Monitoring Varroa Infestations: Washes And Sticky Boards, What Does It Mean?

Sarah Cross, James Sainsbury, Grant Fale, Katrina Bankier, Max Buxton, Tamatea Nathan, Ashley Mortensen

The foundation of any sustainable pest control programme is monitoring the population size of the target pest. Varroa destructor is recognised as one of the honey bee's most damaging pests, and there are several methods available to beekeepers to monitor Varroa infestation rates, including sticky boards and washes. However, each of these methods comes with its own advantages and disadvantages. Sticky boards are easy to use and require minimal colony disturbance, but interpreting the results of a sticky board is not well defined. Washes give a clear indication of the Varroa infestation rate, but they require the collection of a sample of bees from the brood comb and a few steps of processing, typically outside the apiary, before you have the results. We conducted a study where we compared the results of 3 consecutive days of 24-h sticky board collections and an alcohol wash of ~300 bees conducted on 48 colonies in summer 2019 to investigate correlations between the Varroa infestation rates indicated by alcohol washes, 24-h mite fall onto a sticky board, and colony strength. We will discuss those findings and the implications for Varroa management programmes.

Susceptiblity of mānuka and other Myrtaceae associated with honey production to myrtle rust

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Austropuccinia psidii was first found in New Zealand in autumn 2017 and is believed to have spread from Australia via wind-borne spores. During the incursion response Myrtaceae across large areas of New Zealand were surveyed for the presence of myrtle rust. This included high numbers of mānuka (Leptospermum scoparium), of which only very low numbers were found infected. This led to a perception by many that mānuka could be resistant or tolerant. However, the low number infected could be because plants had not been exposed to the pathogen and/or the small leaves and branch tips of mānuka make detection of the pathogen more difficult. Recent susceptibility testing undertaken in Australia on mānuka from New Zealand indicates the level of susceptibility is higher than initially thought. In the short term, the impact might be minimal, but there could be longer-term implications on mānuka health, with plants having to fight repeat infections from this pathogen. Of further concern was the finding that the stems, as well as the leaves, of many mānuka plants in the Australian trials were infected, and analysis of the initial data suggests that leaf resistance and stem resistance to A. psidii are due to different mechanisms. Encouragingly, a number of plants in the Australian trials showed significant resistance to fungal infection of either the leaf or stem. Overseas testing of New Zealand mānuka, kānuka (Kunzea robusta) and pōhutukawa (Metrosideros excelsa) against a different strain of A. psidii in South Africa showed all species were susceptible. Of concern, mānuka flowers became infected and died. Whilst the focus is now on disease management, our border biosecurity fight against myrtle rust is not over. With numerous strains of this pathogen present worldwide, the need to prevent further spread of different rust strains and conserve New Zealand's unique Myrtaceae germplasm is paramount.

The effects of chlorpyrifos-based sprays on honey bees in Central Otago

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Although chlorpyrifos is known to impair neurological function in honey bees, it is the active ingredient in many current-use pesticides. While banned or restricted in several countries, these agrochemicals are still widely used in New Zealand. Because chlorpyrifos accumulation in New Zealand honey bees had not been researched previously, a project aiming to track chlorpyrifos uptake into bee colonies was commenced in Central Otago in October 2016. Five honey bee hives were positioned 65 m from a field of flowering purple tansy (Phacelia tanacetifolia). This crop was then sprayed with Lorsban TM 50 EC, a common-use, chlorpyrifos-based pesticide. This set-up simulated the accidental spraying of a crop already provisioning foragers. To determine whether delayed introduction after spray application measurably reduced chlorpyrifos uptake by bees, three further hive-sets (10 in each) were then introduced to the same site 48, 72 and 96 hr post-spraying. All project colonies had matched sister queens and hive configuration. Prior to field experimentation, samples of nurse bees, pupae, emergent adults and drones were taken from all hives to assess their baseline chlorpyrifos levels. Repeat sample sets were then collected at regular intervals from all hives throughout a three-month period following their move onto the test site. In all treated colonies, chlorpyrifos was found in all sample types in concentrations higher than baseline and, in many cases, higher than levels known to affect honey bee neurofunction. Untreated control colonies exhibited no increases in chlorpyrifos concentrations above baseline. In all spray-exposed hives, chlorpyrifos levels increased steadily for 9-14 days post-exposure before declining gradually over a three-month period to level out at above-baseline concentrations. Five queens sacrificed at the conclusion of the experiment contained chlorpyrifos. Mean winter cluster sizes of colonies introduced before spraying were significantly less than those of controls and colonies introduced after spraying. When fed larval diet spiked with field-matched concentrations of chlorpyrifos, laboratory-cultured bees showed significantly lower rates of successful adult emergence than controls.

