Final Report

Exploration of advanced control and detection methods for varroa mite

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Public summary

The Varroa mite (*Varroa destructor*) threatens honeybee populations globally. This project assists the Australian beekeeping industry respond to varroa by providing information on developing technologies to both monitor and control varroa in the Australian context. Australia was the last major region free from this pest, and this enabled the project to review varroa infestations in other countries and thereby develop a framework to better use control and monitoring techniques in Australia. We also compared the structure of the bee-keeping industry both here and overseas to identify potential challenges to varroa management nationally. Our work identifies potential tools for varroa control and monitoring, and a framework in which to use them, to safeguard both honey production and the essential pollination services bees provide to agriculture.

The project collected information on existing and emerging methods for Varroa mite monitoring, detection and control using a formulated search strategy repeated across many databases that harvested nearly 2000 references and can be repeated to enable the information to remain relevant to the industry. PH22002 also collected information by engaging directly with researchers and stakeholders at meetings. A distilled list of potential techniques, focusing on monitoring techniques and biological and physical control methods, was reviewed at a two-day workshop involving representatives from many aspects of the pollination and beekeeping industries. The workshop bridged the gap between research and practical application, exploring how the techniques could be tailored to suit different beekeeping operations in Australia.

The result is a shortlist of emerging monitoring and control techniques that could be further developed as tools to defend Australia against varroa. Ph22002 also developed a framework that recognized that varroa infestations go through phases, dependent on the amount of varroa moving into hives. The most appropriate control methods depend on the phase of the varroa infestation at hand. During the chronic phase with little movement into hives, techniques targeting varroa reproduction are most applicable. Individual efficacy is not as important as whether the suite of control methods deployed brings the reproductive rate below 1. During an acute phase where many mites enter the hive daily, long-lasting techniques with high efficacy are necessary within the hive, while the best non-chemical response is to stop varroa from entering the hive.

The Industry analysis identified that varroa will probably cause many sideliners to exit the industry because of the added cost. Recreational beekeepers may be more receptive to the benefits of IPM but may be difficult to reach. Alternatively, large commercial operations will be easier to inform about new management techniques but will be more risk-adverse than recreational beekeepers. The commercial operators will also be those most likely to spread varroa through pollination services, so engaging with them is critical.

The information produced by this project provides a comprehensive list of techniques that could be further explored to monitor and manage varroa, along with a structure in which to use those techniques, and recognition of industry challenges in engaging industry with these techniques. The work supports the pollination industry by providing beekeeping with a roadmap and tools to effectively respond to the Varroa mite challenge.

Keywords

Varroa destructor; pollination; Biocontrol; Monitoring; Integrated Pest Management; Australian honeybee Industry; honeybee management.

Introduction

Varroa (*Varroa destructor*) is a parasitic mite that has caused significant damage to bee populations worldwide. The Varroa mite's impact on honeybees includes weakening colonies by feeding on the fat bodies of bees (Ramsey et al 2019), transmitting viruses such as the deformed wing virus, and ultimately leading to the collapse of bee colonies if not managed effectively (Rosenkranz et al., 2010). Internationally, the Varroa mite (*Varroa destructor*) has caused major destruction to the honeybee industry, severely weakening or causing the collapse of most hives if left untreated (Jack & Ellis 2021) contributing to a loss of about 39% of hives from April 2021 to April 2022 in the US alone (Aurell et al 2022, preliminary results). Consequently, varroa is a major threat to pollination services provided by honey bees, which support 64% of Australia's total horticultural production volume (Pollination, RIRDC publ. No.03/077; Hort Innovation 2019) contributing about $ 3.85 billion to agricultural production (*Ibid*).

Australia was the last major beekeeping country free of Varroa, so concern for this pest increased when heightened surveillance (Hort Innovation report 2022 MT16005) enabled the detection of a Varroa mite incursion into Australia in June 2022 (Somerville & Kratz 2022; NSW DPI Primefact -Varroa mites). In September 2023, the attempt to eradicate the outbreak was recognized as unsuccessful, emphasizing the importance of identifying comprehensive management and detection options suitable for Australia.

Prominent methods overseas have focused on pesticides, but sustainable control of this pest demands continuous review as Varroa readily develops resistance to key chemistry (Mitton et al 2022); honey products become contaminated with the insecticides (Presern et al 2020); and some pesticidal control has detrimental effects on the bees (Mullin et al 2010). Even more recent organic pesticides such as formic acid which is a compound found in honey, is problematic, particularly at high temperatures (Jack & Ellis 2021), and so could be a poor fit for Australia.

Given these problems, there has been a move to develop alternative, more sustainable biological and cultural control methods to Varroa management. However, these methods can be both more complex to undertake and to obtain high efficacy, and have encountered various barriers to uptake overseas. An approach developed in other industries that can overcome such barriers and has supported the use of softer pest control options is Integrated Pest Management (Wilson et al 2018), where pests are maintained below economic thresholds using a range of methods that would inhibit Varroa mites developing resistance to one method. As thresholds are a key component of IPM, identifying effective monitoring and detection methods are also critical. IPM provides a framework to support the use of biological and cultural control methods. Without such a framework, developed in consultation with industry, many control methods are unlikely to be effective and uptake would be low.

An effective IPM approach that supports biological and cultural control methods as well as detection techniques is reliant on industry support (Carriere et al 2020, Wilson et al 2018). How the industry is structured, the connectivity between parts of the industry, and the level and type of extension support for apiarists will affect the success of biological and cultural control methods, and therefore needs to be understood.

Project PH22002 aligned with the Strategic Investment Plan for the Australian beekeeping industry, prioritizing sustainable pest management and innovative responses to new threats, with a focus on non-chemical control methods and improved early detection technologies. In particular, it compiled a list of promising biological and cultural control methods as well as detection and monitoring techniques for further research. The techniques were aligned within a framework developed by the project that identified the best way to use these techniques for maximum efficacy. The framework also identified gaps in our knowledge with respect to how varroa invade hives, and the relative importance of different factors in the rate of increase of varroa within hives.

In addition, PH22002 reviewed the influence of industry structure and culture on Varroa control efforts. It compared the Australian industry with those in New Zealand and the USA, identifying operational and cultural practices that will affect the efficacy and adoption of novel Varroa management strategies.

In conclusion, PH22002 represents a significant step forward in preparing the Australian beekeeping and agricultural industries to address the Varroa mite threat. By integrating global insights and a thorough understanding of the industry's unique features, the project has developed a comprehensive and sustainable Varroa management approach, underscored the need for ongoing innovation and adaptability in pest management (Rosenkranz et al., 2010) and established a model for future research and initiatives in the sector.

In particular, PH22002:

- Reviewed global non-chemical control methods and identified how they could be integrated into an Australian IPM framework.
- Developed a shortlist of biological and cultural control methods for further study to improve their suitability for Australia.
- Identified innovative Varroa detection technologies, recognizing the need to detect the rate of varroa intrusions into hives,

and the rate of increase within hives.
- Created practical Varroa management recommendations for beekeepers.
- Assessed the role of industry structure and culture in facilitating or hindering Varroa mite control.

Methodology

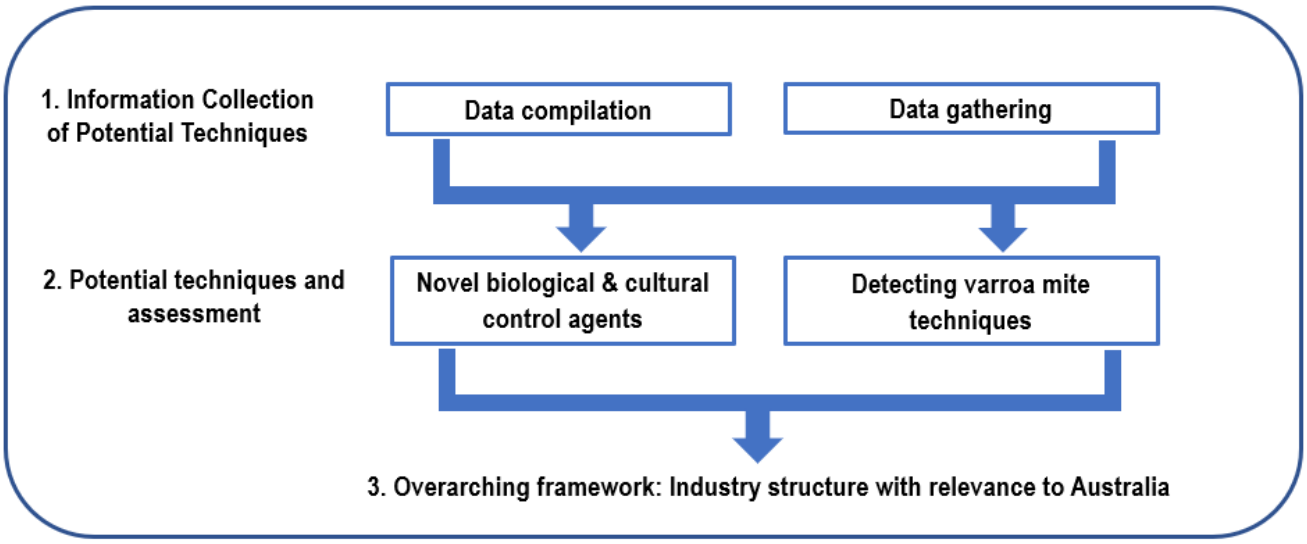

Figure 1. Outlines the project's structure: 1. The initial stage which was collecting the information on potential techniques; 2. The techniques were then be categorized into five sub-categories and reviewed with respect to biological and industry filters. 3. Concurrently, information on the framework of the beekeeping industry both here and overseas was collected, to enable the alignment of the techniques with the structural characteristics of the Australian beekeeping industry.

1. Information Collection of Potential Techniques

Data Compilation

The literature review was undertaken in a quantifiable and repeatable way using the search strategy detailed in (Appendix 1), making it a live document that can be reviewed and built upon in the future using the same protocol. Details of the protocol are provided in (Appendix 1).

Data Gathering

Data gathering (see Appendix 2) for more detail) included the Endnote library review, undertaking a survey of beekeepers (Appendix 3), and direct engagements with researchers and industry partners during national and international conferences, meetings, and industry gatherings (Table 3a and b).

2. Potential Techniques and Assessment

Data Assessment: Each sub-category of control strategies was compiled into separate Excel spreadsheets which were reviewed by the core team at regular meetings during the year. While we focused on monitoring techniques and biological and physical control methods, we also reviewed biochemical and genetic techniques in case they could be incorporated within the targeted methods to improve efficacy. The assessment process involved evaluating each strategy against a set of criteria adapted from Integrated Pest Management principles, focusing on life history stage targeted, effectiveness, whether they required hive modifications, and feasibility in Australian conditions. Our international collaborators and colleagues (encountered at international conferences and workshops) provided global insights and lessons learned with various techniques.

Varroa Workshop: A two-day workshop was organized to bring together stakeholders directly involved in Varroa mite management. It included representatives from all Australian states; the pollination industry; international experts; commercial, sideliners, and recreational beekeepers; biosecurity officers; extension officers; and legislators to get comprehensive feedback on the compiled control and monitoring techniques (Appendix 5, Table I-II). More detail is available in (Appendix 6) Day 1 involved the researchers and focused on reviewing the assembled techniques and looking to see where we could value add. Day 2 included people from all aspects of the industry

Note: To engage with scientists who may have been concerned about IP, we developed and offered a non-discloser agreement.

3. Overarching framework: Industry structure with relevance to Australia

An in-depth analysis of the Australian honeybee industry was conducted through a review of industry documents, websites of associations and statutory authorities, and interviews with 17 industry experts, representatives of grower and packers' associations, and beekeepers' associations at the national and state level, and officials of the state agriculture and primary industries departments. A comparative framework was employed to assess the industry's structure, governance, and Varroa preparedness, with specific attention to regions with climatic similarities to Australia, such as Florida, and the USA. Interviews were semi-structured, allowing for in-depth exploration of topics such as industry resilience, adaptation strategies, and needs for research and support.

Results and discussion

1. Information Collection of Potential Techniques

Data Compilation

Together Ms Megan Gee and Dr Fazila Yousuf gathered a database of nearly 2000 records in the EndNote library. This database was later segregated into five sub-categories for detailed review.

Data Gathering

EndNote Library Review:

The collected database was analysed and segregated and summarized in five sub-categories in Excel spreadsheets, which were developed into charts and reviewed at the varroa workshop. Although the primary objective was to collate information from the past five years, the search extended beyond this period when it was necessary to access original sources or seminal research. A total of 166 databases that were either tangentially related or only briefly touched upon these sub-categories were deemed irrelevant and subsequently excluded from the study.

Table 1: Number of databases reviewed and analysed to generate charts in the five sub-categories.

Sub-Categories	Database
Monitoring and Detection	714
Biological control	339
Physical Control	476
Genetic Control	61
Biochemical Control	244

Beekeeper Survey:

We received responses from 40 beekeepers in Australia and 10 in New Zealand. None had organic certification. Many Australian beekeepers indicated that they typically do not require specific certifications, as they refrain from using chemicals, and thus consider their products to be organically produced even without an organic certificate. Please note this survey was conducted when Varroa was still in the eradication phase and was restricted to few places in NSW. All the beekeepers from New Zealand weren't organically certified because they use different chemicals to manage Varroa and other varroa-related diseases.

In terms of demographics, our analysis revealed a diverse age distribution among beekeepers, with a significant portion (48%) aged 60 years or older (Figure 2a). The gender distribution among respondents showcases the participation of both males and females in beekeeping but males dominate the industry (Figure 2b).

Regarding beekeeper types, our data indicates a mix of commercial, sideliner, and recreational beekeepers, demonstrating the variety of beekeeping practices and commitments (Figure 2c). Approximately 40% of survey respondents are

commercial beekeepers, followed by 36% sideliner beekeepers and 24% recreational beekeepers (Figure 2c). The distribution of the number of hives among beekeepers is categorized into specified ranges for a clearer understanding. Our categorization includes small-scale beekeepers with 1-10 hives (Recreational), enthusiasts with 11-50 hives (Recreational), Sideliners with 51-200 hives, Commercial beekeepers with 201-1000 hives, and some large commercial operations with over 1000 hives. This range demonstrates the diversity within the beekeeping community, from recreational to commercial operations (Figure 2d).

The frequency of hive visits varies across different locations, with a notable portion of beekeepers opting for monthly visits (Figure 2e). Our data indicates that monthly visits are common across NSW, VIC, TAS, and QLD, with varying frequencies also observed (Table 2), including fortnightly visits. This variation reflects the differing management practices among beekeepers in these regions. In New Zealand, all the beekeepers visited their hives every month.

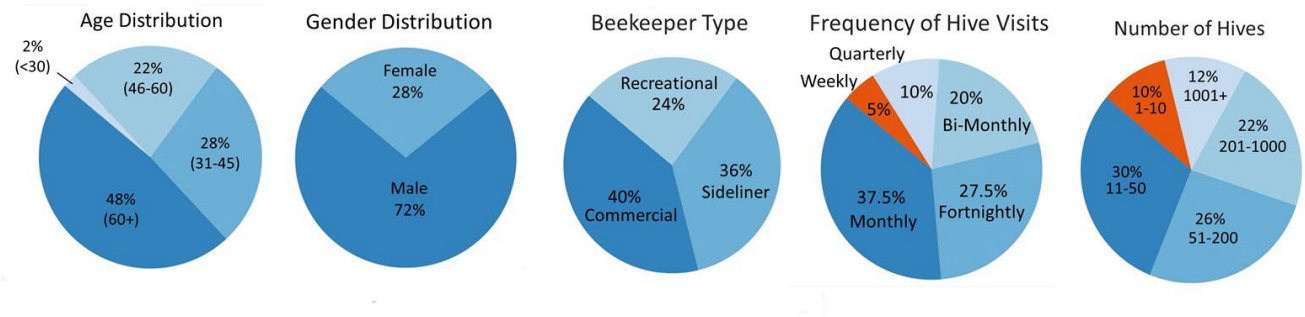

Figure 2: The pie charts offer a comprehensive overview of the characteristics and practices among beekeepers, showcasing distributions based on 'Age', is categorized as 1= <30; 2= 31-45; 3= 46-60; and 4= 60+ years (a); 'Beekeeper Type' (b); 'Gender', F= female and M= male (c); the scale of operations through 'No. of Hives' (d); and the 'Frequency of Hive Visits' (e).

Table 2: Overview of beekeeping visit frequencies across these regions, illustrating the diversity of management practices among beekeepers in different locations.

Location	Bi-Monthly	Fortnightly	Monthly	Quarterly	Weekly
NSW	3	3	6	3	0
QLD	3	6	4	0	2
VIC	1	1	1	0	0
TAS	1	1	2	1	0

Note: Bi-monthly (= every two months)

When the beekeepers were asked the main purpose of their visit to the hives and what activities they would feel comfortable combining, both in Australia and New Zealand beekeepers mentioned that they would go to check queen and colony health as well as for general hive maintenance. Depending on the season they would go to check honey production and for food. About 95% of beekeepers would go and perform multiple activities as required in the same visit (See Q8-9, Appendix 3).

