — impressive! The bees were most efficient at filtering the fungicide propiconazole and somewhat less efficient at filtering the insecticides chlorantraniliprole and diflubenzuron.

Do pesticides applied during almond bloom negatively impact queen development and survival? Yes. Despite the impressive filtering from nurse bees, there are still pesticides that get into jelly and negatively impact developing queens. As seen in Figure 3, the only individual pesticide that negatively impacted queen survival was the insecticide diflubenzuron (purple dashed line); all other individual pesticides did not significantly reduce queen survival. The fact that diflubenzuron was *again* shown to harm developing honey bees (see Wade et al. 2019 for the first documentation) is clear evidence that this insecticide should not be used during pollination of any crop, including almond.

In addition, the combination of chlorantraniliprole, propiconazole, and the adjuvants (green dashed line) also resulted in significantly less queen survival compared to controls. This is an important result for two reasons. First, we know pesticide co-exposures during crop pollination are common, in part because tank mixes of pesticides are common. But unfortunately, when regulatory agencies such as the U.S. Environmental Protection Agency (EPA) assess risk from pesticides, they do not consider risk from co-exposures. The results from Ricke and colleagues are evidence for why this gives an incomplete assessment of risk; co-exposures can result in greater risk to bees compared to single exposures.

Second, when adjuvants were added to the pesticides, they increased mortality of queens. Despite growing evidence that several common adjuvants can be toxic to honey bees, they are widely considered "inert" by regulatory agencies and undergo little testing for bee safety. The EPA does not currently require any toxicological testing on honey bees be done for "inert" adjuvants, nor does it require the specific adjuvants be disclosed on product labels! This is clearly problematic for pollinator safety, especially during California almond pollination where usage of organosilicone adjuvants has increased at least 5-fold in recent years.

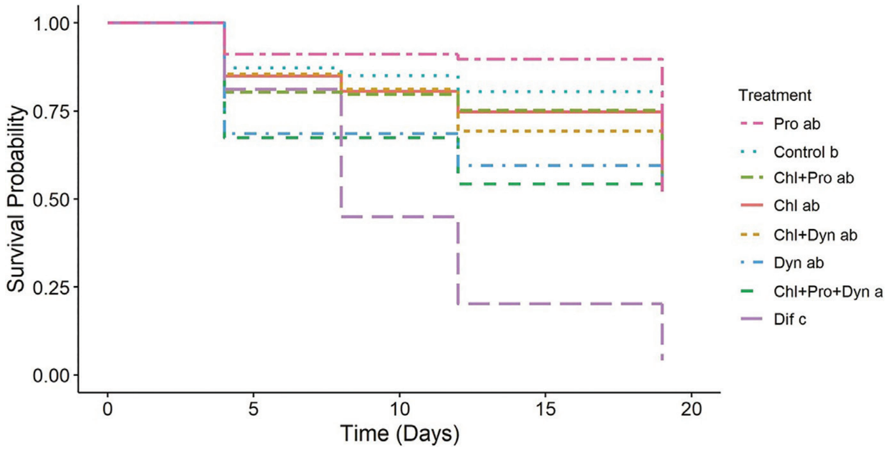

Figure 3. *Kaplan-Meier survival curves for queens reared with each pollen treatment. Data for each chemical were pooled across all trials. Counts of living and dead queens were taken on days 4 (capping), 8, 12 (emergence), and up to 7 days post-emergence (day 19). Letters in the legend indicate significant differences (P < 0.05).*

So, what's the take-home message from the excellent study by Ricke and colleagues? It's pretty simple: Almond growers should avoid using insecticides and adjuvants during bloom. By doing so, 20 billion honey bees (and several thousand beekeepers) will benefit.

Until next time, bee well and do good work.

Scott McArt

*My lab's multi-residue pesticide analysis is open to the public. If you would like to assess levels of pesticides in comb wax, pollen, bee bread, bees, etc, here's a link to our facility's website: **https://blogs.cornell.edu/ccecf/.** As a Core Facility at Cornell, we're not allowed to make a profit. Thus, sample fees cover the cost of consumables, machine time, and labor. Our current price is $90/sample.

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