What if we could make bees immune to pesticides?

Since my lab currently conducts pesticide risk assessments for bees, I sometimes hear feedback along the lines of, "*Scott, it seems like you're always focusing on the problems instead of finding solutions*." While I'm certainly sympathetic to this criticism, I would argue we first need to understand if (or when) there's a problem before coming up with a solution.

But sometimes we already know there's a problem and can focus on solutions. Such is the case for bee exposures to some organophosphate insecticides. Bees aren't exposed to harmful levels of organophosphate insecticides all the time, but they are sometimes, especially during crop pollination. Indeed, a recent worldwide meta-analysis of in-hive pesticide residue studies found that, under current use patterns, five insecticides pose substantial risk to bees: chlorpyrifos, clothianidin, imidacloprid, phosmet, and thiamethoxam (Sanchez-Bayo & Goka 2014). Two of these insecticides (chlorpyrifos and phosmet) are organophosphates.

So, what if we could make honey bees immune to organophosphate insecticides? Specifically, what if we could feed them something that detoxified organophosphates in their bodies before the bees were harmed? And what if this supplement was something that could easily be added to sugar syrup or pollen patties? These are the topics for our forty-third Notes from the Lab, where we summarize **"Pollen-inspired enzymatic microparticles to reduce organophosphate toxicity in managed pollinators,"** written by Jing Chen and colleagues and published in the journal ***Nature Food* [2021].** Full disclosure: I am a co-author on this study.

For their study, Chen and colleagues actually conducted an arsenal of studies. Because of this, it's useful to start with their overall approach. Very simply, the main idea is to feed bees enzymes that detoxify organophosphate insecticides before they're absorbed and harm the bee (Figure 1).

A major trick with this approach is getting the enzymes past the crop (stomach), which is acidic and breaks down enzymes. To do this, the authors needed to load the enzymes into a protective microparticle casing. This can be seen at the left side of Figure 1, where the light blue sphere with the reddish inside is the object of interest. The light blue sphere represents the microparticle casing, the red represents amidohydrolase phosphotriesterase (OPT) enzymes (i.e., the enzymes that detoxify organophosphates), and the dark blue squiggles represent gelatin, which stabilizes everything. If you look from left to right, you can see how the enzyme-loaded

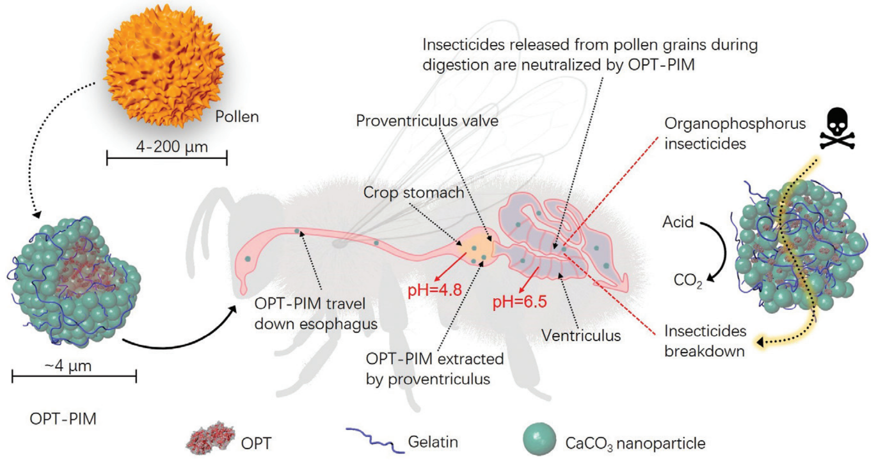

Figure 1. *A schematic of the passage of amidohydrolase phosphotriesterase pollen-inspired microparticles (OPT-PIMs) through a bee digestive tract. Microparticles analogous to pollen grains move into the midgut as they're extracted by the proventriculus, which draws particulates out of the crop stomach. The PIM structure protects the encapsulated enzyme from gastric acidity. PIMs are retained in the midgut to detoxify pesticides as they're released during pollen/nectar digestion.*

