Notes from the Lab:

The Latest Bee Science Distilled

by Kaitlin Deutsch and Scott McArt

Engineered gut microbes combat varroa and DWV in honey bees

Varroa mites. Viruses. For the past several decades, beekeepers have sought to protect their colonies from these two ubiquitous threats that often come together. Not only do varroa mites weaken individual bees by feeding on their fat bodies, they also transmit diseases such as Deformed Wing Virus (DWV).

As everyone reading this column knows, unfortunately beekeepers have limited tools to combat varroa and viruses. We have a couple acids, some essential oils, IPM practices, and a couple synthetic chemical pesticides. To add insult to injury, we know that varroa is already evolving resistance to the synthetic chemicals. So, it's very exciting news that groundbreaking research was just published on a new genetic approach to combat varroa and viruses. In this month's Notes from the Lab, we highlight "**Engineered symbionts activate honey bee immunity and limit pathogens**," published in the journal ***Science*** and authored by Sean Leonard and colleagues at the University of Texas at Austin. Leonard is a PhD student currently working in Dr. Nancy Moran's lab.

For their study, Leonard and colleagues genetically modified naturally-occurring gut bacteria in honey bees to "teach" the bee immune system to recognize and destroy viruses and the varroa mite. They did this by engineering microbes that, once accepted by the host bees, changed bee gene expression and immunity via the production of RNA interference (RNAi) molecules.

A varroa mite, a common pest that can weaken bees and make them more susceptible to pathogens, feeds on a honey bee. Photo credit: Alex Wild/University of Texas at Austin

What does that mean, exactly? Well, RNAi is an important component of the immune system of most animals, including bees and varroa mites. In short, the immune system detects double stranded RNA (dsRNA) molecules, which are produced by viruses (e.g., dsRNA is produced by DWV). Because the dsRNA molecules are an indicator of disease, an immune response is launched to detect and destroy the invaders. The honey bee immune system "learns" the genetic code of that dsRNA molecule and then targets all other molecules with that same genetic code for destruction.

Your immune system (or a bee's immune system) can also be primed so a more effective immune response can be launched (think about those flu shots you get each fall to reduce your chances of getting sick). It had been previously shown that feeding DWV-specific dsRNA to bees prior to exposure to the virus increased lifespan and reduced virus levels in infected bees — suggesting that RNAi could be effective at "silencing" these viruses (Desai et al. 2012). However, the challenge is providing a *constant* source of dsRNA to honey bees that targets the full range of viruses that infect the bees.

This is where Leonard and colleagues' study really breaks ground. Instead of constantly feeding honey bees dsRNA molecules directly, the authors engineered the naturally occurring bacteria in the bee gut to *create* dsRNA molecules. Specifically, they genetically engineered one gut bacterium, *Snodgrassella alvi* (*S. alvi*), to continuously produce dsRNA molecules that prime the honey bee immune system to target DWV and varroa.

But first, the researchers had to ensure that genetically transformed *S. alvi* could survive in the bee gut and produce molecules that would be taken up by bee tissues. To do this, they fed bees *S.alvi* that had been genetically modified to produce "non-target" dsRNA molecules as proof of concept. They found these dsRNA molecules were present in the head, gut, and hemolymph of bees, indicating the molecules were being circulated beyond where the bacteria reside in the gut. Also, the molecules were detected in bee tissues until the end of the 15-day experiment, indicating the dsRNA-producing *S. alvi* were self-sustaining in the bees. Moreover, the genetically modified *S. alvi* strains were shared through social interactions between co-housed bees. Thus, it is possible that only a subset of bees need to be exposed to the bacteria for it to establish in an entire colony (more on this later).

