A promising new antiviral therapeutic for honey bees

Every beekeeper knows varroa is the greatest biological threat to honey bee colonies throughout the world. But in fact, this is only partially true.

Varroa feeds on bee fat bodies, and because fat bodies are essential to the immune system of bees, feeding by varroa weakens bees' immune response. At the same time, viruses such as deformed wing virus (DWV) are transmitted to bees in the process of feeding by varroa. These viruses, in combination with the bees' weakened immune response, are what make a particularly lethal 1-2 punch.

Yet we beekeepers focus almost exclusively on varroa control, not virus control. This is evident when you look through any beekeeping supply catalog, whose pages are filled with anti-varroa miticides such as formic acid (Formic Pro and Mite Away Quick Strips), oxalic acid (Api-Bioxal), hop oils (Hopguard), amitraz (Apivar), and more. Have you noticed there's a conspicuous absence of anti-virus therapeutics in beekeeping catalogs?

Antivirals have been developed for viruses that infect other organisms, including humans. I'm sure everyone has heard of Paxlovid, which many of us have taken over the past few years to treat COVID. I don't think I'll ever forget Paxlovid and its awful metallic taste, which sticks with you day and night while you're taking it!

So, what about antivirals for bees? Is there any evidence that therapeutics can reduce virus levels in honey bees? What are the mechanisms by which antiviral therapeutics might work in bees? And how about their safety; is there any evidence the therapeutics cause unintended harm to bees, or are they safe? These are the topics for the seventieth *Notes from the Lab*, where I summarize ***"Potassium ion channels as a molecular target to reduce virus infection and mortality of honey bee colonies,"*** written by Chris Fellows and colleagues and published in the journal ***Virology Journal*** **[2023].**

For the study, Fellows conducted a series of lab experiments at Louisiana State University under the supervision of Daniel Swale (see Photo 1), followed by field experiments with full colonies at the USDA Baton Rouge lab under the supervision of Mike Simone-Finstrom. They focused their attention on ATP-sensitive inward rectifier potassium (K_{ATP}) channels because these channels have previously been shown to play a major role in viral infection of mammals, flies, and more recently, bees.

In the lab, the authors started by testing whether four K_{ATP} modulators (i.e., their four putative antiviral therapeutics) were safe for bees. The general setup for these experiments is shown in Figure 1, where

Mike Simone-Finstrom

Photo 1 *Senior author Daniel Swale (now located at the University of Florida), with his son Matthew, a promising future honey bee researcher*

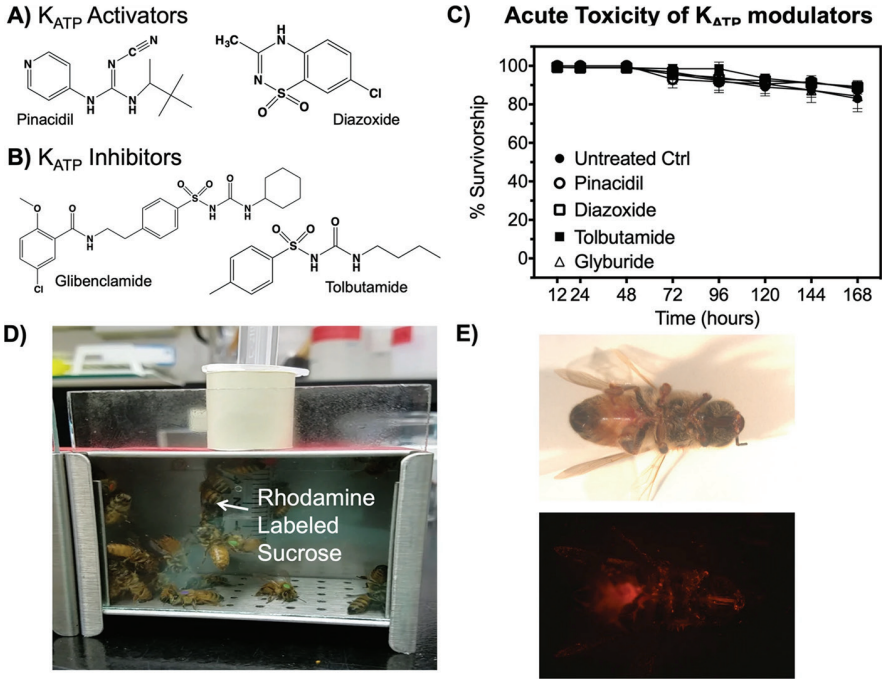

Figure 1 *Structures of the four K_{ATP} modulators (i.e., possible antiviral therapeutic drugs) used in this study and their toxicity to bees. (**A**) K_{ATP} channel activators pinacidil and diazoxide, and (**B**) K_{ATP} channel inhibitors glibenclamide and tolbutamide. (**C**) Survival of honey bees orally dosed with each of the K_{ATP} modulators or an untreated control. Data points represent means +/- SEM. (**D**) Image of caged bees with sucrose solution containing Rhodamine B, a fluorescent tracer, to ensure bees consumed the treatments. (**E**) Representative images of bees under white and fluorescent light that verify individuals fed on the chemical-treated solutions.*