Drone broods are primarily present during the spring-summer season, except regions where similar seasons persist year-round; in such areas, drone broods are present throughout the year.

When Australian beekeepers were asked about the potential costs of finding Varroa in their hives, the majority speculated that expenses could double to quadruple. In New Zealand, beekeepers reported not making separate trips specifically for Varroa, but rather monitoring for Varroa during routine hive treatments. Estimating separate costs for Varroa management in New Zealand was challenging because beekeepers typically combine these visits with other activities.

In response to inquiries about whether they had developed a strategy or action plan for addressing Varroa mites, nearly all beekeepers mentioned they would isolate the affected hives, report to the local Department of Primary Industries (DPI), and follow directives from local government authorities. Regarding the final question about the desire for more knowledge on Varroa, all beekeepers expressed interest in learning about the advantages and disadvantages of using chemicals, including the potential impact on their business. Some also voiced concerns about competing in the international market. All beekeepers were eager to learn about alternative methods to chemical treatments, questioning the effectiveness of such non-chemical approaches in controlling Varroa.

Direct Engagements: Direct engagements provided additional insights into monitoring and control techniques, including the discovery of novel methods and alternative applications of established techniques. Local conferences and meetings were instrumental in acquiring firsthand information from beekeepers and industry professionals (Table 3a). For example, the 'Buzz with Bees' event organized by the Bee Industry Council of Western Australia (BICWA), which serves as the peak industry body for beekeeping in WA, offered valuable insights into the WA honeybee industry and beekeeping business practices. Direct discussions with stakeholders also facilitated engagement with the beekeeper survey, provided a deeper understanding of industry shifts as Varroa management transitioned from eradication to management, and helped in identifying a diverse group of stakeholders for our Project Reference Group (Appendix 4, Table I) as well as contributors to our workshop.

Table 3a: The table summarizes the details of local conferences/meetings attended by members of our team.

Conferences/Meetings/Symposium	Place	Date Attended	Attendee
NSW Apiarists' Association	Penrith, NSW, Australia	18-19 May 2023	Mary Whitehouse Fazila Yousuf & Elizabeth Frost
Tasmanian Beekeeper's Association	Hobart, TAS, Australia	26-27 May 2023	Fazila Yousuf & Elizabeth Frost
Queensland Beekeeper's Association	Toowoomba, QLD, Australia	15-16 June 2023	Mary Whitehouse & Fazila Yousuf
Victorian Apiarist Association Conference AGM 2023	Bendigo, VIC, Australia	5-7 July 2023	Elizabeth Frost
Australian Almond R&D Forum	Robinvale, VIC, Australia	21-22 August 2023	Mary Whitehouse
Buzz with Bees - honey experience and bus tour	Perth, WA, Australia	17 February 2024	Fazila Yousuf
Varroa Emergency workshop	Cairns, QLD, Australia	8 March 2024	Fazila Yousuf
Science meets Parliament	Canberra, Australia	20-21st March 2024	Mary Whitehouse

Research and Engagement with Industry – Group Diversity: The diversity of our project core team, the PRG, and all other participants listed in (Appendix 5, Table II), who actively engaged in our discussions on the various techniques developed across the five categories during our workshop, underscores the breadth of expertise within our group. This diverse composition ensured that our results underwent thorough scrutiny by experts in the field.

2. Potential Techniques and Assessment

Data Compilation and Assessment: The charts created (Sup. 1-3) feature columns that display the relevance of each technique to the life stages of honeybees and Varroa mites, as well as the sensitivity and accuracy of each technique's application. Throughout the chart compilation process, valuable feedback was provided by each team member. Based on this feedback, an additional column was incorporated to underscore the limitations of each technique. Our discussions also led to the identification of gaps in the research, which subsequently inspired the generation of innovative 'blue sky' ideas during our Varroa workshop (Appendix 6). Insight on different techniques was also gathered from local conferences and meetings attended (Table 3a).

International Collaboration: We as a team attended several international conferences and honeybee-targeted workshops and meetings and developed valuable collaborations. The list of conferences/meetings/workshops attended is given below in (Table 3b).

Table 3b: The table summarizes the details of international conferences/meetings/workshops attended by members of our team.

Conferences/Meetings/Symposium	Place	Date Attended	Attendee

The 4th Annual New Zealand Honeybee Research Symposium	Rotorua, New Zealand	28 June 2023	Fazila Yousuf & James Sainsbury
Apiculture NZ's Conference	Rotorua, New Zealand	29-30 June 2023	Fazila Yousuf James Sainsbury & Mark Goodwin
Apimondia International Beekeeping Congress	Chili	4-8 September 2023	Juliana Rangel
Western Apicultural Society International Conference	Calgary, Canada	29 September-1 October 2023	Juliana Rangel
Meeting with Israeli Honeybee and Varroa researchers and representatives of the company ToBee at B. Triwaks Bee Research Center	Rehovot, Israel	6 March 2024	Mary Whitehouse
COLOSS Varroa control and RNSBB Taskforce Workshop 2024	Bilbao, Spain	12-13 March 2024	Mary Whitehouse
COLOSS Training School "Methods for resilience breeding and management"	Zaldivia, Spain	14 March 2024	Mary Whitehouse
Meeting with the company Effect Modelling & Statistics	Hirschberg, Germany	15 March 2024	Mary Whitehouse

Varroa Workshop:
Dr Fazila Yousuf presented charts that were developed after being reviewed and discussed in various meetings by the research team over the previous nine months. These charts outlined research that is currently used for monitoring, detecting, and controlling varroa mites in countries where the mites have been established.

Day 1: The scientists involved in the project used the charts as a tool to review what is known about varroa control and monitoring techniques, and to develop further ideas. The gaps were identified and discussed, and what could be developed further for Australian conditions (See Appendix 5-6 for details).

Day 2: With the presence of key industry and government people the ideas were further discussed. Dr Mary Whitehouse and Dr Fazila Yousuf have given a project overview and discussed how the charts were developed (Appendices 7 and 8). Dr Francesco Stolfi has given a talk on the Australian honeybee industry and comparison with New Zealand and the USA (Appendix 9).

Report on Monitoring and Detection options for Varroa.

This project aimed to review monitoring and detection options that could be relevant to Australia. From our project's survey we developed a chart (Sup. 1) which summarized both the detection methods currently in use and those that are in development that could be adopted for Australia. However, there are different reasons to monitor for varroa, and these reasons affect which monitoring options are most applicable. Reasons to monitor include monitoring to detect varroa, and monitoring to manage varroa.

If the aim of monitoring is to **detect varroa**, then tools sensitive to the presence of varroa are most relevant, while quantifying the number of varroa present is not. Effective tools must both avoid false positives and be highly sensitive to the presence of varroa. However, the choice of tool is also affected by the scale at which detection is required. For example, the scale could be that of a hive, an apiary, or a region.

At the scale of the hive, sticky bottom boards are probably the most effective given limited human resources, although it can take a number of days to get results. Mites on sticky boards mainly correlate with mites emerging from brood in the hive, particularly if no miticide is used, so detection may be delayed until mites are reproducing in the hive. In addition, stickiness must be maintained throughout the sampling period to stop ants removing mites. To detect new incursions, sticky bottom boards combined with a miticide is probably best technique (Owen et al 2021), although their degree of sensitivity is not clear. That is, it is not clear how many varroa need to be in a hive for a mite to be found on the sticky board.

If mites are resistant to the miticide used as a knock down method, sticky board accuracy could be compromised. Alternative knock down chemicals to avoid varroa resistance to miticides, such as tobacco smoke, could be developed, but at this stage because their efficacy is not clear, neither is their effect on bees.

Alternative techniques for detection at the hive scale, such as using odors are currently not sensitive enough (Szczurek et al 2020). Other techniques, such as using AI to identify varroa on mites entering hives may not sensitive enough at this stage to detect when small numbers of mites enter a hive or an area. Likewise, techniques measuring sound. These techniques need to be calibrated with sticky traps to compare their sensitivity. Techniques monitoring hive health would probably only signal an infestation once the hive was heavily infested. All these techniques are costly per hive.

At the scale of an apiary, sampling hives using eDNA techniques to detect the presence of Varroa has potential, but there are challenges of false positives and contamination, although these could be overcome with an effective protocol. Odors could also be used, although the sensitivity of these techniques is not clear. The sensitivity of all new techniques would need to be calibrated to that of sticky bottom boards.

At the scale of a region, remote sensing techniques could be used to identify the most expedient places to put sentinel hives to determine the location of the outbreak. Here testing honey using DNA techniques or sampling honey for varroa biochemical signatures could be effective, as sentinel hives have limited reach (Owen et al 2021). Again, any new technique developed to detect varroa must be compared or calibrated to a sticky bottom board (with miticide strips) to confirm its efficacy.

If the aim of the monitoring is to **manage varroa** once they are in a region or apiary, then measurements that are quantifiable are required. This is particularly the case when using an Integrated Pest Management (IPM) approach and aiming to keep varroa under threshold. Sampling to manage varroa requires less sensitivity but a greater level of accuracy than sampling to detect varroa. To manage varroa, colonies are sampled to identify 1) if the varroa infestation requires a control response, 2) to test if a control response has been successful; or 3) the most appropriate type of response.

Control responses following IPM are required if the pest numbers reach their economic threshold. This is easier to calculate in a pest which attacks the commercial product of a crop (such as the fruit) as greater numbers usually mean more damage. However, pests that reduce the energy available to enable the crop produce that product (those that attack photosynthesis or phloem - the “engine” of the crop) are more difficult to monitor because there isn’t a linear relationship between pest numbers and crop damage. What tends to happen is that the crop initially copes, then productivity slows down until finally the crop dies.

Varroa is a mite, and mites usually attack the engine of the “crop”. Therefore, reducing hive damage requires monitoring the rate of increase of varroa in the hive, as well as the threshold. This will require monitoring hives on consecutive occasions. With other mite pests the rate at which they invade a “crop” doesn’t contribute to the overall number of the pests in the crop, or the rate of increase within the crop. It is the speed at which the pest reproduces within the crop that determines the rate of increase within the crop. Because varroa can experience periods of high re-infestation rates, the rate of increase is not just driven by hive productivity but could also be affected by re-infestation rates. Therefore, determining if the hive is experiencing high re-infestation rates or not will determine the most applicable management technique. Determining re-infestation rates will require monitoring techniques that can identify the rate at which mites enter hives, as well as in-hive mite densities.

Currently, mite washes are the main monitoring technique for varroa control. They dislodge varroa attached to adult bees in hives, enabling them to be counted. This is useful because this is the stage of varroa’s lifecycle that is most exposed and vulnerable to control options. As mite washes quantify in-hive mite densities, it is a good technique to identify if mite numbers are reaching threshold.

However, mite washes don’t identify if the varroa sampled originated from that hive, or travelled to that hive on a bee. Therefore, they can’t be used to determine if the rate of increase in varroa in the hive is driven by mite reproduction or affected by mite invasion.

There are a number of techniques in development that could record the number of mites entering hives. The majority of these involve using AI to visually recognize varroa entering hives on bees. These could be developed into effective tools to measure re-infestations rates. The advantage of these techniques is that once set up they could be monitored remotely, although they are expensive. Further development would require calibrating their accuracy, and identifying biases in recognizing varroa in difficult to see locations.

Monitoring varroa requires repeated sampling and comparing results of the samples. Techniques that make this easier for the beekeeper and are cost effective are key. Modelling mite rate of increase could reduce the amount of sampling required, reducing beekeeper workload and cost. But this would still require information on in-hive varroa numbers, the rate of re-infestations, as well as other hive variables.

Other techniques monitoring in-hive mite densities that could be undertaken remotely (making sampling easier for the beekeeper) include using odor or vibrations to detect varroa. At this stage these techniques are not sufficiently quantifiable. Techniques monitoring hive health could alert the beekeeper to problematic hives, but would not on their own assist with

mite detection, as health is affected by a range of factors, and bees vary in the degree to which varroa affects their health. Therefore health levels may not correlate with varroa hive loads.

In addition, Mite washes also need to be reviewed. For example, there are discrepancies between regions on the amount of time bees should be shaken in a soapy water wash. Current recommendation is 20 seconds. Researchers in Germany argue that bees need to be shaken for 45 minutes to get an accurate assessment of the number of varroa in a hive. Therefore short mite washes may not be a good method for detecting the presence of mites in hives, but may suffice for quantitatively estimating hive numbers. More work is needed to determine if the increased accuracy of longer shakes is necessary when quantifying varroa numbers for varroa control.

Mite "washes" that use CO_2 to knock out varroa from a sample of bees could be used to maintain shorter handling times and accuracy. While highly effective in the laboratory, CO_2 would require training under field conditions because it is harder to use than the other methods. Firstly, you need a lower dose to knock out bees than you need to knock out mites, and the temptation would be to stop the CO_2 flow once the bees were knocked out, which would lead to an undercount of varroa. Secondly, after using CO_2 tanks repeatedly, they become more likely to spit CO_2 "snow" into the bee sample rather than just CO_2 gas. The snow would kill the bees, and its presence may cause the operator to stop before the mites are knocked out.

In conclusion, the most applicable monitoring technique will be determined by whether the stakeholder is monitoring to detect varroa or to manage varroa. To manage varroa effectively the beekeeper needs to know the re-infestation rates, in-hive densities and rate of increase. These factors affect whether or how to control the varroa. Therefore, it may be necessary to use a couple of monitoring methods to gauge re-infestation rates if this is not clear, and hives will need to be monitored regularly to check if a control method had worked, and to gauge the rate of mite increase. Remote monitoring techniques under development may assist and reduce the workload when monitoring hives regularly, but their cost effectiveness is unclear. Modelling mite rate of increase could also reduce beekeeper workload and assist varroa monitoring.

Report on Varroa Biological and Physical Control methods.

This project reviewed biological and physical control methods being developed to control varroa. A detailed list of distilled techniques is provided in (Sup. 2) which also includes potential genetic and biochemical methods for reference.

To be effective, these tools need to be used appropriately within a management regime. Correct management requires recognizing how varroa attacks the hive's productivity. For example, unlike pests that attack the commercial product of a "crop" (such as the fruit) Varroa, like most mites, are "R" strategists with a high reproduction rate that usually attack energy production. With these types of pests, the hive will initially cope, then productivity slows down until finally the hive dies. Therefore, along with an economic threshold, the rate of increase of varroa within the hive is important. If the reproductive rate of increase is controlled or reduces, then the pest does not overwhelm the productivity of the hive and the pest can be suppressed and restricted from reaching its economic threshold.

Normally, the rate of increase within the hive is driven by reproductive success. Suppressing this rate of increase can be achieved by deploying a suite of techniques to suppress reproduction. However, as discussed above in the report on monitoring and detection options for varroa, varroa hot spot sites in Australia are experiencing an "acute" or "invasion" phase, where large numbers of varroa are constantly arriving at clean hives in hotspot locations. Under these circumstances it is not possible to reduce the rate of increase in a hive using control options aimed at reducing the reproductive rate because there is no relationship between today's and tomorrow's mite numbers. One-off control methods also will not work. The only management responses in this situation is to make the hive continuously too toxic for any varroa that do arrive to survive, or to stop varroa entering the hive.

Overseas, most locations where varroa is established normally experience chronic infestations, where the movement of varroa between hives is inconsequential to the rate of increase of varroa within in the hive. Here control can be achieved by reducing reproductive rates of varroa within hives using a suite of non-chemical methods which will be discussed later.

When hives are experiencing high re-infestation rates in the **acute phase**, long lasting within-hive control methods that attack varroa on bees need to have high efficacy. As this project focused on potential biological or physical /cultural control methods that could be developed for Australia, predators were considered as an in-hive control method. The most promising was the pseudoscorpion *Chelifer cancrioides,* which can live in hives and will attack varroa and not the bee brood. However, it is a scavenger, and only attacks varroa dislodged from bees and had no effect on in hive varroa numbers in field trials (R. Van Toor, pers comm). Also, in Australia it is only reported from Tasmania, so currently could not be used on the mainland.

Biological agents that could be long lasting within a hive include entomopathogenic fungi *Metarhizium anisopliae* and

Beauveria bassiana (Sup. 2, Biological Control 2, Category 'Fungi', No. B22) and *Bacillus thuringiensis* (Bt) (Sup. 2, Biological Control 2, Category 'Bacteria', No. B8-B20). Of these *M. anisopliae* is the most advanced with reports that it can remain in the hive for up to 42 days. Bt variants can minimize the negative impact of Varroa mites on colonies without causing adverse effects on adult bees and larvae (Alquisira-Ramírez et al., 2014; 2017) and can kill varroa (Gregorc et al. 2022), but no Bt strain specifically developed for Varroa control currently exists. With both the fungal and bacterial options there are concerns about laws concerning their residues in honey, and the regulatory hurdles that may be required for them to be used. To be effective in the acute stage they would need high efficacy.