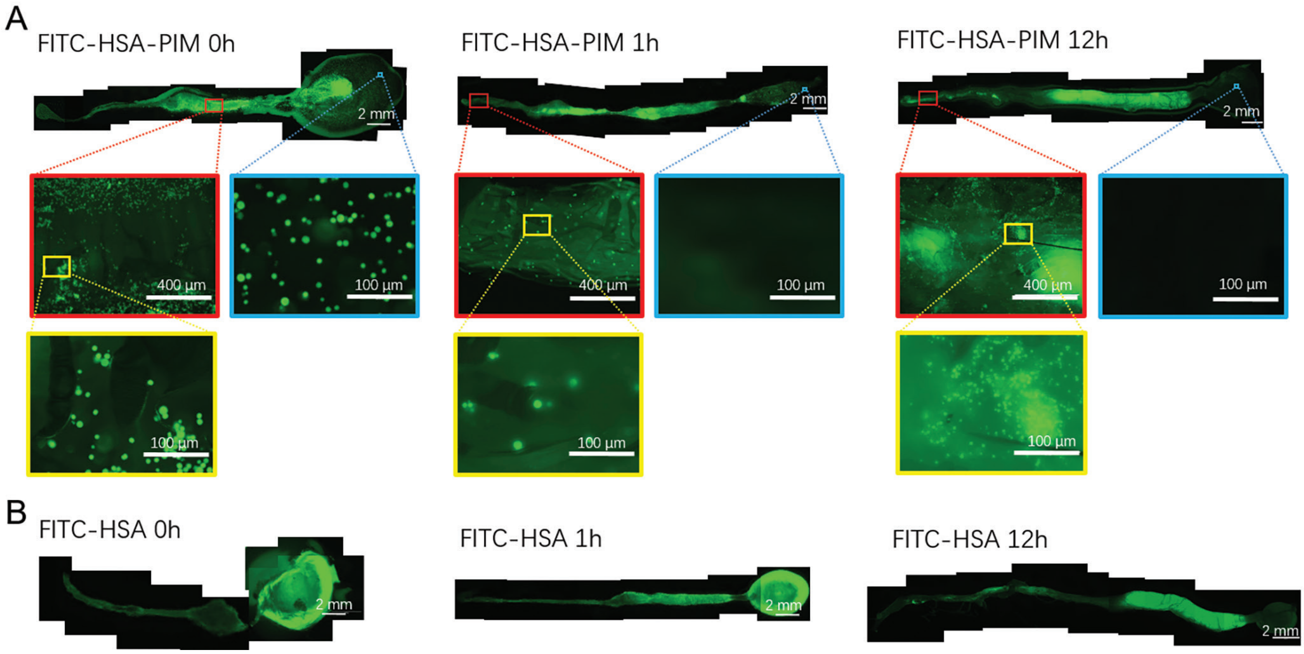

Figure 2. *Tracking of digested pollen-inspired microparticles (PIMs) by fluorescent imaging of bumble bee GI tracts. A) GI tracts following protein-loaded PIM treatment; fluorescence was maintained up to 12 h post-consumption. Microparticle morphology was clearly visible and microparticles were successfully drawn into the midgut (n = 3; relatively brighter background at 1 and 12 h was probably due to protein leakage during digestion). B) GI tracts following protein treatment without microparticles; fluorescence was qualitatively less pronounced compared to PIM treatment, suggesting PIMs improved retention and protection (n = 3).*

microparticle is ingested by the bee, travels through the acidic crop, then is available to detoxify organophosphates in the midgut where digestion and nutrient/toxin absorption occur in bees.

First, Chen and colleagues needed to manufacture a microparticle that was uniform in size and stable enough in sucrose to be fed to bees, while at the same time capable of being loaded with enzymes that could interact with organophosphates. To do this, they combined $CaCl_2$, Na_2CO_3, and gelatin and prepared the mixture with a precise recipe. Next, they added the enzyme (OPT) at various concentrations, eventually finding that 0.5 mg/ml was a sweet spot where paraoxon (an organophosphate insecticide) was rapidly metabolized and the sucrose solution was attractive to bees. They also tested the durability of the OPT-loaded microparticles for metabolizing paraoxon and malathion (both are organophosphate insecticides) across a range of temperatures, pH conditions, and up to 14 days after manufacturing. Finally, they measured acetylcholinesterase (AChE) activity in the presence of paraoxon. Since AChE is inhibited by organophosphates such as paraoxon, high AChE activity indicates effective detoxification.

Since the preliminary experiments were looking very promising, at this stage the authors tested the OPT-loaded microparticles in bees. They started by assessing whether microparticle loading allowed enzymes to remain in bee guts longer than enzymes that weren't loaded in microparticles (Figure 2). Then they dosed bees with paraoxon or malathion and directly tested whether OPT-loaded microparticles protected the bees from these organophosphate insecticides.

So, what did they find? Were the OPT enzymes durably loaded into microparticles? Yes. Loading the OPT enzymes into microparticles made the enzymes much more stable in a range of pH conditions (pH 4.8-7.4) that exist in a bee digestive system. The microparticles also allowed OPT to function for much longer and at higher temperatures (nearly 100% effective at 40 degrees C) compared to OPT that wasn't loaded on microparticles. In addition, fluorescent imaging showed that enzyme-loaded microparticles appeared to be retained in bee guts for longer than enzymes without microparticles (compare the panels in Figure 2A to the panels in Figure 2B).