ABAtE Year 1: Soil samples and phage finding, a progress report.

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American Foulbrood (AFB) is a disease of honey bee larvae and pupae and is caused by the bacterial pathogen *Paenibacillus larvae*. AFB is the most serious disease that infects honey bees and is present in almost all countries where honeybees are found. The aim of my research project is to discover bacteriophages that can be used to prevent AFB infection by destroying *P. larvae* before it can infect the hive. Bacteriophages (phages for short) are simple viruses that kill specific bacteria. Phages are highly abundant in the environment with an estimated 10^{31} globally. Work carried out abroad has shown that phages can be isolated from healthy hives and nearby soil and that these can be used to defend against AFB. In year 1 we used acitizen science approach to collecting 375 soil samples from hives throughout New Zealand. To-date we have found phages in 17 different soil samples and will continue to look for further *P. larvae* specific phages (success rate = 17/375). I will show images of these phages along with isolation notes and phage names. These phages will be genetically sequenced and tested in order to assemble an effective phage cocktail. In the future, this phage cocktail will be tested against both the vegetative and sporulated forms of *P. larvae* in in-lab testing. This project: ABAtE(Active Bacteriophages for AFB Elimination), provides the groundwork study for an innovative approach to naturally protecting NZ beehives against AFB.

Aiding and ABAtE-ing: A Complementary Host Phage Hunt in the ABAtE Project

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American Foulbrood is caused by the bacterial pathogen *Paenibacillus larvae*. Healthy hives can be protected from infection by viruses that kill the bacteria. These viruses, called Bacteriophages or phages for short, are specific to their bacterial host and can destroy it without damaging species that are important to hive health. The ABAtE (Active Bacteriophages for American Foulbrood Eradication) project at Massey University is looking for phages in New Zealand that can act as a prophylactic treatment to protect hives by preventing P. larvae infection. My contribution to the ABAtE project involves culturing and characterising five non- pathogenic bacterial species that are closely related to *P. larvae*, with a focus on their potential to act as alternative hosts in the ABAtE phage hunt. These bacterial strains are all of the Paenibacillus or Brevibacillus genera within the Paenibacillaceae family. Bacteriophages can have either narrow or broad ranges of host specificity, and by discovering viruses which can infect these related bacterial species I may be able to identify some that can also infect P. larvae. Non-pathogenic hosts may also be useful in generating safe bacteriophage lysates for treatment of beehives in the future. By extending the range of phage hosts we are using in the ABAtE project, I hope to contribute to the eradication of American foulbrood in New Zealand.

Using bioactive compounds to protect honeybees against Varroa.

Artemio Mendoza Mendoza, Rudi Marquez, Travis Glare, Martin Laas.

Bioactive compounds have an enormous potential to control pathogen/parasite infestation (e.g. Varroa mites, *Nosema* spp.) of honey bees. However, despite entomopathogens having specificity towards Varroa mite, their implementation as biocontrol agents is limited because of their poor establishment in hives. To overcome this limitation, we are evaluating the effect of bioactive ingredients isolated from these fungi against Varroa without harming bees. In addition, we are evaluating alternative strategies to obtain specific and more potent miticides using chemical biology.

Beekeepers growing bumble bees: a key step towards a new model of pollination service provision

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Honey bees and other insects are responsible for pollinating crops in New Zealand that are worth a total of $5 billion in export earnings. While pollination is a valuable regular source of income for beekeepers, many beekeepers prefer to focus on honey production, which can at times limit the numbers or quality of hives available for pollination. With most crop industries in NZ aiming to double their production over the next decade, our team at Plant & Food Research have been developing new options for crop pollination to meet the needs of these growing crop industries. One option would be to transform how growers and beekeepers think about pollination; specialist pollination service providers could be contracted to provide pollination for crops using a variety of techniques. A key to this concept is the development of alternative pollination options, including the use of managed bumble bee colonies. We've found that bumble bees are highly effective for pollinating kiwifruit under nets, without the associated problems that honey bee colonies have in these enclosed orchards. Currently available commercial bumble bee colonies are significantly more expensive than honey bees, given the higher stocking rates required. We've developed a new bumble bee rearing system which any beekeeper can deploy in their sheds to produce affordable bumble bee colonies for use in orchards and field crops. Our vision is that this system would allow beekeepers who want to focus on pollination services to be able to provide tailored pollination options for growers involving both bumble bees and honey bees, and take a major step towards the concept of Pollination Service Providers.