Physical control methods that potentially could be very effective during an acute phase are those that stop varroa entering a hive (see Sup. 2 control methods, physical control) One method knocks mites off hives at the entrance that then fall into an oil or sticky trap. These traps could be combined with a visual system to count the mites, so that varroa are being monitored and controlled simultaneously. An alternative to an oil or sticky trap is to use *C. cancrioidies* to attack the fallen varroa. To develop techniques blocking mites entering hives requires knowing the type of bee upon which varroa enter hives, and if the bee "preferences" of varroa changes or is influenced by characteristics of the transporting bee or its hive. Currently varroa are known to enter hives through worker drift, by robbing, or by drifting drones. Therefore, techniques to stop drift, robbing or drones could minimize varroa spread, depending on which method was prevalent at a given time. Stopping mites entering hives is an area of research that has great potential.

When varroa are in a chronic phase, the best control methods are those reducing within hive varroa reproductive rates, keeping varroa below the economic threshold. These can include varroa on bees moving into brood, but control methods that target reproduction within the brood chamber have more efficacy. As multiple control methods could be deployed concurrently to reduce reproductive rates, the efficacy of individual techniques within the suite would not need to be high. The aim would be that combined, the control methods reduces the varroa reproductive rate to below 1, driving a gradual decline in mite numbers. Physical/cultural control methods, used in conjunction with genetic control methods, would be most appropriate. As these would be multiple non-chemical control responses, varroa is unlikely to develop resistance to them. Such methods, such as including brood breaks and drone brood traps, are already in use overseas. Nevertheless, research is necessary to identify the timing and management of these techniques for maximum efficacy, reduced costs, and best fit with beekeeper current management procedures within Australia.

For example, summer brood interruption of at least 25 days was found in Europe as effective in reducing varroa numbers and varroa rate of increase, with little economic cost. Could this be effective in Australia? In Europe additional control methods that target varroa on adult bees in hives, such as Oxalic acid, were also deployed after brood trapping for maximum effect. Drone brood traps are also effective, but time consuming. Use of this technique would require identifying when and how to use this technique to maximum effect within the Australian context.

Novel techniques requiring further research that are physical control methods include heating hives, which can kill varroa in brood cells (Sup. 3). Heating could be achieved using heat packs, or by modifying hives to enable them to be heated electronically from within. A caveat is that any technique trying to heat hives will have bees working against it. Work is needed to identify if any of these techniques could be used by large apiaries. Additional research would identify when to time the heating event with respect to mite and colony life histories and the Australian climate.

A physical option not supported is using combs with small cell sizes. The aim of the small cells is to reduce the amount of time the bees are developing, and therefore the amount of time available for the mites to reproduce. Studies suggest this is not effective.

Vibrations are also known to kill varroa. Those involving sound take too long to kill mites, while electromagnetic vibrations may be more effective but are still in the early stages of development. Electromagnetic vibrations could be further explored as a technique to kill varroa.

Although not in the remit, some genetic and biochemical control techniques were reviewed as potential complimentary techniques in combination with biological and cultural/ physical control methods. These are listed in Sup, 2, [control methods, Genetic control and biochemical control methods). Of these, RNA interference (RNAi) is the most researched. RNAi works by disrupting varroa reproduction. The RNAi molecule is fed to bees, and is passed onto varroa when they feed on the bees. The molecule disrupts varroa reproduction. Although it has been researched for at least 20 years in three different continents and owned by three different companies, no commercial product has been developed. The RNAi is effective in the lab, but the molecule degrades quickly under field conditions. Its effectiveness may also depend on whether the varroa is feeding on Hemolymph or fat bodies. Recent work from Europe suggests that varroa feed on fat bodies of adult bees during dispersal and hemolymph of developing bees during reproduction (R. Bahreini, COLOSS 2024 presentation). Future work on RNAi would need to look at RNAi transfer from both Hemolymph and fat bodies, and focus on developing novel means to stabilize the gene under field conditions. The contact details of people currently researching this product are in (Sup. 3).

Any improvement in the genetic resistance of bees to varroa would compliment biological and control methods. Despite massive efforts over 30 years, there has only been limited success, mainly focused on local resistance. Nevertheless, there is variation in the ability of hives to detect and remove varroa infected brood that can be exploited locally. One technique affective in New Zealand could be easily tested here. It involves a genetic marker for Varroa Sensitive Hygiene behaviour (VSH). There is an adenine/guanine (A/G) single nucleotide polymorphism of which the G allele is associated with Varroa Sensitive Hygiene behaviour. Researchers in New Zealand (Sainsbury et al 2022) have found that queens carrying two copies of this gene have hives that are more resilient to varroa colonies with lower levels of varroa. Queens in Australia could be screened for this marker and tested to see if their workers in Australia have heightened Varroa Sensitive Hygiene behaviour. If effective, using these queens could compliment physical control methods to reduce varroa reproduction rate. However, the cost of testing the queen of each hive could be a challenge for large commercial operations.

In conclusion, the type of control method and its efficacy is dependent on factors affecting the rate of increase within the hive. Effective control methods for acute infestations include those that stop varroa entering hives, and those that are very efficacious at killing mites on adult bees within hives continuously. Non-chemical techniques to stop varroa entering hives have great potential, although more needs to be known about how varroa enter hives (what type of bee is used by varroa), whether the choice of bee changes, and whether varroa are more attracted to some hives than others. Non-chemical techniques that attack varroa on adult bees in hive are all in preliminary stages of development, and include the fungus *Metarhizium anisopliae*, where research is more advanced, or potentially a variety of the bacteria *Bacillus thuriengiensus* (Bt), although our research did not uncover such a Bt in development for varroa. Their usefulness in an acute infestation would depend on their efficacy.

In hives experiencing chronic infestations, economic thresholds are important, and the aim is to maintain a low varroa reproductive rate to stop varroa from crossing those thresholds. Control methods targeting brood reproduction are the most efficacious, but they can be supported by control methods targeting varroa on adult bees. A suite of techniques deployed concurrently would be particularly effective if they keep the varroa rate of increase below 1, causing the population to decline. Therefore, individual methods used to control varroa in chronic infestations do not need to have high efficacy, but in combination they need to keep reproduction below 1. In chronic infestations what is critical is knowing when to administer control methods and what control combinations are effective. Therefore, research is needed to identify when to use known techniques and how to combine them to suppress varroa reproductive rate in Australia. Other control methods that could be developed are methods to heat hives, or electronic vibrations. For chronic infestations, research is needed to identify when to use known and developing techniques and how to combine them to suppress in hive varroa reproductive rates in Australia.

3.Overarching framework: Industry structure with relevance to Australia

Report on the structure of the industry

Integrated Pest Management (IPM) provides a framework in which to use new technologies or practices most effectively. However, the ease at which information on these new technologies or practices can be disseminated and the incentives for uptake by beekeepers are affected by the structure of the industry. The structure of the industry refers to the social, economic, and political settings that affect the choices of individual beekeepers about the adoption of an IPM framework and its tools (including control techniques, surveillance and monitoring) (Appendix 6). The elements of industry structure relevant to our analysis are listed in the table below based on three separate dimensions: The nature of the resource in terms of economic value and location, the characteristics of the producers, and the governance system, focusing in particular on the network relationships linking governmental and non-government actors with regard to the exchange of information and funding (adapted from Ostrom 2007):

Table 4: Components of the Industry Structure

Nature of the Resource	• Economic value and ease of entry • Location
Characteristic of Producers	• Socio-economic attributes • Mental Models • Norms
Governance System	• Network Structure (Information sharing and funding)

In what follows we discuss these elements and for each, we provide our assessment of their impact on the potential for IPM adoption, summarized as positive, negative or mixed, with an emphasis on the impact of current developments.

Nature of the resource

Beekeeping is an industry with low barriers to entry and exit relative to other agricultural industries. as it requires little investment. This incentivizes a short-term orientation that gives greater weight to short-term profitability than the long-term sustainability of the resource, especially for new entrants, which has negative implications for the adoption of IPM.

The economic value of beekeeping for beekeepers is in the value of products (mostly honey) and pollination services. The value of pollination services for beekeepers has increased in recent years relative to the production of honey (Clarke and Le Feuvre 2021). The impact of this development is negative. This is for two reasons:

· Less dependence on honey for income means that beekeepers are less susceptible to requirements for low chemical residuals in honey that come from honey packers and from some international trade partners (in particular the European Union)

· Increased economic importance of pollination services means increased mobility of hives and thus increased risk of spread and more difficult surveillance

About location, beekeeping is overwhelmingly concentrated in New South Wales and Victoria (Clarke and Le Feuvre 2021). On the one hand, this facilitates surveillance; on the other, it also facilitates spread. The impact on IPM adoption is thus mixed.

However, location also has a positive impact about the relative geographical isolation of the Northern Territories, Western Australia, and Tasmania from the core of the beekeeping industry, which reduces the risk of spread and facilitates surveillance of the movement of infected bees.

Characteristics of producers

Australia lacks a large-scale survey of beekeepers. However, anecdotal evidence suggests that Australian beekeepers are older relative to countries like New Zealand and the United States. They are therefore likely to be relatively averse to the adoption of new approaches to pest management, with negative implications for the adoption of novel IPM techniques.

At the same time, these are often multi-generational operations with norms emphasizing family heritage and environmental stewardship, with positive implications for IPM adoption.

The vast majority of beekeepers fall in the recreational category (50 hives or less), while large operations (more than 1000 hives) are rare. The following table summarizes the size distribution of New South Wales, Queensland, and Victoria, where the majority of hives are concentrated in Australia, comparing it to New Zealand and the United States (Florida):

Table 5: Size distribution of the beekeeping industry

New South Wales, Australia		**Queensland, Australia**		**Victoria, Australia**		**New Zealand**		**Florida, USA**	
≤ 50	91%	≤ 50	95%	≤ 50	96%	≤ 50	89%	≤ 100*	77.8%
> 1000	**?**	> 1000	0.25%	> 1000	?	> 1000	14%	> 1000	3.3%

*≤ 100 hives is the threshold for classification of recreational beekeeping in the United States

Sources:

NSW, VIC: Clarke, Michael and Danny Le Feuvre (2021)

QLD: Queensland Department of Agriculture and Fisheries (2023)

NZ: New Zealand Ministry for Primary Industries (2022)

FL: Court et al., (2022)

The very high share of recreational beekeepers has mixed implications for the adoption of IPM. On the one hand, contrary to commercial operations, recreational beekeepers do not contribute to the risk of spread through pollination services; furthermore, they are likely to be especially sensitive to certain advantages of IPM compared to chemical controls, particularly in terms of impact on the environment and of toxicity. However, the large number of recreational beekeepers and the fact that they are not easily accounted for despite being subject to mandatory registration (this now obtains for all states after Tasmania recently introduced mandatory registration for all beekeepers), means that surveillance and extension are particularly difficult.

We expect the share of sideliners to decline and that of large operators to rise as Varroa gets established in Australia, reinforcing the current trend for the increase in the average size of commercial operations (Clarke and Le Feuvre 2021). This is because Varroa increases the costs of beekeeping. Hence, smaller operators will find it more difficult to stay in the market, while the fact that their income is not entirely dependent on beekeeping will facilitate their exit. Conversely, economies of scale will incentivize the agglomeration of commercial operations and possibly the entry of large multinational operators, as has been the case in New Zealand.

We expect the impact of these developments to be mixed. A lower number and a larger size of operators will facilitate surveillance and extension activities; however, larger businesses tend to move their hives for pollination over longer distances than smaller ones, thus increasing the risk of contagion (van Dijk, Gomboso and Levantis 2016).

Governance system

The government and non-government organizations play an important role in enabling the dissemination of information about new tools for varroa control and monitoring to beekeepers. In particular, they support surveillance and biosecurity measures and can provide extension services on how to perform IPM and relay its advantages. Understanding the role of these organizations and how they interact is important for identifying the best way to deliver information on new techniques for varroa control, and how best to combine these techniques.

The Australian Constitution states that the governance system for agriculture is shared between the Commonwealth and states and territories.At the Commonwealth level, Plant Health Australia, AgriFutures and Hort Innovation are statutory authorities depending on combined government and industry funding (in the form of industry levies). Plant Health Australia supports surveillance and biosecurity. AgriFutures and Hort Innovation are Rural Research and Development Corporations whose remit include extension services.

At the state/territory level, responsibility lies with agriculture and primary industries departments. State and territory departments are the first point of contact for individual beekeepers, particularly through biosecurity agents. Biosecurity agents are in a position to also provide extension services, including information on IPM, but their potential impact is limited by their low numbers and by the fact that beekeepers may predominantly see them as regulatory enforcers rather than providers of impartial information.

All these entities work closely together and with universities and industry associations representing growers and beekeepers. They thus constitute an effective network for the distribution of information between levels of government (Commonwealth and states/territories), between state and territory governments and between government and industry, thus creating positive conditions for IPM adoption.

A point of concern is the structure of funding, which works against the provision of long-term extension services and thus has a negative impact on IPM adoption. This is because, while the Commonwealth authorities provide grants to government or industry actors, grants run only for a limited number of years. State and territory departments could in principle provide long-term support for extension activities, but their funding comes from the general budget of the state and territory governments and thus must compete with a plethora of other interests calling on government funding.

On the industry side, the Australian Honeybee Industry Council (AHBIC) represents the industry nationally, both for commercial and recreational beekeepers. Its members include the state beekeepers' associations as well as the Crop Pollination Association and the Honey Packers and Marketeers Association of Australia. At the same time, AHBIC is an industry partner of Plant Health Australia In other words, it operates as a 'nested enterprise' between different levels of government and between private and government actors (Ostrom 1990), and it is thus uniquely placed to act as a trusted bilateral conduit of information between the industry, universities, and government authorities.

Industry Comparisons between Australia, New Zealand and Florida (USA).

Below we summarise our assessment of the impact on IPM of the various elements of Australian beekeeping. We also report where we found significant differences between Australia and the industry in New Zealand and Florida (USA).

Nature of the resource

- Economic value and ease of entry: Negative
- Location: Mixed (concentration in NSW and VIC), positive (geographic isolation of NT, TAZ, WA)

International comparison: Conditions for IPM adoption deriving from the economic value of honey are better in New Zealand than in Australia, due to the significance of specialty honey (Manuka honey) and to the fact that, contrary to

Australia and Florida, honey production is expected to remain more important for beekeepers than the provision of pollination services.

Characteristics of producers

- Socioeconomic attributes: Mixed
- Mental models: Negative
- Norms: Positive

International comparison: Operators are larger in New Zealand and Florida than in Australia. However, it is unclear whether this difference may be significant in terms of IPM adoption in Australia, since as discussed above, size has mixed implications for the adoption of IPM.

Governance system

- Information sharing: Positive
- Funding: Negative

International comparison: It is under the governance dimension that Australia presents the best potential for IPM compared to New Zealand and the United States. In the case of New Zealand, broad administrative reforms in the 1980s reduced the scope of government action in the economy. With regard to the beekeeping industry, this had several implications. The government no longer pays for biosecurity personnel, and the industry has not stepped up to take the place of the government. The national beekeepers' association has been in discussions with the government to start a formal partnership for biosecurity, but no agreement has been signed yet.

Moreover, both in New Zealand and the United States the national beekeepers' associations do not have the established and formal links with government agencies and other industries (specifically packers and growers) that AHBIC has. They are therefore significantly worse placed than AHBIC to operate as a 'nested enterprise' connecting governmental and non-governmental actors with regard to the distribution of both information and funding.

Special cases:

Specific considerations can be made regarding Tasmania, Western Australia and the Northern Territory, with the caveat that the industry is smaller, and in the case of the Northern Territory much smaller, than in the rest of Australia.

We expect Tasmania and Western Australia to be particularly good locales for IPM adoption. They are favoured by their geographical isolation from the rest of the country; moreover, both produce specialty honeys (respectively leatherwood and jarrah honey), for which low chemical residuals is an especially important marketing asset. Western Australia has also an especially dynamic state beekeepers' association. Moreover, it is the only state that forbids the use of antibiotics, thus indirectly providing a regulatory incentive for beekeepers to adopt IPM.

Conversely, we expect difficulties for IPM adoption in the Northern Territory. While the Northern Territory benefits from its geographical isolation, the beekeepers' community is fragmented (there is no beekeepers' association) and many beekeepers come from non-English speaking communities, which makes extension efforts more difficult.

In summary, the structure of the Australian industry presents both obstacles and opportunities for the adoption of IPM. It also presents factors likely to have mixed effects (the socio-economic characteristics of producers and the difference in the features of the production localities).

With regard to the obstacles, beekeeping as an industry is relatively easy to enter and exit, which favours a short-term orientation that runs counter to the potential greater complexity and longer-term horizon of IPM. Moreover, the recent emphasis on pollination makes the surveillance element of IPM more difficult. The age structure of producers is also likely make the adoption of new frameworks problematic, as is the episodic nature of available public funding.