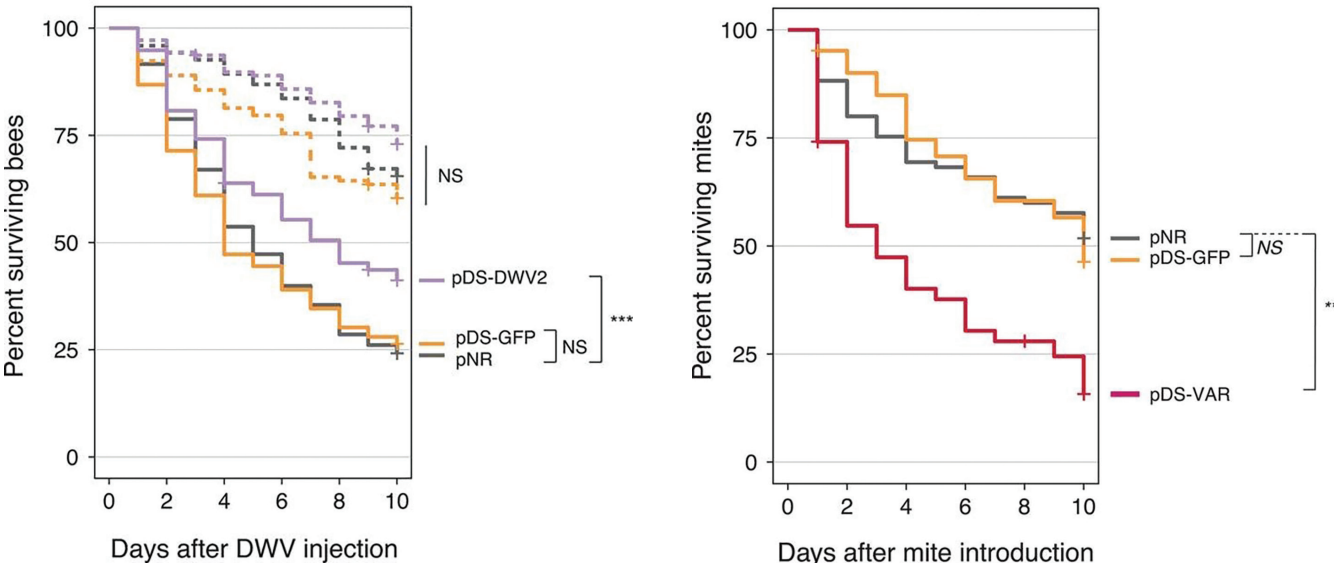
OK, so we've got bees with genetically enhanced gut bacteria producing dsRNA molecules that are able to reach all parts of the bee and remain present for over two weeks. Now, how can this technology be used to protect bees? The next step was using this tool to target viruses and varroa. Deformed Wing Virus is a widespread RNA virus and infections cause wing deformity and reduced lifespans in workers. "Silencing" DWV and other viruses via RNAi has been proven to improve bee longevity and health, but the dsRNA must be continuously provided to the colony for the bees to be protected (see Hunter et al. 2010 to read more about large-scale field application of dsRNA to combat Israeli Acute Paralysis Virus [IAPV]).

So, the researchers engineered the *S. alvi* bacteria to produce dsRNA molecules that matched the genetic code of DWV. To test its effects, they injected DWV into bees that were previously fed bacteria continuously producing DWV-specific dsRNA, as well as control bees without genetically modified bacteria. The bees with genetically enhanced microbes producing DWV-specific dsRNA were 36.5% more likely to survive the 10-day experiment than control bees (see Fig. 1).

Wow, knocking down specific viruses sounds great. But varroa transmits lots of different viruses, all with unique genetic codes. What about knocking down the mites themselves? Because they harbor and transmit diseases, varroa levels in a colony often predict virus prevalence in a colony. Varroa mites have been associated with not only DWV, but also other harmful viruses such as IAPV and Black Queen Cell Virus (BQCV), among others. So, it makes sense that the authors' next step was trying to engineer bacteria that target not just the DWV genetic code, but the varroa mite itself.

To do this, the researchers took advantage of the *mite's* immune system, as these parasites also rely on RNAi to fight invaders. The researchers created a new strain of dsRNA-producing *S. alvi* bacteria that matched the genetic code of crucial genes in the varroa mite. Because varroa feeds on bees, it ingested the molecules being produced within those bees — including the varroa-specific dsRNA. These molecules triggered the mite's immune system to target all molecules with the same genetic code for destruction — *even though the genetic code was its own*!!

Yes, you read that correctly. The varroa-specific dsRNA successfully tricked the mite's immune system so it



(L) Fig. 1 *Symbiont-produced RNAi can improve honey bee survival after viral injection. Survival curves of bees monitored for 10 days after injection with DWV (solid lines) or phosphate-buffered saline controls (dashed lines). Bees inoculated with pNR (no dsRNA control), pDS-GFP (off-target dsRNA control), or pDS-DWV2 (dsRNA matching the genetic code of DWV) and then injected with phosphate-buffered saline controls showed no significant change in survival (dotted lines). However, when injected with DWV, bees inoculated with pDS-DWV2 (solid purple line) showed 36.5% greater survival compared with bees inoculated with pNR or pDS-GFP (solid black and yellow lines, respectively).* **(R) Fig. 2** *Symbiont-produced RNAi kills varroa mites feeding on honey bees. Survival curves for varroa mites that fed on bees colonized with engineered S. alvi for 10 days. Varroa that fed on bees inoculated with pDS-VAR (dsRNA matching the genetic code of varroa, red line) showed greater mortality than bees inoculated with pNR (no dsRNA control; black line) and pDS-GFP (off-target dsRNA control; yellow line).*

attacked and destroyed itself. Indeed, the effects on mite mortality were striking. In experiments where mites were allowed to feed on honey bees with varroa-specific dsRNA, the mites were 70% more likely to die than the mites fed on control bees (See Fig. 2)!