Management of giant willow aphid in New Zealand
An update on the concluding Sustainable Farming Fund project

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The giant willow aphid, a relatively new pest in New Zealand, is negatively impacting apiculturists by harming willows, causing crystallised honey in beehives, and triggering greater numbers of pest wasps. We have been working on management of giant willow aphid for the last three years. This project has focussed on biological control and willow resistance, as safe and sustainable methods for long term management. The project is drawing to a close, and we have achieved the identification of two willow cultivars demonstrating resistance to the aphid, and completed host specificity testing of a potential biological control agent. This agent is a parasitoid (a natural enemy) from California that has been imported into containment in New Zealand. Based on our host specificity test results, which showed zero parasitism in all other aphid species tested, we believe this parasitoid is safe to release into the New Zealand environment and will offer some relief from the damaging effects of giant willow aphid. We are therefore applying to the Environmental Protection Authority for permission to release the parasitoid, and we seek beekeeper participation to support our proposal. If the outcome is positive, the first releases should be able to take place in 2020. This presentation will also cover results of the 2018 survey conducted with beekeepers, “Giant willow aphid: How is it impacting you?”.

A DNA sequencing method for determining the floral origin of Honey

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Honey is a vital source of revenue for New Zealand apiculture industry. Consumer demand for honey of specific floral origin is growing due to the perceived health benefits of these products. However, the botanical composition of different kinds of honey is difficult to determine due to the inherent problems in identifying specific pollen and chemical markers. These difficulties in identification have led the consumer to question the authenticity of single origin honey. In recent years, DNA has been used to identify the different types of pollen in honey to alleviate the need for expert visual pollen identification. Unfortunately, the high concentrations of inhibitors in honey mean DNA extraction techniques rely on large initial sample sizes and long extraction methods; these techniques require large volumes of sample and are difficult to adapt to automated cost-effective formats.

We have developed a novel high throughput DNA sequencing test that can determine the floral composition of honey. We use 4-methyl-morpholine N-oxide monohydrate (MNNO) a compound commonly used in the textile industries to dissolve polysaccharides, to pull apart the outer walls of pollen to allow the purification of high-quality DNA. Using this compound, we have developed an automated technique that can extract pollen DNA out of as little as 0.1 mL of honey (as opposed to the current volumes of ~1 ml). This quality pollen DNA can be sequenced and the identity and quantity of the different pollens present in the honey determined. The ease of use, small initial sample size and relatively short extraction time adds to the repertoire of current techniques in the field. This method could open new avenues of research to develop a low-cost test for honey producers, which ensures consumer security and satisfaction.

Understanding bee behaviour, including the effects of temperature, on their ability to defend their hives from intruders as tested on the German wasp, Vespula germanica.

Sean Foster, Bee-IQ Solutions

Field research was undertaken in the Auckland region in a heavily wasp infested area between July 2018 and October 2018. We needed to evaluate our hypothesis that the current makeup of our commercial Langstroth hives were contributing to the colonies exposure and therefore vulnerability to being overcome by hive intruders.

In order to undertake this work we modified 2 beehives with glass floors and video recorded what was happening both at the external entrance and inside the hive. Based on these observations and our interpretation of bee and wasp behaviour we developed simple prototypes of our current HiveGate. Over the research period we fine-tuned both the design and mechanical use of the prototypes, as well as observing additional responses that will positively affect overall colony health.

Are Your Varroa Treatments Working? They Mite Bee!