However, Australia also provides unique opportunities for the success of IPM. The key one is the conduciveness to information sharing of the governance structure, particularly with regard to the involvement of beekeepers in information sharing through the institutionalised cooperation between public information sources (statutory authorities, universities), private actors (packers and growers) and the national beekeeper association. This aspect also appears to set Australia apart from international peers such as New Zealand and the United States, where the scope for cooperation between governments and universities on the one hand and industry operators on the other is more limited.

Outputs

Table 6. Output summary

Output	Description	Detail
Reports	Beekeeper's online survey was completed. Data relevant to the varroa monitoring, detection, and biological control was gathered and recorded in the Endnote library. Beekeeper's engagement through association meetings and conferences. Monitoring and detection methods and Biological control methods were reviewed, discussed during the varroa workshop. Blue Sky ideas were generated. Varroa Workshop conducted.	Provided to Hort Innovation via Milestone 102-104, 01 July 2023- 02 February 2024.
Final Report	Summarizing all the activities of the project.	18 April 2024.

Industry articles		
Output	**Description**	**Detail**
Industry article	Does Australia have varroa mite predators?	The article highlighted the potential of using pseudoscorpions, a biocontrol agent against Varroa mites. https://extensionaus.com.au/professionalbeekeepers/does-australia-have-varroa-mite-predators/ (Appendix 10)
Industry article	Chemical-Free Varroa Control Methods for Australian Recreational Beekeepers	The article focused on non-chemical control methods, tailored to the needs and contexts of recreational beekeepers in Australia. The Australasian Beekeepers, March 2024 (Appendix 11)

Note: The selection of topics and magazines was based on a preliminary survey to identify areas of high interest and information gaps within the beekeeping community.

Scientific articles		
Output	**Description**	**Detail**
Scientific paper (Review)	Varroa Control and Management Strategies: What Works and What Does Not in the Australian context.	In preparation for submission to Trends in Parasitology.
Scientific paper	A structured approach to monitoring and managing Varroa	In preparation for Submission to Pest Management Science.
Scientific paper	Honeybee Industry Structure: Australia vs New Zealand and USA.	In preparation for Submission to Apidologie.
Beekeeper/Researcher's Engagement		
Output	**Description**	**Detail**

Conference Talk	Varroa pest management gaps: how IPM could provide a robust framework	Dr. Whitehouse presented a talk" at the NSWAA conference in May 2023, and at the Bee Meeting (University of Sydney, NSW) in July 2023 (Table 3a and Appendix 14).
Poster Presentation	Varroa pest management gaps: how IPM could provide a robust framework.	Dr. Whitehouse presented a poster at the Australian Almond R&D Forum at Robinvale, VIC in August 2023 (Table 3a).
Conference Talk	Safeguarding Australia's Bees: The Quest for the Best Varroa Mite IPM Solution	Dr. Yousuf presented a talk at the 4th Annual New Zealand Honeybee Research Symposium at Rotorua in June 2023 (Table 3b and Appendix 15).
Media activities		
Output	**Description**	**Detail**
Radio Interview	Discussed Integrated Pest Management and the potential of non-chemical control methods for varroa mites.	Dr. Whitehouse participated in a live one-hour radio interview with Jenni McLeod from the Bee collective, on 30 May 2023. (Appendix 12).
TV interview	SBS World News Discussed the threat of varroa mite and the need for a multi-pronged approach to manage this pest.	Dr. Whitehouse was interviewed as part of SBS World News (21st September 2023) (Appendix 13). https://www.sbs.com.au/news/video/australia-gives-up-fight-to-eradicate-bee-killing-mite/xwsv7wure?cid=newsapp:socialshare:copylink
Workshops		
Output	**Description**	**Detail**
Varroa workshop	A Varroa Workshop was organized at Macquarie University as part of the requirement of the current project.	The Varroa Workshop hosted attendees from Australian government departments (DAF), State departments (NSW DPI, NT DPI), Beekeepers from different states, AHBIC CEO, and international researchers from New Zealand and USA. For further details see (Appendix 5, 6; Table II)
QBA Varroa mite Workshop	Queensland Beekeeper Association organized a workshop in Cairns for their recent incursion of *Varroa jacobsoni*.	Dr. Yousuf attended the workshop in Cairns and gained an understanding of the current situation in Queensland. She also engaged in discussions about exploring various options for Varroa mite monitoring, detection, and control. The importance of establishing accurate thresholds for Varroa mite presence was also a topic of conversation with the CEO of QBA. The workshop took place on Friday, 08 March 2024, in Cairns.
COLOSS Training School "Methods for resilience breeding and management"	The workshop taught non-chemical control and monitoring techniques of varroa	Dr Whitehouse attended the workshop in Zaldivia, Spain (14 March 2024).
Other Activities		
Output	**Description**	**Detail**
NSW Apiarists'	Varroa pest management gaps: how IPM could	Dr. Whitehouse and Dr. Yousuf attended the conference. Dr.

Association	provide a robust framework	Whitehouse presented a talk at the NSWAA conference, Penrith, NSW, Australia (18-19, May 2023).
Tasmanian Beekeeper's Association	The purpose was to engage with the beekeepers and stakeholders and discuss the project's objectives and know their perception about Varroa mites.	Dr. Yousuf and Ms. Frost attended the meeting in Hobart, TAS, Australia (26-27 May 2023).
Queensland Beekeeper's Association	The purpose was to engage with the beekeepers and stakeholders and discuss the project's objectives and know their perception about Varroa mites.	Dr. Whitehouse and Dr. Yousuf attended the meeting in Toowoomba, QLD, Australia (15-16 June 2023).
The 4th Annual New Zealand Honeybee Research Symposium		Dr. Yousuf and Dr. Sainsbury attended the Research Symposium. Dr. Yousuf presented a talk, in Rotorua, New Zealand (28 June 2023).
Apiculture NZ's Conference		Dr. Yousuf, Dr. Sainsbury and Dr. Goodwin attended the conference, in Rotorua, New Zealand (29- 30 June 2023).
Victorian Apiarist Association Conference AGM 2023		Ms. Frost attended the meeting in Bendigo, VIC, Australia (5-7 July 2023).
Australian Almond R&D Forum		Dr. Whitehouse attended the meeting in Robinvale, VIC, Australia (21-22 August 2023).
Apimondia International Beekeeping Congress		Dr. Rangel attended the meeting in Chili (4-8 September 2023) (Table 3b).
Western Apicultural Society International Conference		Dr. Rangel attended the conference in Calgary, Canada (29 September-1 October 2023) (Table 3b).
COLOSS Varroa control and RNSBB Taskforce Workshop 2024		Dr. Whitehouse attended and presented a talk at the conference in Bilbao, Spain (12-13 March 2024) (Table 3b).
Western Australia BICWA event		
Buzz with Bees		Dr. Yousuf attended the full-day event in Perth, WA (18 February 2024), and gained information about the Western Australian beekeepers and industry.

Outcomes

Table 7. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
A prioritised shortlist of biological, cultural and detection methods ready for further testing both overseas and in Australia where possible.	1.Manage European Honey Bee 1.1 Bee health	We have compiled comprehensive lists of potential monitoring, detection, and control methods—including biological, genetic, physical, and biochemical approaches (submitted to Hort Innovation in MS104) These would be accessible for public use. We have also provided a shortlist of techniques in development.	Supplementary material 1, 2, 3
An understanding of international research into Varroa mite detection and control particularly using biological and cultural methods	1.Manage European Honey Bee 1.1 Bee health	We attended numerous international conferences and workshops to understand varroa research overseas. In particular, the COLOSS workshop was very informative	Appendix 16 Table 3b of overseas conferences
An understanding of how pest control in hives operates within the industry framework both in Australia and overseas, thereby learning from previous international failures in technology uptake.	1.Manage European Honey Bee 1.3 Educating stakeholders	We have written a report on the industry framework in comparison to that overseas, which is included in the final report	Results and Discussion; 3. Overarching framework; Report on the structure of the industry
Improved awareness of alternatives to pesticide control of Varroa mite and the economic advantages of avoiding the development of resistance.	1.Manage European Honey Bee 1.1 Bee health	Our work has provided a framework to target the use of specific monitoring and control techniques that improves their efficacy and thereby provides an economic advantage. The varroa workshop engaged with stakeholders from all aspects of varroa management, improving awareness of this approach	Varroa workshop (Appendix 5-6) Talks at other conferences (Table 3a and b).
Enhance the honey bee industry's defense against Varroa mite, therefore supporting Hort Frontiers Pollination fund Strategic Investment Plan (2020-2025): 1.1 Improving management of European Honey Bee for pollination / Future-proof against exotic pests and diseases	1.Manage European Honey Bee 1.1 Bee health	The project has supported the pollination industry by identifying monitoring and control options for varroa that could be used in Australia. It also has identified a framework in which to use these techniques for greatly efficacy and control	Final report 190

Monitoring and evaluation

Table 8. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
Has the Varroa project, through its horizon scanning process, identified future control techniques that are available and suitable for industry uptake?	Yes, the project has generated extensive new knowledge on future control techniques for Varroa mite management, documented through presentations, workshops, magazine articles, and milestone reports. This information is summarized in Output Table 6.	It is crucial to provide ongoing updates on the latest advancements in Varroa control methods to ensure the industry has access to the most current and effective strategies. Identified gaps in knowledge about Varroa control require further investigation. We plan to address these gaps in a subsequent follow-up project, pending its approval, to continuously enhance Varroa management practices.
Has the Varroa project addressed the needs and concerns of industry, particularly beekeepers, in relation to Varroa mite management? Has the project identified economically and environmentally sustainable varroa management and monitoring tools?	Yes, through an extensive literature review and thorough discussions with core project members, we have identified methods that are well-suited to the Australian context, addressing both the needs and concerns of the industry and beekeepers regarding Varroa mite management. Yes, the project has identified various potential methods tailored to the different stages of Varroa mite infestation and the lifecycle of honeybees. The suitability of these methods for different types of beekeepers, including recreational, sideline, and commercial, has also been assessed, ensuring both economic and environmental sustainability.	Regular engagement with beekeepers to update and refine Varroa management methods based on their experiences and feedback. Continuous evaluation and adaptation of Varroa control strategies to incorporate the latest scientific findings and technological advancements. Development of updated educational resources and training programs tailored to the diverse needs of beekeepers, promoting best practices in Varroa management. Ongoing assessments of the economic viability and environmental sustainability of Varroa management tools to ensure they are cost-effective and eco-friendly. Exploration of emerging technologies and innovative approaches, such as precision apiculture and AI-driven tools, for more efficient and effective Varroa management.
Has the project targeted levels of engagement with industry and Hort innovation been achieved throughout the Varroa project, ensuring their active involvement and input in the project activities and decision-making processes?	Yes, the project successfully engaged with the industry and Hort Innovation throughout its duration, ensuring active involvement and input in all project activities and decision-making processes. Key results were communicated to beekeepers, the wider industry, and stakeholders through various channels, as detailed in Table 6 (Output Table). Regular updates were also provided to Hort Innovation through the quarterly submission of milestone reports. A significant highlight was the organization of a targeted Varroa	Expanding the use of digital platforms, such as webinars and social media, to increase the reach and frequency of project updates and engage a broader audience within the industry. Establishing regular collaborative meetings or forums that bring together researchers, beekeepers, and industry representatives to foster a continuous exchange of ideas and experiences related to Varroa management. Strengthening and extending

	workshop, where project achievements were presented and extensively discussed (details provided in Appendix 6).	partnerships with research institutions and industry bodies to ensure ongoing collaboration and support for future projects and initiatives addressing Varroa mite and other challenges.
Were engagement events undertaken with DPI?	Yes, we maintained regular engagement with the DPI NSW through Ms. Elizabeth Frost. Information was also disseminated to other state DPIs during our PRG meetings and the Varroa workshop.	Broadening the scope of engagement to include more representatives from various state DPIs to enhance regional insights and contributions. Establishing formal feedback channels with DPI representatives to gather and incorporate their insights into project activities and outcomes more effectively. Exploring opportunities for joint initiatives or collaborative projects with DPIs to address shared concerns and leverage collective resources and expertise. We are doing it for our follow-up project.
Has engagement with the PRG been utilized to extend engagement to the industry?	Yes, engagement with the PRG was effectively utilized to extend outreach to the broader industry. Throughout the one-year project duration, we organized two meetings. During these meetings, we shared our research findings and engaged in discussions, while also inviting their feedback and suggestions. Additionally, these findings were disseminated to the wider industry through the Varroa workshop, a combined event that also saw participation from PRG members.	Utilizing interactive online platforms for workshops and meetings to enhance participation and accessibility for all industry members, including those in remote areas.
Did the planned collaboration with New Zealand institute of Plant and Food Research, and Texas A&M University (USA) take place?	Yes, we established fruitful collaborations with the New Zealand Institute of Plant and Food Research (PFR), including working with Dr. James Sainsbury, Ms. Meegan Gee, and Emeritus Professor Mark Goodwin, a former research leader at PFR and an expert in honeybee pollination. In the United States, we collaborated with Associate Professor Julianna Rangel at Texas A&M University.	We have advanced strong relationships with all our collaborators, which we aim to further enhance and extend in our upcoming follow-up project proposal.
What efforts were undertaken by the project to enhance efficiency in its execution and achieve project goals in a streamlined manner?	The team members had clear defined roles that enhanced efficiency, but were also given flexibility in how they achieved their goals. This approach facilitated swift responses to new findings, challenges, and opportunities, ensuring the project remained on the cutting edge and	Organizing cross-industry workshops that bring together stakeholders from related fields to explore interdisciplinary approaches and innovative solutions to common challenges faced in Varroa mite management. Establishing knowledge-sharing

	relevant. A major part of this project was locating and reviewing the large volume of literature associated with varroa management. This was achieved efficiently by using the protocol outlined in methods that streamlined the analysis. Through active and ongoing engagement with stakeholders, including industry partners and research collaborators, we ensured that the project remained aligned with industry needs and benefited from a diverse range of expertise and perspectives. Regular meetings with core team members, alongside updates during PRG meetings and Varroa workshops, ensured effective communication and collaboration. Collaborative tools were employed to keep all involved parties well-informed and actively engaged.	initiatives, such as webinars and online forums, to disseminate project learnings more widely and gather diverse perspectives from beyond the immediate project stakeholders. Working with beekeepers to identify practical applications of our findings.

Recommendations

- Highlighted list of recommendations is provided as a table in SUP 3.
- Make management strategies more effective by developing models to identify thresholds and to model the effect of varroa population dynamics on hive vitality and survivorship (that can incorporate new information as it comes to hand)
- Test techniques developed overseas that reduce varroa rate of increase for their applicability in Australia, including factors like cost and time.
- Engage with industry to identify how to incorporate new approaches into Australian beekeeping.
- Engage with industry while testing technique combinations for varroa management necessary in the chronic stage.
- Monitoring to detect Varroa is quite different from monitoring to manage varroa, and they require different techniques. Identify the sensitivity of current methods to detect varroa within a hive and a region to manage spread. For example: How many varroa need to be present in a hive before it is classified as varroa infested?
- Check the efficacy of current quantifying methods to monitor varroa, such as various forms of mite washes.
- Develop new detection techniques, particularly those that quantify varroa entering hives. Ideally this could be combined with methods to stop varroa entering hives.
- Recognize that varroa infestations go through acute and chronic phases, and that this affects what control methods will work. Develop methods to identify if a hive or apiary is in an acute or chronic phase.
- The acute phase requires techniques that kill mites once they enter hives or stop mites entering hives. Research biological techniques that can remain in hives for many days that can kill mites that have entered hives. If these have low efficacy, they could still be effective in the Chronic phase.
- There are currently very few options to stop mites entering hives. Research identifying how varroa are entering hives would assist in developing techniques to stop varroa entering hives. Research to stop varroa entering hives

is critical.

- Use the charts on control techniques to identify new non-chemical control methods for future development.
- Test for the presence of a Varroa Sensitive Hygiene marker gene on queens, that when present on New Zealand queens affords that hive 30% greater resistance to varroa. Test if this finding is transferable to Australia.

Refereed scientific publications

None to date. Manuscripts in preparation.

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Intellectual property

Because we have collected information that may not be in the public space, we need to protect the IP of third parties with which we were engaging. We have developed non-disclosure agreements for this purpose. But as to date we do not have any project IP or commercialization to report.

Acknowledgements

The authors wish to express their gratitude to all the beekeepers who participated in the online survey. Their responses have provided valuable initial insights into beekeepers' perceptions of the Varroa mite and their attitudes towards the use of chemicals for its treatment, as well as their overall satisfaction within the Australian context.