Alright, let me pick my jaw up off the floor. This sounds too good to be true. What's the catch? And what does this mean for beekeepers? As with all genetically modified organisms, there must an extended period of further testing and regulatory review to determine its safety before it is used outside the laboratory. So, don't expect there to be packets full of genetically engineered bacteria for sale tomorrow. Of key importance is determining if the bacteria can survive outside the honey bee gut, and if there's risk of transmission to non-target organisms. The dsRNA produced by the bacteria have been specifically designed to target the genetic code of either the virus or the varroa mite, limiting the potential for non-target effects. And as far as we know, this strain of *S. alvi* can only survive and colonize the digestive tracts of honey bees — not any other bee or insect. But further work must be conducted to assess this possibility before the technology leaves the lab.

Leonard and colleagues' study potentially places an exciting future tool in a beekeepers' arsenal for the ongoing battle against the varroa mite and the diseases it transmits. (See Fig. 3 for a summary of the key findings described above.) Perhaps bees can be protected for long periods of time once genetically engineered bacteria are established in the gut. That possibility is particularly exciting, since all current treatments for mites are short-term and require repeated applications that come with their own costs to bees (and beekeepers).

In addition, another exciting aspect of the technology is its adaptability (pun intended). Indeed, because of the way in which the protection works, resistance in the viruses or mites is rather unlikely to evolve, or if it does evolve, is easily overcome by just changing the target sequence to match the new virus/mite strain.

Finally, because honey bees are eusocial organisms that interact closely with each other, it may be possible to feed genetically modified bacteria to a subset of a colony and have it shared among workers so the entire colony is protected. The authors point out that their study does not address this yet; more research is needed to determine whether the genetically modified bacteria can be shared among bees and persist for longer than two weeks.

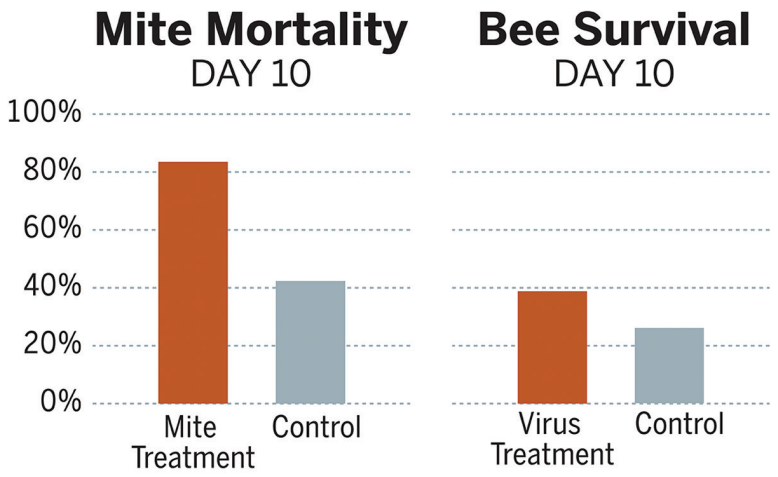


Fig. 3 *Varroa mites feeding on bees treated with the mite-targeting strain of bacteria were about 70% more likely to die by day 10 than mites feeding on control bees. Meanwhile, another set of bees treated with the strain of bacteria targeting the deformed wing virus were 36.5% more likely to survive to day 10 after exposure to the virus compared to control bees.* Credit: University of Texas at Austin

Overall, this new study by Leonard and colleagues is tantalizing — and maybe not just for honey bees. There is ample evidence to suggest that honey bee viruses are "spilling over" into native bees and other pollinators. Recent work demonstrates that wildflowers and bumble bees near apiaries have higher prevalence of viruses, including DWV, compared to locations without a nearby apiary (Alger et al. 2019), indicating that honey bees may be driving disease patterns in the broader pollinator community. By protecting honey bees against varroa and its associated viruses, it is possible that the genetically engineered bacteria may also provide protection for wild pollinators as well. We're excited to see how this technology continues to develop in the (hopefully not-too-distant) future!

Until next time, bee well and do good work,

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