Grant Fale, James Sainsbury, Sarah Cross, Katrina Bankier, Max Buxton, Tamatea Nathan, Ashley Mortensen

The New Zealand beekeeping industry requires effective Varroa destructor control to be sustainable. New Zealand Beekeepers primarily rely on three synthetic chemical miticides, amitraz (Apivar®), flumethrin (Bayvarol®) and tau-fluvalinate (Apistan®) to manage Varroa infestation rates in their honey bee colonies. There are ongoing concerns that Varroa will develop resistance to these chemicals in New Zealand, as has been observed elsewhere in the world. This study assessed the effectiveness of Apivar®, Bayvarol®, and Apistan® in reducing Varroa infestation rates in manged honey bee colonies in the central North Island. Mites per 300 bees were calculated before and after calendar autumn application, and compared with the Varroa infestation rates of untreated colonies.

Giant willow aphids affect the spring flowering and growth of willow trees

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Abstract

Willow trees are key sources of spring pollen and nectar for honey bees, but in recent years they have been affected by the presence of the giant willow aphid. This aphid forms dense colonies on the stems of willow trees, producing large quantities of honeydew that blacken the stems and leaves with the growth of sooty mould. The effect of the aphid on the spring flowering and growth of willow trees was assessed in a nursery field trial at Massey University, as part of a MPI Sustainable Farming Fund project on the management of the giant willow aphid.

A wide range of willow species and hybrids were included in the field trial, including willow cultivars planted for soil conservation, such as 'Tangoio', 'Moutere' and 'Booth', and willow cultivars selected for spring pollen and nectar by Trees for Bees. The willow trees were planted as 20 cm length cuttings in paired rows, with one row of trees sprayed with insecticide to control the aphids, and the other row of trees infested with aphids.

During the first growing season (2017-2018), the aphids had no effect on the growth of the willow trees. But during the second growing season (2018-2019), the aphids had a noticeable effect on the spring flowering, and growth of the willow trees. In susceptible willow cultivars, the spring flowering was delayed, and the period of flowering was extended, and there was a reduction in tree growth. In resistant willow cultivars, there was no effect of the aphids on spring flowering or tree growth.

Planting resistant willow cultivars provides an effective way of managing the giant willow aphid, and ensuring a source of spring pollen and nectar for honey bees.

Host-mediated differences in quantity and chemical composition of honeydew of the giant willow aphid, *Tuberolachnus salignus*

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The giant willow aphid, *Tuberolachnus salignus* (GWA) is a voracious phloem feeder of willow stem and deposits copious amounts of honeydew, providing an energy-rich source for other organisms from different tropic levels. When melezitose-containing GWA honeydew is foraged by honeybees, it can negatively affect bee's health and make honey extraction difficult. Thus, information on the willow clones, which contributes to minimal content of melezitose in honeydew, is urgently needed to solve honeydew-related problems in the apiculture industries. As different willow clones can possess different chemical composition of phloem sap, both the quantity and quality of excreted honeydews can differ among willow clones on which the GWA feeds. In this study, we investigated the effect of willow clone differences in the quantity and sugar composition of the GWA honeydews. The significant variation in the number of droplets and amount of honeydew of GWA was observed from 3 hour samples collected from different willow clones. The GWAs that fed on the NZ 1184 clone deposited the highest amount of honeydew, while the lowest production was observed on PN 249, PN 714, PN 220, PN 751, PN 747 and NZ 1040, and no honeydew was deposited from the clones PN 376 and NZ 04-106-073 that are resistant to the GWA. Although the total sugar content of GWA honeydew from 13 willow clones were not statistically different, differences in the percentage composition of glucose, fructose, sucrose and melezitose were observed. Linear discriminant analysis showed that the GWA honeydews collected from willow clones have distinctive sugar compositions. Overall, these results demonstrate that the quantity and sugar composition of GWA honeydew from different willow clones varies, reflecting the different chemical composition of the willow clones.

Optimising Best Practices For Stonefruit Pollination

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Plant & Food Research's Pollination & Apiculture team, with co-funding from Summerfruit NZ, have been investigating the pollination of stonefruit crops for the last four years (2014-2017). This work to date has demonstrated that honey bee visitation rates to flowers in plum and apricot orchards are generally lower than recommended rates, and has documented significant variability in fruit set rates in orchards. Polleniser compatibility and temperature effects on pollen tube growth are less likely to be an issue than the variation in pollinator activity between days, sites and with varying weather conditions. Comparative research was conducted on cherries in Central Otago in spring 2018 to address the effects of temperature on fertilization and the use of best-practice honey bee pollination. Here, we will discuss the variation in fruit set and pollinator activity in cherry orchards and whether or not current best-practice honey bee pollination is correlated with a higher fruit set.