Additionally, the authors extend their thanks to all the members of the Project Reference Group for their dedication in attending meetings, both online and in person. Their feedback and suggestions have been instrumental in shaping the work conducted in this project. We are particularly grateful to Steve Fuller and Danny Le Feuvre. We would also like to thank the beekeeping industry for engaging in this project and for providing sage advice.

Appendices

Tables in Appendices

Supplementary files.

Sup. 1	Chart of Varroa Mite Monitoring and Detection Methods
Sup. 2	Charts of Varroa Mite Control methods
Sup. 3	New Potential Monitoring and Control Charts

Appendix 1 – the protocol to compile the literature review.

To compile data, an initial assembly was undertaken by a specialist affiliated with Plant and Food Research New Zealand (PFR), which was later refined by Dr. Yousuf. All the collected data were systematically organized within an EndNote library. The literature search was conducted using a multifaceted strategy, as detailed below:

Web of Science (WoS) All Databases: Search 1: A broad search using the keywords "mite*" combined with either "control*" or "treatment*" yielded 19,503 results, which were not individually verified.

Search 2: The terms "ectoparasite*" and "mite*" along with "control*" or "treatment*" produced 9,670 unverified results.

Search 3: A proximity search ("ectoparasite* near/3 mite*") combined with "control*" or "treatment*" narrowed the results down to 196.

Search 4: Focusing on the last five years, "ectoparasite*", "mite*", and "control*" or "treatment*" provided 796 results, with 117 examined in detail.

Search 5: A refined search for reviews using "acaricid*", "miticid*", or "ectoparasite* and mite*" with "control*" or "treatment*" and "review*" yielded 740 results, which were checked and saved.

Search 6: Another five-year-oriented search using the same keywords resulted in 3,033 entries. Exclusions were made to omit irrelevant research areas, resulting in 1,185 entries that were thoroughly checked.

Excluded research areas: Search 7: Keywords related to Varroa mite monitoring and economic aspects were searched, producing 784 results, with 302 selected for closer examination.

Public Environmental Occupational Health or Genetics Heredity or Science Technology Other Topics or Food Science Technology or Nutrition Dietetics or Anatomy Morphology or Forestry or Business Economics or Engineering or Biophysics or Health Care Sciences Services or Anthropology or History or Sociology or Construction Building Technology or Surgery or Fisheries or Education Educational Research or Physics or Obstetrics Gynecology or Government Law or Energy Fuels or Dentistry Oral Surgery Medicine or Information Science Library Science or Geriatrics Gerontology or Cardiovascular System Cardiology or Respiratory System or Hematology or Psychology or Anesthesiology or Marine Freshwater Biology or Medical Laboratory Technology or Water Resources or Neurosciences Neurology or Mathematics or Mathematical Computational Biology or Computer Science or Pediatrics or Endocrinology Metabolism or Materials Science or Instruments Instrumentation or Geography or Urology Nephrology or Allergy or Automation Control Systems or Polymer Science or Public Administration or International Relations or Communication or Family Studies or Social Issues or Acoustics or Architecture or Area Studies or Critical Care Medicine or Emergency Medicine or Film Radio Television or History Philosophy Of Science or Legal Medicine or Nursing or Otorhinolaryngology or Paleontology or Philosophy or Psychiatry or Radiology Nuclear Medicine Medical Imaging or Thermodynamics or Archaeology or Development Studies or Electrochemistry or Geochemistry Geophysics or Geology or Medical Informatics or Optics or Orthopedics or Rheumatology or Social Sciences Other Topics or Women S Studies.

CAB Abstracts and Other Databases: For Varroa control, treatment, and management, CAB Abstracts yielded 419 results, WoS provided 574, and CCC contributed 415 results, although the latter could not be downloaded.

SciFinder: Searches related to novel Varroa mite control strategies exceeded 1,000,000 hits, with "mite treatment and control" within the last five years leading to 14,997 entries. A thorough review of 1,300 of these entries resulted in 51 selections.

USDA Publications: A query for Varroa-related publications resulted in 115 entries, with the first 60 (up until 2018) being reviewed and 18 selected.

Google Scholar and Google: A series of searches for novel controls, treatments, and detection methods related to Varroa mites since 2019 and from 2018 to 2023 were conducted, resulting in several pages of results being reviewed and a final selection of articles made after removing duplicates.

Russian Citation Index and World Wide Science: Exploring Varroa-related literature from 2018 to 2023 in the Russian Citation Index led to 671 results, with the first 300 reviewed and 65 selected after deduplication. Worldwide Science yielded 849 deduplicated entries, with 200 reviewed, all of which were already included in the library.

Open Science and Europe PMC: Searches in these databases for Varroa-related literature from 2018 to 2023 resulted in a small number of selected, highly relevant articles after reviewing the first few hundred results and removing duplicates.

Additional Searches: In June 2023, further searches were conducted in WoS for literature connecting Wolbachia with Varroa, bees, mites, or pseudoscorpions, resulting in 437 results, with 310 selected. Searches for Varroa and "formic acid*" over the last five years in WoS and Google Scholar were also performed, yielding a select few after removing duplicates.

Note: Until 20 January 2024, additional research efforts were directed towards understanding various aspects of biological, genetic, physical, biotechnological, and biopesticide control mechanisms for Varroa mites. This endeavor resulted in the identification of 141 records, out of which 89 were selected for their relevance to Varroa control. This phase of the research extended the investigation period beyond the initial five-year timeframe to incorporate seminal works and foundational research data.

Appendix 2 – Data gathering methods

EndNote Library Review: This phase involved a systematic review of literature compiled in the EndNote library, conducted by Dr. Yousuf. The inclusion criteria for literature were relevance to Varroa mite control, peer-reviewed status, and publication date within the last 5 years. Exclusion criteria included non-peer-reviewed articles and those not directly related to the control methods of interest. The literature was then categorized into five predefined areas: Monitoring and Detection, Cultural and Mechanical Control, Biological Control, Genetic Control, and Bio-chemical Control, based on the content's primary focus.

Beekeeper Survey: A survey was designed to capture Australian beekeepers' perceptions of the Varroa mite problem. The survey included both closed and open-ended questions (Appendix 3), covering aspects such as awareness, impact, control practices, and barriers to effective management. The survey was distributed electronically via beekeeping associations and social media platforms using QR code, with efforts made to ensure a representative sample. Ethical considerations, including informed consent and anonymity, were addressed in the survey introduction.

Direct Engagements: Structured interviews were conducted with researchers and industry partners during conferences, meetings, and industry gatherings, focusing on current practices, challenges, and innovative solutions in combating Varroa mites. Participants were purposively selected based on their expertise and involvement in Varroa mite control efforts. We also held informal discussions with researchers, industry leaders, and politicians about varroa management and the varroa incursion.

Appendix 3

Beekeeper's survey questionnaire: Page 1

Beekeeper Survey Varroa IPM in Australia

Dr Mary Whitehouse
Senior Research Scientist
Applied Bioscience, **Macquarie University**
Mary.whitehouse@mq.edu.au
Office: +61 2 9850 8143

Complete this form and leave it on the table.

Use the **QR code** to access the online version.

Just 5 min of your time can help save our bee future.

This survey would help researchers develop the best possible varroa control strategy for Australian beekeepers. **We will not share your details with any other organisation.**

1. **What is your age:**
   - ☐ <30
   - ☐ 31-45
   - ☐ 46-60
   - ☐ 60+

2. **Gender?**
   - ☐ Male
   - ☐ Female
   - ☐ Other

3. **Where are you based (to understand local climate limitations)? Do you move your hives during winter?**

4. **Do you have organic certification?**
   - ☐ Yes
   - ☐ No

5. **How many hives do you manage?**

6. **Are you a: Recreational** or **Sideliner** or **Commercial beekeeper? (Please circle)**

7. **How many times a year do you visit the hives for beehive management.**

8. **What is the main purpose of your visits?**
   - ☐ 1. To ensure the queen bee is healthy and productive
   - ☐ 2. To check the health of the colony. Any pests or diseases?
   - ☐ 3. To check honey production
   - ☐ 4. Swarm prevention
   - ☐ 5. Hives maintenance
   - ☐ 6. To ensure the bees have enough food resources
   - ☐ 7. Nutrient supplement (e.g. Sugar syrup)
   - ☐ 8. Requeening

Beekeeper's survey questionaire: Page 2:

☐ 9. All of the above

9. **Which of the hive-jobs above are you comfortable combining on your visits (indicate using the numbers above)?**

10. **When are drone broods present in your hives?**

11. **If you had to make an extra trip to your bees for varroa control, approximately how much would it cost ($) (taking into consideration travel / time / potentially extra labour)?**

12. **Have you developed a strategy or action plan for addressing varroa mites if they were found in your hives? If yes, could you briefly describe your planned approach?**

13. **What would you like to know about varroa control?**

14. **Would you be comfortable sharing your contact details?**

Please note, this information will only be used for research purposes and will not be shared with any other organisations or individuals.

If so, please provide:
Name: _____
Email: _____
Phone: _____

Beekeeper Survey

https://www.surveymonkey.com/r/87CHC3N

Appendix 4
The first Project Reference group meeting.
Held on 16 August 2023. (8. pages)

Page 1

Table I: Project Reference Team Members

Name	Role and Organization
Dr Mary Whitehouse	Project Leader, Macquarie University
Dr Fazila Yousuf	Project Delegate (Postdoctoral research fellow), Macquarie University
Dr Francesco Stolfi	Team member, Macquarie University
Dr James Sainsbury	Team member, NZ Institute of Plant and Food Research
Dr Mark Goodwin	Team member, NZ Institute of Plant and Food Research
Ms Megan Gee	Team member, NZ Institute of Plant and Food Research
Dr Mark Harvey	Team member, Western Australian Museum
Dr Juliana Rangel	Team member, Texas A&M University, USA
Ms Elizabeth Frost	Team member, NSW DPI
Mr Danny Le Feuvre	PRG_CEO of AHBIC
Mr Steve Fuller	PRG_ Commercial Beekeeper (NSW) and past NSW Apiarists' Association president
Mr Neil Bingley	PRG_ President of NSW Apiarists' Association Inc.
Ms Shannon Mulholland	PRG_ Biosecurity Officer, NSW DPI
Dr Leigh Pilkington	PRG_ Director emergency Management, Biosecurity and food safety, NSW DPI
Dr Cameron Jack	PRG_ Varroa IPM Expert-University of Florida
Ms Kellie-Ann Robinson	PRG_ Owner of 'The Thriving Hive-Urban Beekeeper'
Mr Lindsay Bourke	PRG_CEO of Tasmania Beekeeper's Association
Mr Michael Finey	PRG_ Extension Officer, Department of Industry, Tourism and Trade, NT
Mr Tim Preusker	PRG_ Property Manager-CMV Farm Keiths Grove
Mr Onyeka Nzie	R&D Manager-Hort Innovation

First Project Reference group meeting

Date: Wednesday 16 August 2023 (10-11am AEST)
Meeting chair: Dr Fazila Yousuf

Purpose: First PRG (Project Reference Group) meeting for the Hort Innovation funded project 'Exploration of Advanced Control and Detection methods for Varroa mite (PH22002).

Apologies for Absence

- **Team members:** Elizabeth Frost, James Sainsbury, Phil Taylor, Maciej Maselko, Fei Liu, Megan Gee
- **PRG members:** Neil Bingley, Leigh Pilkington

Meeting attendees

- **Team members:** Mary Whitehouse, Francesco Stolfi, Juliana Rangel, Mark Goodwin, Mark Harvey
- **PRG members:** Onyeka Nzie, Danny Le Feuvre, Cameron Jack, Lindsay Bourke, Steve Fuller, Shannon Mulholland, Kellie-Ann Robinson, Michael Finey, Tim Preusker

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Meeting chair started the meeting with

Meeting Agenda:

Introduction of core team members.

Introduction of PRG members.

Overview of the updated "terms of reference" document.

Discussion on the project background and objectives.

Updates on what the project has achieved in the last three months.

Q&A or suggestions session.

Chair requested team members and PRG members to introduce themselves.

Mary Whitehouse: Project leader, senior research scientist at Macquarie University, has background in behavioural Ecology and for the last 20 years she has been working on integrated pest management in cotton where this technique of combining different control methods has been extremely effective.

Fazila Yousuf (Chair): Postdoctoral research fellow with a background in IPM, her main focus and interest are in biological control.

Francesco Stolfi: Senior lecturer at Macquarie University, involved in analysing the institutional structure of the honeybee industry. With regards to Varroa issue of surveillance and condition that facilitate the adoption of IPM for varroa. Has spoken to beekeeper's associations, biosecurity officers and other actors involved with Varroa in Australia.

Juliana Rangel: Professor of Apiculture, Department of Entomology, Texas A&M University. Her research program focuses on the biological and environmental factors that affect the reproductive

First Project Reference group meeting

quality of honeybee queens and drones, the behavioral ecology and population genetics of unmanaged honeybees.

Elizabeth Frost: Works for DPI NSW in Tocal, has a background in bee husbandry. She is involved in National Honeybee Genetics Improvement Program and the NSW Varroa Mite Emergency Response. Her role in this project is to bridge our team with the beekeeping industry and do reality check.

James Sainsbury: Team leader at New Zealand Plant and Food Research in the bee biology department. Renowned for translating research into practical applications. His role is to provide strategic guidance and play a key role in shaping the project's outcomes and supporting Postdoc Research fellow.

Mark Goodwin: He used to be the research team leader New Zealand Plant and Food Research for the 30 years. He has experience with honeybee topics, dealt with various bee diseases, and he is a pollination biologist. He was involved in both the incursions of varroa mite in the North Island and the South Island of New Zealand.

Phil Taylor: Head of Applied Bioscience at Macquarie University. He is an expert in sustainable pest management. His role is to offer institutional support and guidance for this project.

Maciej Maselko: Leads a synthetic biology research group at Macquarie University, an expert in insect genetic engineering, especially CRISPR methodologies. And his role in this project will be reviewing the genetic modifications we are considering for biological control in this project.

Fei Liu: Organic chemist with a passion for the intersection of chemistry and biology. She has a strong background in synthetic chemistry and chemical proteomics. She collaborates with Australian proteomic analysis facility at Macquarie University and her role in this project would be to land her expertise to evaluate methods targeting chemical communications in bees and varroa mites.

Mark Harvey: Curator of arachnids at the Western Australian Museum, specializes in pseudoscorpions.

Megan Gee: Also, from New Zealand Plant and Food Research. She has compiled all data related to varroa mite control in EndNote library.

Onyeka Nzie: He is an R&D manager for this project at Hort Innovation. He was very excited to see a lot of people with a range of expertise. Very happy with this research and support that we are getting ready should varroa become a problem here in Australia.

Danny Le Feuvre: He works for AHBIC, the national representative body for the honeybee industry. He is a commercial beekeeper for 15 years and prior to that, he was a research economist in broad acre cropping, so very familiar with IPM options and chemical usage.

Cameron Jack: He is an Assistant professor at the University of Florida. He has been researching for about a decade on honeybee and Varroa and specifically his research is related to varroa control biology.

Lindsay Bourke: He is the President of the Tasmania beekeepers for the last 12 years. He was a chairman of AHBIC for seven years. And he was there in the old days when Mark Goodwin came to Australia many times. He attended 19 of his lectures to tell us how they are going to stop varroa in South Island in New Zealand. Unfortunately, they weren't successful. But he said that we will try and help in Australia.

First Project Reference group meeting

Neil Bingley: He is the current president of New South Wales Apiarists' Association.

Steve Fuller: He is the commercial beekeeper and president of the crop pollination association Australia. He is also a honeybee and pollination panel member for AHBIC and Agrifutures.

Leigh Pilkington: He is the director of emergency Management, Biosecurity and food safety at NSW DPI.

Shannon Mulholland: Her substantive role is in DPI NSW in plant biosecurity preparedness team. So, she spent most of her time preparing for incursions and then responding to incursions. So, at the moment she is the state deputy incident controller for varroa mite. She is interested to see what the research projects are investigating and delivering and seeing if there's any way that DPI NSW can incorporate those immediate methods into the response or in a preparedness capacity. If the response is unsuccessful in eradication, what other options DPI has for transitioning to management. So, she was appreciative of being involved in this project.

Kellie-Ann Robinson: She is an urban beekeeper. She supports quite a lot of hobbyist beekeepers and have some connections with some of the local clubs, Queensland based. She was interested in how pest management plays out for recreational beekeepers.

Michael Finey: He is an extension officer for the Northern Territory Department of Industry, Tourism and Trade. He has previously worked in biosecurity roles, and familiar with IPM from a few different perspectives. So, he was happy to provide this project any support from his end.

Tim Preusker: He is the property manager, and he manages CMV almond orchards. His involvement is relevant because almond is the main industry where honeybees are used for pollination.

Meeting chair discussed and went through the updated PRG terms of reference document. Updated version of ToR is attached with meeting summary note.