bees were kept in cages and allowed access to sucrose feeders containing K_{ATP} channel activators (pinacidil or diazoxide), K_{ATP} channel inhibitors (glibenclamide or tolbutamide), or an untreated control. The sucrose solution contained a fluorescent tracer to monitor that the bees were consuming the treatments (see Figure 1, panels D & E) and survival was monitored over two weeks.

Next, the authors tested whether K_{ATP} channel modulation impacted virus replication in bees. To do this, they inoculated bees with Israeli acute paralysis virus (IAPV), IAPV plus one of the K_{ATP} channel activators (pinacidil), or an untreated control, and monitored IAPV levels and bee survival over two weeks.

In addition, they assessed a possible mechanism for how K_{ATP} channels slow virus infections: by modulating the production of reactive oxygen species (ROS). To do this, they conducted a suite of experiments with a chemical that causes the production of ROS in bees (paraquat), a K_{ATP} channel activator (pinacidil), a K_{ATP} channel inhibitor (tolbutamide), and either inoculating with IAPV or collecting bees from low-varroa or high-varroa hives (i.e., bees that were experiencing low or high virus pressure).

Finally, the authors conducted a manipulative field experiment to see if one of the K_{ATP} channel activators (pinacidil) could reduce virus levels in full colonies of bees. To do this, they compared the levels of seven viruses — deformed wing virus A (DWV-A), deformed wing virus B (DWV-B), black queen cell virus (BQCV), Lake Sinai virus 1 (LSV1), Lake Sinai virus 2 (LSV2), and IAPV — among untreated control colonies, colonies that were inoculated with viruses, and colonies inoculated with viruses while being treated with pinacidil (see Photo 2). They also used a slick dead-bee collection apparatus placed in front of the hives, which gathered dead workers that were removed from the hive by undertaker bees (see Photo 3).

Mike Simone-Finstrom

Photo 2 *Lead author Chris Fellows applying a therapeutic antiviral treatment to a honey bee colony at night under red light*

So, what did they find? Were the four putative antiviral therapeutics safe for bees? Yes, at least in terms of survival of individual bees over two weeks. As can be seen in Figure 1, panel C, there was no difference in survival between bees fed a control sucrose solution and bees fed large doses of each K_{ATP} channel modulator. In addition there was no difference in the number of dead bees found in the dead-bee collection apparatus between control colonies and colonies exposed to pinacidil (Photo 3).

Were the K_{ATP} channel modulators therapeutic? In other words, did they reduce virus levels in bees? Yes. The authors tested this question with pinacidil, finding that bees consuming pinacidil had much lower levels of IAPV compared to bees that didn't consume pinacidil. More importantly, nearly four times as many bees survived IAPV inoculation if they consumed pinacidil compared to no consumption of pinacidil. This is a very promising result!

What's the mechanism by which pinacidil reduced virus levels? Probably by regulating the production of reactive oxygen species (ROS), which subsequently interfered with virus replication. Several experiments revealed that bees treated with paraquat, which induces the production of ROS, were better equipped than untreated bees to combat IAPV and other viruses. This effect was amplified when bees also consumed pinacidil, highlighting its possible role in the regulation of antiviral ROS. Further, consumption of pinacidil caused bees to increase activity of the enzyme glucose oxidase, which has been linked to social immune function in honey bees.

Was pinacidil effective at reducing virus levels in full colonies in the field? Yes, and this is the second very promising result. Colonies inoculated with viruses while being treated with pinacidil had much lower levels of

Chris Fellows

Photo 3 *Dead bee traps in front of the experimental hives used in the field study*

DWV-A, DWV-B, BQCV, and LSV2 compared to colonies inoculated with viruses but not receiving treatment (see Figure 2). In fact, the virus levels in the pinacidil-treated colonies were equivalent to control colonies that didn't receive virus inoculation. In other words, treatment with pinacidil essentially reset all viruses to background levels.