Could the OPT-loaded microparticles decrease organophosphate toxicity in bees? Yes. Preliminary experiments in the lab showed that OPT-loaded microparticles were ~90% efficient at detoxifying paraoxon (measured via AChE activity) and more efficient at detoxification than OPT on its own (Figure 3A,B). So, the next step was to expose bees to organophosphates via contaminated pollen and see if feeding them OPT-loaded microparticles in syrup rescued them from negative effects of these insecticides (Figure 3C).

Chen and colleagues conducted three experiments with bees. The first experiment dosed bees with an acutely lethal concentration of paraoxon in pollen (50 µg/g). While only 25% of unprotected bees survived over 12 hrs, nearly 70% of bees that were fed OPT-loaded microparticles survived, and ~35% of bees fed OPT that wasn't loaded on microparticles survived (Figure 3D).

Next, the authors conducted 10-day survival studies with lower concentrations of paraoxon and malathion that are known to occasionally be experienced by bees in crop pollination contexts (15 µg/g and 750 µg/g pollen, respectively). When bees consumed pollen contaminated with paraoxon, they died after 4 days, on average (Figure 3E, orange line). Survivorship did not increase significantly when bees were fed OPT that wasn't loaded in microparticles (purple line), but 40% of bees survived for a full 10 days when they were fed