Project Background: Mary Whitehouse has given project background "So as everybody is aware, Australia is the last Varroa free beekeeping continent. But recently we have had an incursion. And although we are still aiming to eradicate this incursion, there is a possibility that at some stage varroa will arrive in Australia. So our aim is to help Australia to have the best control and monitoring techniques in place. Now overseas, control methods have been largely focused on pesticides that might be the major cause varroa resistance to chemical control. And so now people are looking more at alternative methods, sustainable biological cultural control methods. But these are more complex and more difficult to undertake. They also require quite a bit of integration of using different techniques and monitoring carefully to make sure to understand how effective your techniques have been. So one way to actually help with great different techniques is to use an integrated pest measurement approach. And that's why it's really great that we've got people both around in New Zealand and the U.S. who have had experience with these techniques overseas. And also within our group here who cannot have someone background in this technique of how we could use it. Now the an effective IPM supports biological control methods as well as detection really requires industry support. So working closely with industry is vital for this work and making sure that what we are actually doing is relevant to the different parts of the industry. So when we're developing a system and developing tools, we're not only taking into consideration the different types of beekeepers, because not all tools will be relevant for all beekeepers, which is why in our group we've got commercial beekeepers. I think we've got part-timers as well or side liners and also hobbyists because different techniques will be relevant for different groups. We need to be considerable, consider the different types of climates and habitats that we're looking at beekeeping within

First Project Reference group meeting

Australia. So we have people from all over Australia as part of our group to try and cover that off as well. We also need to make sure that we're looking at trying to attack the varroa mites at different stages. So there's the stages when it's moving around, when it's on the bees and when it's in the brood chamber. Getting it when it's in the brood chamber is the hardest one. But we need to keep those three areas of life cycle in mind when we're looking at these different monitoring control techniques to try and make sure we're capturing different things. So our approach will not be a one-size-fits-all, it will be taking into all these different considerations. And we are in an ideal position because we can learn from our people involved who have come from other countries and really know what they're talking about. So that's a background on where we're going. We're in terms of the actual techniques. We're trying to get the most recent ones and what's just on the horizon and also how they can be modified for the Australian systems."

Project objectives: Meeting Chair discussed project objectives.

1. Do horizon scan to identify emerging trends, potential future developments, and novel approaches.
2. Review globally the status and availability of non-chemical control methods and how they would operate within an IPM framework within Australia.
3. Identify and understand innovations in Varroa detection technology. Early detection is critical for an effective IPM approach to Varroa mite management.
4. Review previous failures in technology uptake, building on existing knowledge. There are two parts to this aim.
    (i) identify what characteristics of the control method reduced or enhanced its uptake.
    (ii) identify how industry structure and culture helped or hindered Varroa mite control.
5. Develop a shortlist of biological and cultural control methods and systems to be studied further and identify what work would be required to improve their fit in Australia.

Meeting chair has given updates on completed activities of the project.

"So, since the project started on 19th of April, we have managed to attend all the beekeeper's conferences in all the different states. Also attended and presented in an international ApiNZ symposium and conference in New Zealand.

Juliana has also attended some international conferences in USA and going to attend one in Chile.

The purpose of attending all these meetings and presenting over work is to understand what the problems beekeepers are are facing and how we can use this information to develop our plan in Australia.

Talked with the beekeepers, researchers, and industry people about bee industry and potential varroa mite threat. Designed three surveys (Beekeeper's, Researcher's, and Industry surveys).

So far, we have completed 50 beekeeper's surveys, but we are looking for more survey's to be completed.

We have also completed some Researcher's surveys.

In addition, we have gathered around 2,000 papers related to honeybees and the Varroa destructor. For now, our focus has been primarily on research from the past five years, although we might expand our scope if necessary. Our collection includes peer-reviewed research articles, reports, and

factsheets. The main thrust of our research will be to identify the various control methods/techniques and to determine why some were successful while others were not. Based on this existing knowledge, we aim to develop or propose modified ideas and/or improvements.

Currently, literature review is underway. Focus is one biological control. Broadly there are five categories into which our research will be divided, and biological control is one of them.

Then the second research category would be to look at different genetics' options in honeybee and varroa control.

Last Question and Answer session:

Chair open the floor for Q&A and suggestions.

Mary Whitehouse talked about next PRG meeting would be the part of a workshop which would be held on 22-23 January 2023. Meeting confirmation and invitation will be sent out in November 2023.

In the next meeting different categories of varroa monitoring and control will be discussed. Attendees can either attend the meeting virtually or in person.

For virtual attendees we will set up 'Virtual Sheldon' (From Big Bang Theory https://www.youtube.com/watch?v=WjWfur9at2s&ab_channel=TBS). Where each person will be on a separate computer and will have a physical presence in the room on a monitor. The monitor can be moved around so that people can go off and talk in groups and discuss things and then come back.

Mary Whitehouse is looking forward to the meeting and she thinks that we have a nice diverse group where we should get some really interesting ideas out of it.

We should get some really interesting ideas out of it.

Mark Goodwin suggested if we could do the meeting in New Zealand.

Mary Whitehouse replied that it is a good idea, but budget would be our main constrain. But she said that we can have a look at that idea.

Onyeka Nzie asked the question about the biological control that the **research fellow** has already done "what are the major findings you found and what's the progress like in terms of being able to control varroa mite?."

Research fellow/Chair replied that she hasn'y completed the biological control category. She is working on it currently. She mentioned that her understanding so far is that biological control agents work well in controlled environments but when it comes to field the success rate is not very high. That is mainly because of the high (33-35˚C) within hive temperature. But there is growing research that how we can artificially stress some of the potential microorganisms so they can tolerate hive conditions well. Ofcourse there are several steps involved in optimizing the control agents. There is a lot of potential in this category.

And when it comes to Australia, we have all these different geographical regions and we have to see that which agent is suitable because some might work in temperate regions and others might not.

It's very interesting, and of course, and it's a softer option as compared to chemicals and it can be the part of integrated pest management system. Biological control on its own might not be enough but can work really well in combination with other controls methods.

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Mark Goodwin mentioned that it's always going to be a challenge to find things. He said that they even taught our beekeepers, they got varroa, they did two day workshops for commercial beekeepers trying to teach IPM. But it wasn't succesful. He further mentioned that if we did a survey around the world at the moment, it's probably 99% chemical control for varroa and that's the real challenge here, that only 1% into something usable.

Research fellow/Chair said "That's right. I totally agree with you, Mark, but the thing is this that I think we have some opportunity in Australia because here varroa is not yet established. So, we still have that opportunity to incorporate some of these softer control methods and if the beekeepers start using them at the very initial stage, then, you know, we can minimize the use of chemicals. So there is a hope for some of these methods.

Mary Whitehouse "Yeah, that's quite interesting. Thank you for that. I think, it important to include that what's empowering some of the control methods or what's stopping them from being using what further research can be done. I think it's a good thing. We have people from USA who have also tried biological control and it's not working. Maybe they can give us insight for their insight as to why it's not working.

Research fellow/Chair said that we are only at the early stages and got more exploring to do in this category. There could be some benefit in looking into other mites if time permitted.

Mark Goodwin said that the threshold has been lowering over time in terms of number of mites that need to be in the hives and the number of mites that you can tolerate. Which is interesting and scary**.** Once the deformed wing virus in New Zealand.

Mary Whitehouse so you think that was the thing that triggered the drop, not something else?

Mark Goodwin As far as we know, but other countries are reporting colony death at much lower levels than what we've got here even. So, we've got some viruses or something else in play.

Juliana Rangel mentioned that some interesting studies looking at the epidemiology of the deformed wing virus and certain strains are kind of taking over other ones. So, there's the wing virus A, B and C, probably others. And there's some areas where they are seeing more of one strain over the other. And it seems to be at least in some studies correlated with Varroa levels. But yeah, in terms of Varroa being more or less, let's say virulent, but it's not a virus, but more prevalent. Not so much except for people who are studying genetic lines of bees that are more behaviourally adept to combating Varroa. And of course, they are seeing more and more my populations becoming resistant to Amitras, which is like our number one line of defense at the moment. So that's kind of scary.

But I know **Cameron** and other people in the US are working hard to develop or test new chemical products that can, hopefully, be used even when varroa become more tolerant or resistant to Amitras.

Danny Le Feuvre said that "Just to put some context around it. So, there is another big project happening, Mark G, which is an AgriFutures project with a different group of researchers and for our experts that are looking at global chemical usage and organic and synthetic as a first line of defense and looking at use patterns and how that's working in resistance. So, this project (Hort Innovation project) is really focused on alternative approaches IPM, non-chemical, technological, cultural, mechanical approaches to varroa knowing that we do have this other project that is going to deep dive on the chemical both synthetic and not. So, it's about not duplicating the work that we've got going on and using this project to complement that other project that is occurring. So, this project will have, in any IPM program, chemicals will be involved. But what we don't need is this project to

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deep dive on the chemical usage and patterns because that's being done by a separate project. So, we're not going to have out of this project the Bible for varroa control, right? This is going to be one piece of the puzzle that we put together with another project and probably there's a whole suite of projects happening in this space now. And so, that'll be a case of putting all these projects together, making sure it ground truths and works for that geographic location and putting together best practice for that. So, the aim out of this project is not to have the single best practice document, but it is to deep dive on those non-chemical IPM strategies to see how we can use that to feed into the bigger picture.

Overall meeting summary from Q&A session:

The meeting revolved around research efforts to combat the Varroa destructor mite, a significant threat to honeybees. The primary focus is on non-chemical control methods, although globally, chemical control remains dominant. In controlled settings, biological control agents show promise with >70% efficacy, but their performance drops in the field, highlighting the need for adaptation. Australia's unique position without established Varroa colonies offers a potential testing ground for some of these alternative control's options. Despite the emphasis on non-chemical methods, chemicals will still play a part in integrated pest management.

Other projects are also ongoing which are focussing on chemical control options. So it is important not to duplicate efforts.

Appendix 5
Varroa workshop: Overview, agenda and attendees

Within this project, we engaged with the pollination industry and apiarists to ensure the developed methods were well-received and adopted. Our engagement included representatives from all Australian states; the pollination industry; international experts; both commercial, sideliners, and recreational beekeepers; biosecurity officers; extension officers; and legislators to get comprehensive feedback on the compiled control and monitoring techniques as detailed in (Sup. 1-2). These discussions were aimed at gauging the industry's receptiveness to various control methods and identifying opportunities for enhancement.

The Varroa workshop served as a key platform for broader engagement, inviting participants from diverse organizations and departments involved in honeybee management across Australia, as well as international experts from the USA and New Zealand. The comprehensive list of participants and details of the Varroa workshop is available in (Table II). This inclusive approach ensured a rich exchange of ideas and fostered collaborative relationships, essential for the successful implementation and adoption of Varroa control methods within the industry.

Day 1: The focus was on reviewing the list of potential control and detection methods. Dr. Maciej Maselko, A/Prof. Juliana Rangel and Dr. James Sainsbury played crucial roles in assessing the scientific feasibility of developing these methods in future projects, particularly those involving molecular approaches. This day also served to identify gaps in the current control options and assess the potential for further research to address these gaps.

Day 2: Key industry figures, including Danny Le Feuvre, Steve Fuller, Neil Bingley, and many other key people were invited to provide their insights on the proposed research methods and their applicability within industry constraints and structural limitations. Dr. Francesco Stolfi presented a comparison between the Australian industry structure and the USA and New Zealand, highlighting potential challenges within an IPM framework. The group engaged in discussions about the control and detection methods in the context of the industry's structure, aiming to pinpoint areas for future research.

The workshop concluded with researchers and industry representatives identifying immediate and long-term research initiatives. A shortlist of promising control methods and detection techniques was developed, considering their efficacy for various Australian beekeeping communities and the need for further development. Additionally, the workshop aimed to outline the requirements for a comprehensive IPM plan to prepare the Australian beekeeping industry for potential Varroa mite establishment.

Varroa workshop: Agenda and Attendees

Varroa Workshop Monday 22nd January 2024

Terminology	
Attendees	-People physically at the conference
Virtual Sheldons	-People attending remotely via a laptop
Partners	-People assisting Sheldons: pushing Sheldons connecting them to power, making sure their voice is heard
Participants	-Everyone at the workshop

Program	
9:00-9:45	Partners Tea, Coffee, Biscuits available
9:45-10:00	Welcome and Introduction Participants divided into 2 groups - choose a facilitator
10:00-10:50	Generating ideas for targetted Charts: Biological Control, Physical/Cultural Control, Detection Methods
10:50-11:00	Quick morning tea - muffins
11:00-11:50	Generating ideas for targetted Charts: Biological Control, Physical/Cultural Control, Detection Methods
11:50-12:30	Review Genetic Control and BioChemical Control charts
12:30-1:30	Lunch
1:30-2:30	Change groups, review the A3 ideas for the 3 targeted Charts
2:30-3:10	Industry structure - Francesco's talk -Focus on Detection
3:10-3:30	Afternoon tea
3:30-4:40	Reviewing ideas - final ranks of A3 ideas per targeted chart
6:00	Dinner

Day 1. 22nd Jan 2024 - Purpose: The scientists involved in the project reviewed the monitoring and management techniques, excluding those involving synthetic chemistry and focusing on those using biological, physical, and cultural control methods. The aim was to identify existing gaps and determine what could be further developed for Australian conditions.

Varroa Workshop Tuesday 23rd January 2024

Terminology	
Attendees	-People physically at the conference
Virtual Sheldons	-People attending remotely via a laptop
Partners	-People assisting Sheldons: pushing Sheldons, connecting them to power, making sure their voice is heard
Participants	-Everyone at the workshop

Program	
9:00-10:00	Meeting People, setting up Sheldons Tea, Coffee, Biscuits available
10:00-10:300	Participants introduce themselves
10:20-10:35	Mary -Welcome and Introduction
10:35-10:45	Fazila- Background on the charts
10:45-11:00	Morning tea
11:00-11:15	Francesco: Industry Structure
11:15-12:15	Reviewing Ideas: Genetic Control Biochemical Control
12:15-1:00	Lunch
1:00-2:45	Generating ideas for targetted Charts: Biological Control, Physical/Cultural Control, Detection Methods
2:45-3:00	Afternoon tea
3:00-3:30	General discussion on managing varroa - where the industry feels the challenges lie
6:00	Dinner Lachlan's restaurant, 99 Talavera Road in the MGSM Executive Hotel

Day 2. 23rd Jan 2024 - Purpose: The Project Reference Group and stakeholders collaborated with the scientists to critically review and further discuss the monitoring and detection, and management techniques (biological, physical, genetic, and biochemical control).

Varroa workshop: Attendees

The list of all attendees representing different states in Australia and international researchers from New Zealand and the USA. Workshop Day 1: Team members and Day 2: Team members, PRG members and Federal government representative.

Table II: List of attendees-Varroa workshop

Name	Role and Organization	Attendance	State, Country
Dr Mary Whitehouse	Project Leader, Macquarie University	Day 1 & 2 (in Person)	NSW, Australia
Dr Fazila Yousuf	Project Delegate (Postdoctoral research fellow), Macquarie University	Day 1 & 2 (in Person)	NSW, Australia
Dr James Sainsbury	Team member, NZ Institute of Plant and Food Research	Day 1 & 2 (in Person)	Hamilton, New Zealand
Ms Elizabeth Frost	Team member, Tocal Agriculture college, NSW DPI	Day 1 & 2 (in Person)	NSW, Australia
Dr Juliana Rangel	Team member, Texas A&M University, USA	Day 1 & 2 (in Person)	Texas, USA
Dr Mark Harvey	Team member, Western Australian Museum	Day 1 & 2 (Online)	WA, Australia
Dr Mark Goodwin	Team member, NZ Institute of Plant and Food Research	Day 1 & 2 (Online)	Hamilton, New Zealand
Dr Maciej Maselko	Team member, Macquarie University Team of leader of sister project on honeybees PH22000	Day 1 & 2 (in Person)	NSW, Australia
Dr Soo Jean Park	Chemical ecologist, Macquarie University	Day 1 & 2 (in Person)	NSW, Australia
Dr Anu Jayaweera	Postdoc in a sister project (PH22000) on honeybees with Dr Maciej Maselko.	Day 1 & 2 (in Person)	NSW, Australia
Ms Megan Gee	Team member, NZ Institute of Plant and Food Research	Day 1 (Online)	Hamilton, New Zealand
Dr Francesco Stolfi	Team member, Macquarie University	Day 2 (Online)	NSW, Australia
Dr Michelle Taylor	Team Member, NZ Institute of Plant and Food Research	Day 2 (Online)	Hamilton, New Zealand
Ms Ashley Zamek	R&D Manager-Horticulture Australia	Day 2 (in Person)	NSW, Australia

Mr Danny Le Feuvre	PRG_CEO of AHBIC	Day 2 (in Person)	SA, Australia
Mr Steve Fuller	PRG_ Commercial Beekeeper (NSW) and past NSW Apiarists' Association president	Day 2 (in Person)	NSW, Australia
Mr Neil Bingley	PRG_ President of NSW Apiarists' Association Inc.	Day 2 (in Person)	ACT, Australia
Dr Cameron Jack	PRG_ Varroa IPM Expert-University of Florida	Day 2 (Online)	Florida, USA
Ms Kellie-Ann Robinson	PRG_ Owner of 'The Thriving Hive-Urban Beekeeper'	Day 2 (Online)	QLD, Australia
Mr Lindsay Bourke	PRG_CEO of Tasmania Beekeeper's Association	Day 2 (Online)	Tas, Australia
Mr Michael Finey	PRG_ Extension Officer, Department of Industry, Tourism and Trade, NT	Day 2 (Online)	NT, Australia
Mr Tim Preusker	PRG_ Property Manager-CMV Farm Keiths Grove	Day 2 (Online)	Vic, Australia
Ms Tara Needham	Department of Agriculture, Fisheries and Forestry, Biosecurity, Canberra	Day 2 (in Person)	ACT, Australia
Ms Zoe Nix	Department of Agriculture, Fisheries and Forestry, Biosecurity, Canberra	Day 2 (Online)	ACT, Australia

Note: Workshop details are given in the report below (Appendix 6).