Well, this sounds excellent. Should I use pinacidil on my bees? While the study by Fellows and colleagues is very promising, there are still several steps that need to happen before we should all go out and buy pinacidil and use it on our bees. And let's be clear, these steps are in place for a very good reason: We all want to be sure there are minimal negative side-effects of taking a drug! Additional clinical trials are needed to make sure this is true. In addition, while pinacidil is clearly promising as a therapeutic, additional studies could directly compare its effectiveness to other K_{ATP} channel modulators. Who knows, maybe there are other K_{ATP} channel modulators that hold even more promise!

We should also zoom out, think about the big picture, and evaluate when and why an antiviral therapeutic should be applied to a colony. In many cases, a miticide is still probably the best course of action. Applying an antiviral therapeutic without a miticide won't get rid of varroa, which will continue to parasitize our bees, weaken their immune systems, and transmit viruses.

But how about applying a miticide *and* an antiviral? This could be an excellent tactic, since typically we're applying miticides when varroa has reached a threshold at which viruses are worryingly elevated in the bees. In this case, antiviral therapeutics could be considered the 2nd part of a 1-2 control punch (the miticide being the 1st part). There's certainly some poetry in a 1-2 control punch neutralizing the menacing 1-2 punch from varroa and its associated viruses.

More research is clearly needed on these topics and others. But for now, let's appreciate that Fellows and his colleagues at Louisiana State University, University of Nebraska, and the USDA Baton Rouge lab are working to put some highly innovative tools in beekeepers' hands. For that, we (and our bees) should all be appreciative.

Until next time, bee well and do good work.
Scott McArt

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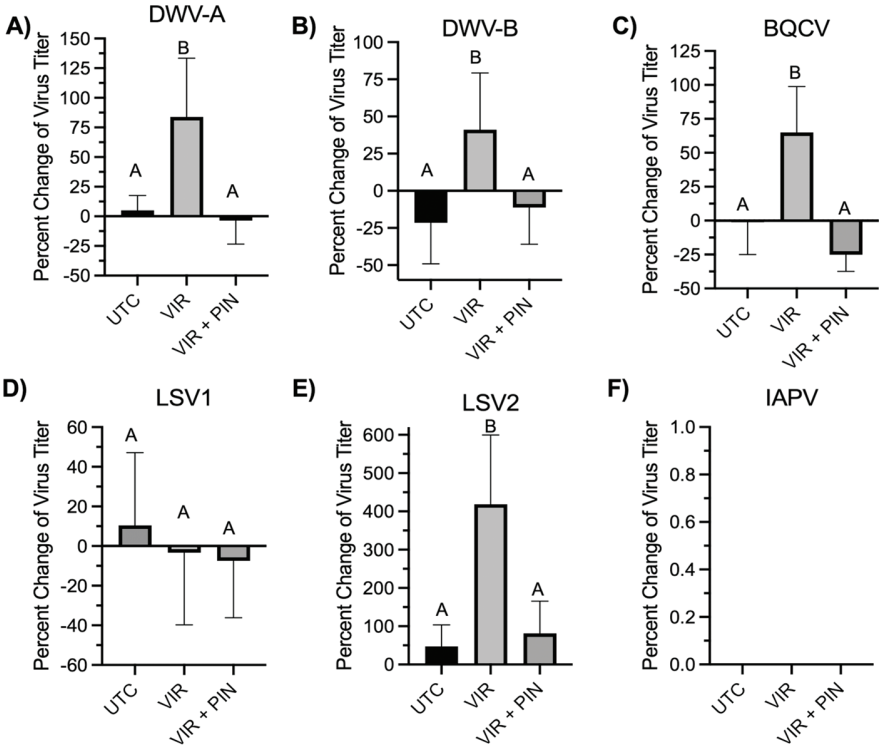

Figure 2 *The effect of pinacidil treatment on virus infection in emerging bees in inoculated colonies. Viruses screened include (***A***) DWV-A, (***B***) DWV-B, (***C***) BQCV, (***D***) LSV1, (***E***) LSV2, and (***F***) IAPV. Bars represent average (n = 10 colonies) percent change in virus titer among emerging bees between initial and post-treatment timepoints +/- SEM. Bars not labeled by the same letter within each virus group represents statistical significance (P < 0.05). Groups include untreated control (UTC), virus only (VIR), and 2 mM pinacidil + virus (VIR + PIN).*

References:

Fellows, C. J., M. Simone-Finstrom, T. D. Anderson and D. R. Swale. 2023. Potassium ion channels as a molecular target to reduce virus infection and mortality of honey bee colonies. *Virology Journal* 20:134. **https://doi.org/10.1186/s12985-023-02104-0**

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