Developing safer, selective insecticides to preserve honey bee health

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Most currently used pesticides in agricultural applications contain broad-spectrum chemicals, which are harmful to a wide range of insects. However, the majority of insects are not pests, and with concerns about global insect declines and the impact this will have on ecosystem health1, there is a need for more environmentally friendly insecticides that have selective action against major pests while preserving the health of beneficial insects.

Honeybees often top the list of beneficial insects, given that thirty per cent of global agricultural systems are reliant on pollination by bees. However, the health of honeybees is increasingly under threat due to the spread of pests and diseases, including the parasitic mite Varroa destructor and other in-hive pests such as the small hive beetle. Broad-spectrum pesticides used in agriculture also contribute to declines in pollinator health2.

The recent arrival of the mite Varroa destructor on Australian shores has caused widespread concern to the beekeeping and horticultural industries, leading to a hive euthanasia program and feral colony baiting in the affected regions as part of the eradication response. This event was a stark reminder of the importance of maintaining current, effective pest control strategies for mites.

Pesticides used to control mites elsewhere in the world are dwindling in efficacy due to the development of chemical tolerance3, and novel control methods are urgently required to safeguard the pollination and horticultural industries. In particular, a selective pesticide that was harmful to Varroa but safe for honeybees would provide a valuable weapon in our arsenal of strategies to combat mites.

How are new insecticides discovered?

In the past, insecticides were identified serendipitously via toxicity screening, with the aim to find chemicals that were lethal to insects, but not to mammals, fish or birds. Once identified, as long as a chemical effectively killed pests, little attention was given to *how* that chemical killed pests.

Insecticides generally work by entering the insect via the gut, respiratory system or through their hard, shell-like external cuticle, and binding to a specific protein 'target', causing that target to malfunction in some way. Often, the protein target of early insecticides was not known until the insecticide stopped working, usually due to a genetic mutation in the target protein that led to insecticide resistance.

Many targets have been identified by studying insects with resistance mutations, but by this stage it is too late – once resistance develops, any insecticide with a similar chemical structure is likely to be ineffective. We now know that most broad-spectrum insecticides are neurotoxic, affecting a range of different protein targets in the peripheral and central nervous systems of the insect, causing paralysis and death. However, in principle any target that provides an essential function to an insect is a potential avenue for insecticide discovery.

Designing pesticides that kill one group of insects but not others is a relatively new area of research, but it draws upon the principles of modern drug discovery that are used to identify new pharmaceuticals. Such methods combine toxicology and chemistry with structural biology, protein biochemistry and genomics, to identify molecules that bind to promising biological targets. The key difference to this approach – target-based drug discovery – is that we already know the target. In the insect world, an ideal target is one that performs an essential biological function, but is both

absent in vertebrates (so that it's safe for animals such as mammals, fish and birds) and also subtly different between insects, providing an avenue to selective insecticides that are safe for 'bystander' insects. Once a target is in hand, high throughput screening of an extensive library of chemicals is performed to identify candidate molecules, which are then optimised to increase affinity and selectivity.

Our research at the University of Sydney, led by Prof Joel Mackay, Prof Ron Hill and Dr Emily Remnant and funded by Hort Innovation and a generous philanthropic donation, aims to identify a selective pesticide that targets the Varroa mite but is harmless for honeybees. Although mites are distinct from insects (they are arachnids, like spiders and ticks), they contain similar biochemical pathways that can be targeted by insecticides. However, these processes are slightly different in honeybees, and we are looking at these differences to find a pesticide that kills mites, but not bees.

In our work to date, we have identified a promising target that is part of the insect and mite hormonal system. Insect hormones regulate many major developmental processes that affect insect reproduction, development and behaviour. These hormones bind to protein receptors that vary subtly in structure between insect groups, providing an opportunity for selective pesticide design. We have now developed an efficient, large-scale process for making our target receptor, and have recently completed our first screening of drug fragments on the Varroa mite hormone receptor. Excitingly, we discovered a number of molecules that interacted with the receptor, and we are now in the process of validating these 'hits'.

At the same time, we have been working to determine differences in the three-dimensional shape of the Varroa and honey bee hormone receptor proteins, to aid us in the search and design of molecules that can exploit those differences. This year we have already made significant progress towards determining these shapes and we hope to have them locked down in the next six months, which will provide a significant boost to our efforts.

Finally, we are concurrently targeting another pollinator pest – the small hive beetle, which thrives in the humid conditions of Australia's east coast. While current beetle traps are effective, they incorporate a broad-spectrum neurotoxic insecticide, fipronil, which is highly toxic for bees if they are exposed.

For example, if a hive takes on water and floods the trap, fipronil can be released into the hive, killing bees. Our aim is to generate beetle-specific chemicals using our hormone-receptor strategy that can be used safely within the hive environment.

Overall, we hope that this new approach to insecticide discovery will allow safe, selective and effective pest control, while maintaining the health of honey bee and improving outcomes for the beekeeping and pollination industries.

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