Appendix 6

Varroa workshop: Report

On Monday and Tuesday, the 22nd and 23rd of January, we held a workshop as part of our Hort Frontiers project titled "Exploration of advanced control and detection methods for Varroa mite". The aim of the workshop was to review currently used and researched monitoring and management techniques, excluding those involving synthetic chemistry and focusing on those using biological, physical and cultural control methods.

On Day 1 the scientists involved in the project reviewed the techniques, while on Day 2 the Project Reference Group joined the scientists to critically review the techniques. In all there were 24 people involved in the workshop, including 10 online. To encourage engagement with the participants online, we trialled a novel approach in which each online participant joined the meeting with their own separate zoom link. This enabled them to engage with others at the meeting independently and have their own physical presence in the room. On Day 1, when we had only four people online, this was effective (Figure 1,2) but on Day 2, when there were nine virtual people, they had too much difficultly hearing each other as autonomous units, so they were combined into one zoom meeting. Unfortunately, this made it harder for some of that group to be heard. Enabling virtual people at meetings to be autonomous helps them to contribute; but unfortunately, the technology, at an affordable price, isn't quite there yet.

Figure 4. Physical and virtual participants at the varroa workshop on Day 1.

Figure 5. Dr James Sainsbury (New Zealand Plant and Food Research) interacting with Dr Mark Harvey (Western Australian Museum) who attended the meeting virtually.

Figure 6. Dr Mary Whitehouse (Project leader, Macquarie University) leads discussion on the monitoring and detection chart with the project's core research team.

The participants on Day 2 included a diverse array of people from every state and territory and from all aspects of the bee keeping industry (Figure 4). For example there were researchers from universities, state, and museum research institutions both from Australia and overseas (including three people from New Zealand and two from the United States); commercial, slideliner and recreational (hobbyist) beekeepers; beekeeping industry leaders, both national and state; representatives from state government bodies and from a federal regulatory body (the Department of Agriculture, Fisheries and Forestry); a representative from the pollination industries and from Hort Innovation. This ensured that the workshop captured the perspectives of all aspects of beekeeping with respect to varroa control methods.

Figure 7. Physical and virtual participants (on laptop) at the varroa workshop on Day 2

Top (L-R): Dr James Sainsbury (New Zealand Plant and Food Research), Ms Elizabeth Frost (DPI, NSW), A/Prof Juliana Rangel (Texas A&M University, USA), Dr Mary Whitehouse (Project leader, Macquarie University).
Bottom (L-R): Mr Neil Bingley (President of NSW Apiarists' Association Inc.), Mr Danny Le Feuvre (CEO, AHBIC), Mr Steve Fuller (Commercial Beekeeper (NSW) and past NSW Apiarists' Association president), Ms Tara Needham (Department of Agriculture, Fisheries and Forestry, Biosecurity, Canberra), Ms Ashley Zamek (R&D Manager-Horticulture Australia), Dr

Fazila Yousuf (Research fellow. Macquarie University), Soo Jean Park (Chemical Ecologist, Macquarie University).

In preparation for the workshop, Dr Fazila Yousuf distilled information from nearly 2000 papers to develop charts outlining researched and currently used control and monitoring methods of varroa. These charts had been reviewed and discussed in various meetings by the research team over the previous 9 months. At the beginning of Day 2 she presented a talk outlining how she chose which techniques to include in the charts.
On Day 1 the scientists involved in the project used the charts as a tool to review what is known about varroa control and monitoring techniques, and to develop further ideas. The aim was to identify where the gaps were, and what could be developed further for Australian conditions.
On Day 2 these ideas were further discussed. After an extensive review of the Biological Control methods, the group concluded there were no clear avenues through which predators could, at this stage, assist with varroa mite management in the hive. However, attacking varroa using the fungi *Metarhizium anisopliae* could be effective, but that it was important that the correct species of *Metarhizium* should be trialled. Using variants of *Bacillus thuringiensis* that specifically attacked mites were also seen as promising but would take more time to develop. With both the fungal and bacterial options there was concern about laws with respect to their residues in honey, and the regulatory hurdles that may be required for them to be used.
The discussions on promising Physical / Cultural control methods included using brood pheromones to lure varroa into specially constructed varroa traps. However, regulatory challenges were highlighted with respect to the use of synthetic pheromones. Heating hives to kill varroa was also discussed, particularly if that could be done in combination with no brood or re-queening periods, to make it more effective. This technique was seen as more applicable for recreational beekeepers who only had a few hives to manage. Techniques to stop varroa entering the hives were also discussed as these have major management ramifications.
Genetic and biochemical control methods were reviewed in reference to how they could link to Biological and Physical / Cultural control methods. Under Genetic Control we identified simple genetic markers for Varroa Sensitive Hygiene as low hanging fruit that could be easily tested and activated in Australia. There was discussion on how large commercial growers could use this given the cost of testing a queen for each hive. Another area of discussion was RNAi work. RNAi work has been undertaken for at least 20 years in three different continents and owned by three different companies. We concluded that the stability of RNAi under field conditions appears to be critical challenge with this technique. We also discussed different ways gene modifications could be used, and whether Australia was ready for that approach. The regulatory requirements for the manipulation of genes also seemed difficult.
The biochemical chart demonstrated how widely people had experimented in terms of plant extracts that could be used to control varroa. Most extracts seemed to reduce Varroa numbers, although most of the tests were undertaken in the laboratory. This was a common complaint about the research. Often methods that appeared promising in the lab were ineffective in the field. The importance of field-testing techniques was emphasised in the workshop.
We also reviewed monitoring, detection, and surveillance techniques for Varroa. A large part of this discussion focused on the different meanings of these three terms, and their relevance to varroa management. Surveillance was defined as operating at a regional level to identify whether varroa had entered a region or not. Here the presence /absence of varroa was important. Detection could occur at a regional scale, or it could refer to identifying which hives within an apiary had been infested with varroa. Detection would again use tools detecting presence or absence. Monitoring, however, is used more directly to manage hives with varroa; both in order to know when to control for varroa, and to test if a control treatment has been effective. Here, the number of varroa in each hive is important, and that number needs to be quantifiable between hives and over time.
The charts showed that there were more opportunities to develop methods to detect the presence or absence of varroa in hives than to quantify varroa numbers. The presence /absence opportunities included using odour, vibrations, or sampling honey for varroa DNA or biochemical changes.
With respect to monitoring, the most reliable methods are those currently being used. All of these require taking a sample of bees from the hive and removing the varroa using either sugar shakes, soapy water, or alcohol. The only alternative being developed is using CO_2 to knock down the varroa from a sample of bees. While highly effective in the laboratory, CO_2 would require training under field conditions because it is harder to use than the other methods. Firstly, you need a lower dose to knock out bees than you need to knock out mites, and the temptation would be to stop the CO_2 flow once the bees were knocked out, which would lead to an undercount of varroa. Secondly, after using CO_2 tanks repeatedly, they become more likely to spit CO_2 “snow” into the bee sample rather than just CO_2 gas. The snow would kill the bees, and its presence may cause the operator to stop applying CO_2 before the mites are knocked down.
There was a lot of research on using cameras to look for varroa on mites entering hives. However, the accuracy of these methods was variable, and it was unclear how this could be quantified if they were used as a monitoring technique. In addition, as they were looking for varroa on bees entering hives, the technique may not be indicative of the varroa being generated by the hive.
This discussion linked to the presentation by Dr Mary Whitehouse at the beginning of Day 2 recounting the type of varroa

infestations that a region could experience, and how that relates to the management of varroa. Control methods to reduce varroa numbers in a hive will be effective if varroa numbers in a hive are increasing through reproduction. If varroa numbers are increasing because of repeated heavy reinfestation, then one-off hive-level control methods will have limited effect, restricting control methods to those that are long-acting or by repeatedly re-applying control.

Knowing which method is driving an increase in varroa numbers could help with hive management. Consequently, there could be an opportunity to identify if varroa population growth is due to reinfestation or reproduction by comparing the number of varroa obtained by sampling in the hive (using, for example, alcohol washes) with number obtained by checking bees entering a hive (using cameras). This would dictate how often control methods would need to be re-applied, or whether another approach to manage varroa is needed.

Another aspect of the talk presented by Whitehouse was the importance of extension. It is one thing to have an extensive tool kit of control methods, but if you don't know how or when to use a tool, it is of no use. Producers need to have someone with whom they can consult about control methods that are relevant to their apiaries.

This point was emphasized in the third talk at the beginning of Day 2 by Dr Francesco Stolfi on beekeeping industry structures in New Zealand, the United States and Australia. He discussed how the lack of ongoing support in New Zealand and the United States had increased those countries' challenges in tackling varroa. He saw that the engagement of the Australian beekeeping industry in the varroa response was a major advantage that would help Australia manage varroa in the long term.

Appendix 7
Varroa Workshop: Talk given by Dr Mary Whitehouse.

Varroa causing major challenges for Australia

Range of situations:

No Varroa → Varroa going ballistic ▪ ▪ ▪ ▪ ▸ Varroa established

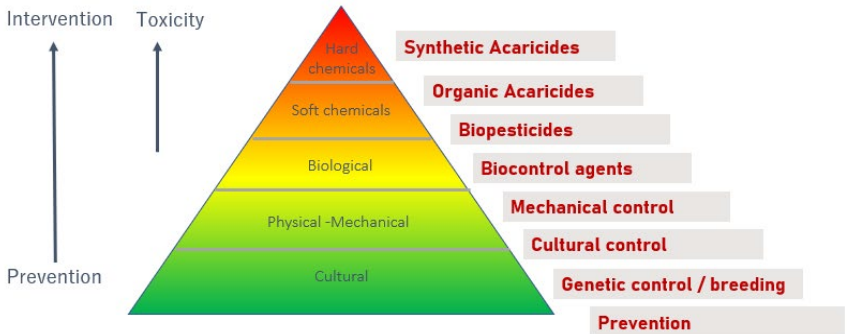
Horticultural industry will become more reliant on managed hives

Beekeeping needs to be sustainable and profitable

Recommended approach: Integrated Pest Management –IPM

https://www.dpi.nsw.gov.au/emergencies/emergency/biosecurity/current-situation/varroa-mite-emergency-response/managing-your-hives-with-varroa

Integrated Pest Management



AIM: manage pests below economic threshold

Multi-pronged approach

Economic threshold

Recommended treatment thresholds by colony phase

- Varroa % is the number of Varroa mites per 100 adult bees (adapted from Honey Bee Health Coalition 2022 by E. Frost)
- In brackets (or number of mites found) is the **total mites found in an alcohol wash, soapy water wash, or sugar shake of 300 worker bees or ⅓ cup**

Colony phase	Wait - immediate control not needed	Urgent - Control immediately
Dormant (broodless period)	Under 1% (less than 3 mites found)	Over 1% (3-5 mites found)
Population increase (typically spring)	Under 2% (5 or less mites found)	Over 2-3% (6-9 mites found)
Peak population (typically summer)	Under 2% (5 or less mites found)	Over 3% (9+ mites found)
Population decrease (typically autimn)	Under 2% (5 or less mites found)	Over 2-3% (6-9+ mites found)

https://www.dpi.nsw.gov.au/emergencies/biosecurity/current-situation/varroa-mite-emergency-response/managing-your-hives-with-varroa

Multi-pronged approach

- **Different seasons**
- **Different brood developmental stages**
- **Different Varroa developmental stages**
- **Different climates**
- **Different types of beekeepers**

Range in types of control methods

Their use supported and integrated within the industry

Aim of the workshop

Assist with the multi-pronged approach

Identify potential novel control and monitoring techniques for Australia

Focusing on Cultural, Physical/Mechanical and Biological methods

The workshop is part of the process to create potential solutions

Step 1: Generate Ideas

Step 2: Refine Ideas

Appendix 8
Varroa Workshop 23 Jan 2024. Talk given by Dr Fazila Yousuf.

Exploration of advanced control and detection methods for Varroa mite

Varroa workshop

Fazila Yousuf
Applied BioSciences
Fazila.Yousuf@mq.edu.au

23 Jan 2024

Two-Pronged Research Approach: Engagement and Exploration

- **Phase 1 (End April 2023)**
- Conferences/meetings
- Knowing honeybee industry
- Industry and stakeholders
- Beekeepers
- International beekeepers (NZ)
- Researchers
- Completing survey

- **Phase 2 ((End July 2023)**
- Literature from the last five years.
- Create an EndNote database.
- Current record is 2000.
- **Horizon scan: what the future look like.**

Categories

1. **Monitoring/Detection**
2. **Physical control** → Cultural, Mechanical
3. **Biological Control** → Predators, Bacteria, Fungi
4. **Genetic Control** → Secondary metabolites
5. **BioChemical**

The work is ongoing and will be updated as new information becomes available.
Please inform me if you identify any gaps or have suggestions for improvement.

Appendix 9

Varroa Workshop 23 Jan 2024. Talk given by Dr Francesco Stolfi (presentation pre-recorded).

Dr Francesco Stolfi's talk on the Australian honeybee industry and comparison with New Zealand and the USA. Presented to all in person and online attendees on Varroa workshop Day 2.

Summary:
Francesco's talk at Macquarie University highlighted the impact of risk aversion on the adoption of Integrated Pest Management (IPM) in beekeeping, emphasizing the visibility of costs versus the less tangible short-term benefits. He discussed how the size of beekeeping operations influences risk aversion and the accessibility of information, with larger operations being more risk-averse but also easier to reach for educational efforts. Francesco stressed the importance of trusted information sources, including biosecurity officers and peers, and the proactive role of beekeeping associations in Australia compared to other countries. The talk concluded with the significance of science and trust in promoting IPM adoption.
Key message: Information from trusted sources can increase IPM uptake by making beekeepers less risk-averse, and that trusted sources are biosecurity officers, peers, and beekeepers' associations.

Presentation link:
https://macquarie.zoom.us/rec/share/nbnp9CP32PdggdW_TDhgqIEOjPs1_gheCZjOeaobVN_yX5XsR5JiwJdjaoYW7wik.jyoiGN36Z9um97vG

Transcript:
Slide 1: This talk aims to give a social science view regarding the adoption of IPM.
Slide 2: So, what effects risk aversion with regard to IPM adoption. On the one hand, we have the assessments, the assessment of the cost, benefits connected to the use of IPM and on the other, the information available.
Now problem is that cost, tends to me more visible in terms of manpower cost and the potential lower effectiveness of IPM compared to epic chemical methods in the short term. Conversely benefits and to be less feasible and these can be listed as the fact that IPM is less damaging to the environment.

- It reduces personal exposure to chemicals.
- It's less damaging to bees.
- It lowers residues in bee products and avoids resistance to chemical control methods.

These last 3 are specifically the economic benefits of IPM that are at the core of the decision-making process of profit-oriented operators.
With regard to information. What is key is access to trusted source of information, not only on the technical side of using IPM on the metals but I will stress on the economic implications of adopting IPM.
Now a key point that I want to make in this presentation is that both the assessment of cost benefits enhance the extent of risk aversion to IPM adoption and the reachability, and then the enhance the access to information depend on the size of beekeeping operations.
Slide 3: So let me first briefly point out that in Australia, as in other countries, such as New Zealand or United States, the beekeeping industry has a very skewed size distribution. The overwhelming majority of beekeepers have fewer than 50 colonies. Large operator above 1,000 are really a very small minority of the industry.
Slide 4: Now the point that I would like to make again is the importance of science in with regard to affecting risk aversion to IPM adoption.
On the one hand, because cost is more visible and more short-term than the economic benefits of IPM. The more operators are motivated by profit considerations that is the greater their size, the more they will tend to be risk averse towards IPM adoption.
On the other hand, larger operations are also easier to reach for all actors involved in distributed in information, and hence in providing accurate assessment of the cost benefit balance.
The key point here, then, is that size of the keeping operations increases both importance of the productive motive and the reachability of beekeepers, and hence it both positively and negatively affects this conversion and this creates an opportunity for action, as the next slide will show.
Slide 5: As we just said, large operations are the most risk averse. They are also, however, the easiest to reach. So, increasing in size from, roughly speaking, hobbyist to sideliners, to commercial we can expect risk aversion towards IPM adoption to increase, however, reachability also increases with risk aversion.
Now the point is clearly to reduce risk aversion through information activities, expansion activities. And from what we just said, we can expect this information activity to be to offer more bang for the buck, so to speak, to be more effective. as the size of a beekeeping operation increases as we move from hobbyist to sideliners, to commercial operators. In other words, information has the greatest impact on changing behaviour when directed at large corporations.
Slide 6: Now, the other side of providing information is the sources of information. Trusted sources of information.