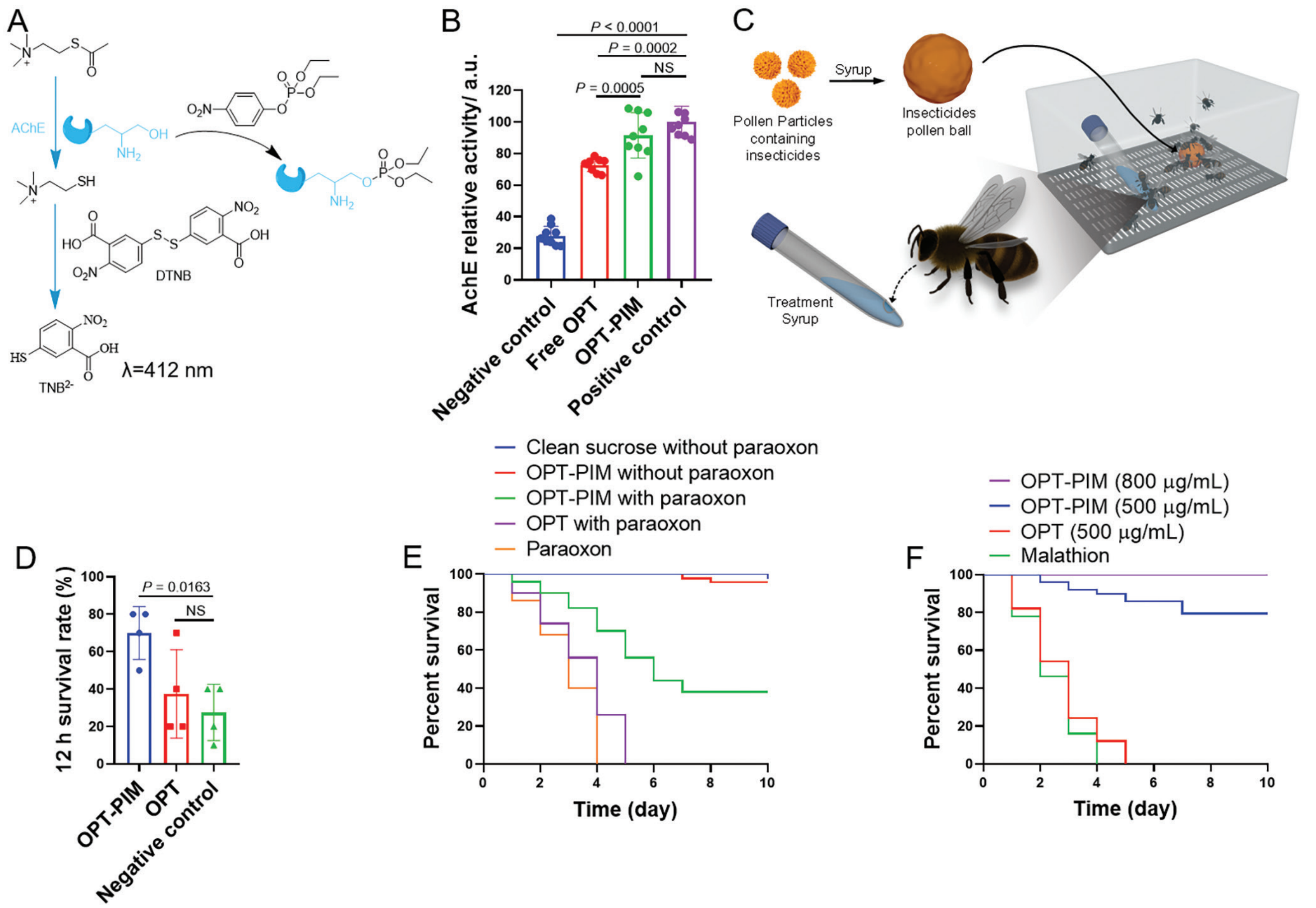

Figure 3. *Characterization of OPT−PIM efficacy through acetylcholinesterase (AChE) activity and bee survival experiments. A) The formation of thiocholine from acetylthiocholine through AChE cleavage can be characterized using DTNB. DTNB and thiocholine react to form TNB2−, the absorbance of which can be measured at 412 nm. B) Relative activity of AChE from homogenized honey bees when incubated in 0.5 mM paraoxon or DI water (the positive control) and treated with samples of free OPT, OPT−PIM and DI water (the negative control). For this experiment, n = 9. The positive control is homogenized honey bee cells without any paraoxon treatment; the negative control is homogenized honey bee cells treated with paraoxon but no free OPT or OPT−PIMs treatment. C) The apparatus for determining mortality following contaminated pollen ball consumption against PIM treatment in syrup. D) The survival rate of bumble bees following acute exposure to paraoxon (50 µg/g pollen) over 12 h when treated with 500 µg/ml OPT treatments (n = 40). E) Exposure to paraoxon (15 µg/g pollen) over 10 d (n = 50). F) Exposure to malathion (750 µg/g pollen) over 10 d (n = 50). Data are presented as means and error bars represent the standard deviation.*

OPT-loaded microparticles (green line). In other words, the OPT-loaded microparticles greatly improved bee survival in the face of paraoxon exposure. In addition, there was no difference in survival between unexposed bees that were fed clean sucrose vs. sucrose and OPT-loaded microparticles (red and blue lines), indicating the microparticles themselves are safe for bees.

Finally, Chen and colleagues conducted a similar experiment with malathion, finding even more promising results. When bees consumed pollen contaminated with malathion, they died after 4 days, on average (Figure 3F, green line). However, when bees were fed OPT-loaded microparticles while consuming malathion-contaminated pollen, survivorship increased to 80% after 10 days when they were fed a 500 µg/ml OPT-PIM solution (blue line) and 100% of bees survived when they were fed an 800 µg/ml OPT-PIM solution (purple line). In other words, the OPT-loaded microparticles at the 800 µg/ml concentration provided bees 100% protection in the face of malathion exposure.

Well this is interesting. Does this mean I should feed my bees OPT-loaded microparticles? That's an excellent question. If you anticipate your bees may be exposed to harmful levels of organophosphate insecticides via crop pollination or some other exposure event, it may be worth considering. Along these lines, the second author on this paper (James Webb) has started a company and is actively working with beekeepers to test the OPT-loaded microparticles (and related technologies for other pesticides) in the field. If you're interested, check out his company and get in touch with him: **https://www.beemmunity.co/**

Is this technology good or bad for sustainability in beekeeping and agriculture? That's a loaded question. Since I became aware of the results summarized in this study, I have discussed this topic with numerous people. On the one hand, this technology clearly stands to improve the health of managed pollinators such as honey bees. Fantastic! But at the same time, it would be very difficult — perhaps impossible — to feed OPT-loaded microparticles to all non-target organisms in agricultural contexts. For example, ~100 species of wild bees pollinate apple in New York, and we know those bees are sometimes exposed to harmful levels of organophosphate insecticides (Centrella et al., 2020; Zhao et al., in prep). If managed honey bees have access to OPT-loaded microparticles but wild bees don't, does that leave the wild bees out in the cold? Good question.

Another question is, would making honey bees immune to pesticides encourage farmers to increase pesticide usage? Pesticides cost money, so farmers have financial incentive to use as little as possible. And I've never met a farmer who wants to harm bees. So I'm hopeful adoption of these new technologies would not increase pesticide usage. But I'm not a psychologist with a crystal ball, so I don't know.

As new technologies such as OPT-loaded microparticles become available, these are important questions regarding sustainability in beekeeping and agriculture. What do you think?

Until next time, bee well and do good work.

Scott McArt

Reference:

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