First. we are talking about biosecurity officers both in United States and in Australia. They very often double, not only as enforcers but with a role of providing information to be keepers. And here 2 points are important.

1. Long-term relationships matter. The degree of trust towards biosecurity officers depends on the extent of the relationship that they have over time with beekeepers. Secondly, that as much as possible it would be useful to separate information from enforcement again or trust from trust issues.
2. Secondly a second source of trust information can be peers through learning from peers observing what peers do, and critically also overall transparency in what other beekeepers are doing in order to avoid concerns about cheating.
3. Finally, beekeepers, associations both at the commonwealth and the state level.

Beekeepers Associations are typical so-called nested enterprises which in the social science literature refer 2 groups or actors that can act as trusted intermediaries between industry operators and operators outside of the industry. In this case, between beekeepers and external actors, such as government agencies and universities.

Now, in this context. what is the specific situation of Australia. With regard to relationship with bio security officers? Australia is in a good position internationally compared to the United States and New Zealand, because like the United States it can avail itself beekeepers can avail themselves of the support of biosecurity officers.

This is not the case, for instance, in New Zealand, were, already, in the 1980s. The government made significant accounts to the budget for biosecurity officers.

Secondly. and this is really a point of difference with regard to Australia, New Zealand, and by now also, the United States has taken a very and the beekeeping of association has taken a rather reactive role, probably with regard to Varroa, because probably Varroa has been there for many years. In this country conversely, both of the Commonwealth and under State Level beekeeper associations have been very proactive in devising responses to Varroa.

Slide 7: So, in conclusion. have 2 key take-home points.

Size matters, and it's an opportunity for action, and, secondly, trust matters, and Australia is internationally well placed to mobilize it.

Thank you for your attention.

Appendix 10
Article 1: The potential biological control agent, (a predatory mite) can be used against Varroa mites in Australia.

Above left: A very young pseudoscorpion eating a varroa mite. Image courtesy of Sam Read, PFR
Above right: An adult pseudoscorpion eating a varroa mite next to a parasitised honey bee drone pupae. Image courtesy of Robert Lamberts, PFR

Ron is linked to an international group led by Mary Whitehouse (NSW Department of Primary Industries /Macquarie University) interested in identifying tools that could be used against Varroa mite in Australia should the unthinkable happen. This team includes Elizabeth Frost (NSW DPI), Juliana Rangel (Texas A&M), and Mark Harvey (Western Australian Museum). Mark, who is a world expert on pseudoscorpions, said that *Chelifer cancroides* are in Tasmania, but haven't yet been reported from the Australian mainland. Our next step is to see if these critters are on the Australian mainland, or if other Australian pseudoscorpions could be effective against Varroa mites.

This is where you could help.

If you find a pseudoscorpion near your hive (or your house or chicken pen as they hang out there too) please photograph it with your phone, and send the picture to Mark Harvey (E: mark.harvey@museum.wa.gov.au; or 0407 553 567). If possible, capture the critter (it won't bite or pinch), put it in a zip-lock plastic bag or small jar, and pop it in the freezer (with the date and location where you caught it). Freezing it means that later, if it is a relevant species of pseudoscorpion, it can be formally identified and have its DNA assessed. The photographs will help us find out if these critters are on the mainland, or if there are other useful pseudoscorpions out there that could be called into service if necessary.

Managing Varroa mite, should it evade eradication in Australia, will require a range of tools. Predators that take out Varroa mites could be part of the pest management toolbox. This survey is an initial step to increase our preparedness.

Does Australia have Varroa mite predators?

Published - 4 November 2022 By Dr. Mary Whitehouse, NSW Department Of Primary Industries

Do our bees have useful associates that attack Varroa mites? – you can help.

Above: A honey bee next to an adult Chelifer cancroides (pseudoscorpion). Image courtesy Robert Lamberts, PFR

With the threat of Varroa mite looming large, it might be helpful to see what defences our bees may have in or around their hives. Overseas where insecticide resistance in Varroa mite is a concern, researchers have been testing Varroa mite predators that are compatible with bees.

One candidate is the pseudoscorpion ~~Chelifer cancroides~~. This critter has been studied by New Zealand bio-protection researcher with beekeeping experience, Ron van Toor (Plant & Food Research, NZ (PFR)). The pseudoscorpion isn't an actual scorpion (it has no sting) but it is a distant relative, and readily attacks Varroa mites – even when it is a small juvenile.

Ron's work has shown that pseudoscorpions have no interest in bees or their young, and live comfortably in modified hive bottom boards from where they enter the hive to search for mites. The bees tolerate their presence. The questions are, could they be a tool to counter Varroa mites and where are they in Australia?

SEARCH...

RECENT POSTS

WHAT ARE THE BEST TOOLS TO EVALUATE HONEY BEE HEALTH?

CAN PROBIOTICS HELP WITH BEE HEALTH AND BEHAVIOUR?

HAZARD REDUCTION BURNS: CAN THEY BE DONE BETTER?

INTRODUCTION TO INTEGRATED PEST MANAGEMENT FOR BEEKEEPERS

OLD COMB = A LESS PRODUCTIVE COLONY

RECENT COMMENTS

CHRISTINE ON BRAULA FLY (WALK)

PETER MOBBS ON BRAULA FLY (WALK)

KEVIN OH ON PROPOLIS: AN EMERGING MARKET

DUNCAN ON OLD COMB = A LESS PRODUCTIVE COLONY

DUNCS HONEY ON DO HEALTHY BEES MAKE BETTER HONEY?

https://youtu.be/y1zdancXRDg

More information

Acknowledgements:

This article was peer-reviewed by Elizabeth Frost and Nadine Chapman.

Appendix 11

Article 2: Discuss chemical-free varroa free methods for recreational beekeepers in Australia.

THE AUSTRALASIAN Beekeeper

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Copy Deadlines
Mon, 22nd March 2024 (for April 2024 issue)
Mon, 19th April 2024 (for May 2024 issue)
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CHEMICAL-FREE VARROA CONTROL METHODS

FOR AUSTRALIAN RECREATIONAL BEEKEEPERS

ARTICLE BY BY FAZILA YOUSUF[1], MARY WHITEHOUSE[1,2], JULIANA RANGEL[4], MARK HARVEY[5], FRANCESCO STOLFI[6], ELIZABETH FROST[7], MARK GOODWIN[3]

This article explores non-chemical approaches that have been tried overseas for managing Varroa. While not yet tested for Australian conditions, these techniques could assist hobbyist beekeepers manage Varroa and potentially reduce the need for insecticides.

Among the many threats to honey bees, the incursion of *Varroa destructor* (Varroa) stands out as a formidable foe to the Australian beekeeping industry. The situation has worsened since Varroa established and began its rapid spread in New South Wales, with some bee keepers experiencing heavy hive infestations in just a couple of days. In light of this expanding infestation, the NSW DPI have developed a comprehensive plan advising beekeepers on how to treat their hives in a manner that aligns with the currently recommended chemical management plans (Frost 2023, DPI NSW).

While miticides can minimise the rapid spread of Varroa and quickly reduce Varroa populations, these harsh chemicals come with costs and potential negative impacts that some recreational beekeepers may want to avoid. While there will be some situations when chemical control will be necessary to keep Varroa at bay, this article explores alternative, practical methods that have been used overseas that could be useful for Australian recreational beekeepers.

Honey bee with Varroa mite (photo: Mark Goodwin).

How do Varroa Enter Hives?

Varroa commonly enter hives by attaching themselves to drones or workers who drift between hives, rob neighbouring hives, or by jumping onto bees visiting flowers. Hive colonisation can be very fast, dramatic, and difficult to control in areas newly invaded by Varroa. However, in areas where Varroa is more established, minimising drifting can reduce mite infestation. Drift can be reduced by keeping hives separated or arranged in a horseshoe pattern with the entrances pointing outwards, and by making each hive look distinctive. If a colony is already infested, softer options can maintain Varroa levels below the Varroa threshold.

How Do I Estimate the Varroa threshold?

The Varroa threshold is the number of Varroa inside a hive that is likely to damage colony health, or likely to do so before the hives are next visited and inspected, often causing colony death if left untreated. It is usually measured as a percentage, or the number of Varroa per 100 adult honey bees. Overseas, thresholds have been dropping over time. Other countries have also found that the destructiveness of the Varroa varies with the season and with the honeybee colony stage. In particular, the same percentage of Varroa per honeybee is more destructive in winter than in Spring or Summer because the mites affect honeybee survivorship over winter. The current Varroa

threshold in Australia is based on the threshold used across North America, which recommends control at greater than 2-3% infestation during spring, greater than 3% infestation during summer, greater than 2-3% infestation during autumn, and greater than 1% infestation in winter if there is a brood less period (Table 3, Frost 2023, DPI NSW). In late spring and summer regular monthly Varroa checks are advisable.

How Do I Sample for Varroa?

Determining the presence of Varroa is the first step in their control. The sugar shake method is an effective, reliable, quick way to check for Varroa in hives. Its major drawback is that it is not effective in high humidity and some workers that have been shaken in the sugar may be killed. In highly humid environments the alcohol wash or soapy water wash methods are recommended for mite surveillance. To determine Varroa levels using the sugar shake method, shake adult honey bees from at least three brood frames into a tray or hive lid, ensuring that the queen is not present. It is better to sample honey bees from the brood nest area rather than from honey supers because Varroa are more likely to be found on nurse bees that are feeding the brood.

Shake the honey bees down to a corner of the tray/lid and use a ¨cup measure to scoop along the bottom side of the tray to collect 1/2 cup of bees, or 125ml in volume (the equivalent to about 300 honey bees). Cover the jar with a mesh to prevent honey bees from escaping and add about 1 tablespoon (10 g) of icing sugar to it by rubbing it through the mesh lid. Gently roll the sugared honey bees for 60 seconds, set down the jar and leave for a few minutes, gently roll again, and then shake the jar upside down, vigorously shaking it above a white tray or into a shallow dish of water. The powdered sugar will dislodge the Varroa from the bees, allowing the mites to pass through the mesh while the bees remain in the jar. Varroa can then be counted on the tray; however, if there is a lot of sugar, use a fine mesh sieve to remove the excess sugar or use the shallow dish with water method which will dissolve the icing sugar. Most honey bees will survive and can return to the hive. This method is cost-effective, quick, simple, and less harmful to bees, making it ideal for recreational beekeepers (See NSW DPI Tocal College video:).

To calculate the infestation rate, divide the number of Varroa by the number of honey bees and multiply by 100. For example, 6 mites in a sample of 300 honey bees equal a 2% infestation rate.

What Do I Do If My Hive Is infested With Varroa?

Recreational beekeepers can try several methods that have been used overseas to manage Varroa:

1- Drone Brood Trapping: A Natural Approach

Drone brood trapping is a highly effective method used extensively in Europe. Varroa preferentially infest drone brood because drones take longer to develop, enabling Varroa to produce more offspring per brood cell. Drone production may occur in Australia from early Spring through Autumn, depending on environmental conditions, so this practice has a potential wide window of use. In spring, place a drone frame in the centre of each brood box to encourage the queen to lay unfertilised eggs in the frame. As soon as most of the drone

Varroa in a cell (photo: Mark Goodwin).

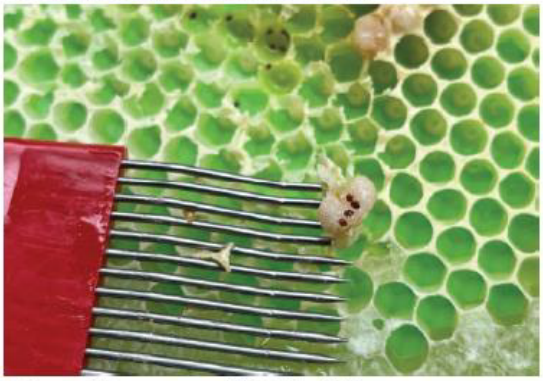

Figure 1: Drone pupae infested with Varroa. Credit: Kirra Hughes.

cells are capped and before any adult drone emergence, remove the drone frame from the hive and replace it with an empty drone frame. It is important to remove the drone frame before the drones emerge (24 days from egg to emergence), or else extra Varroa will also emerge (Figure 1).

Destroying capped drone brood can be done a number of ways. First, beekeepers can manually uncap drone cells using an uncapping fork to remove drone pupae, wash out any remaining Varroa in the cells with pressurised water, shake the frame dry and store or return it to the hive. Alternatively, beekeepers can freeze the frames for 2-3 days to kill the Varroa, scrape the frozen drone brood off the frame and dispose of it, before returning them to the hive. Drone brood trapping disrupts the Varroa life cycle and is generally safe, though it may slightly reduce honey production. In regions where drone brood is produced year-round, it is necessary to insert drone foundation frames monthly for this technique to work well.

2- Ventilated bottom boards: A subtle Tool

There is conjecture over the effectiveness of ventilated (screened) bottom boards to reduce Varroa numbers. These boards allow dislodged Varroa to fall out of the hive, preventing them from re-entering the hive and re-infesting

the brood. They can be used in both warm and cold regions. In colder climates, adding a solid tray at the bottom of the ventilated bottom board can provide necessary insulation, although some honey bees can regulate hive temperature effectively. While ventilated bottom boards can significantly reduce mite numbers, the effect is subtle (no more than 11-18%) and this subtlety may be lost during times of Varroa invasion and expansion. Ventilated bottom boards can reduce mite population growth but are not a solution on their own and should be used in conjunction with other control methods.

3- Powdered Sugar Sprinkling: Promoting Grooming Behaviour

Powdered sugar sprinkling is a dual-action method, but its effectiveness overseas has been disputed, and its usefulness in Australia, particularly in humid areas, is doubtful. In fact, it should be avoided in very humid conditions as sugar can form clumps and may also stick to the honey bees, reducing its efficacy and impairing the bees' movement. The technique works by encouraging bees to groom themselves and each other, dislodging Varroa in the process, and by making it harder for Varroa to attach to the bees. This method is more effective when there is no brood and when used in combination with ventilated floorboards or sticky mats.

Sprinkling powdered sugar between the brood nest frames of the hive is potentially a simple way to reduce mite populations, however significant repeated applications are necessary and it should only be attempted in dry climates. Always check mite numbers a day or so after using sugar sprinkling to check its efficacy. If there is little or no effect, use a different control method. This technique is unlikely to be useful for most Australian beekeepers.

4- Resistant Australian Honey Bee Queens: A Future Strategy

Checking for Varroa-resistant honeybee queens in Australian stock could be the way of the future. A naturally occurring heritable trait in honey bees, the "Varroa Sensitive Hygiene" (VSH) trait, limits the survival and reproduction of Varroa mites in colonies. There is an adenine/guanine (A/G) Single Nucleotide Polymorphism (SNP) located on chromosome 9 at the nucleotide position 9224292 of the honey bee genome (assembly Amel 4.0). The G allele of SNP 9-9224292 is associated with VSH behaviour (Tsuruda et al 2012). Researchers in New Zealand (Sainsbury et al 2022) have found that queens carrying two copies of this gene have hives that are more resilient to Varroa colonies with lower levels of Varroa.

This gene is naturally occurring in honey bee populations, although how to harness this knowledge for Australia is unclear. Currently, the best approach for Australian beekeepers is to select for Varroa resistance traits (once they get Varroa) by recording pedigree, phenotyping for Varroa resistance trait(s), and implementing controlled breeding through queen bee artificial insemination or geographic or temporal control of mating. Although the presence of the Varroa resistance traits (such as VSH, or others such as grooming or mite-biting behaviours, removal of Varroa-infested larvae, uncapping and recapping brood behaviour) does offer some resilience against Varroa, other methods of Varroa control would still be necessary in a colony with these genes.

Queen bee and workers (photo: Mark Goodwin).

In summary, drone brood trapping is the most effective, currently available non-chemical Varroa control tool that can be used by recreational beekeepers who do not want to use chemicals for Varroa control. Regardless of the non-chemical or chemical approach used, it is important to check your Varroa numbers every month, especially in the Spring, to protect your honey production and pollination bees, and autumn when Varroa numbers are increasing, honey bee numbers are decreasing, and overwintering bees are being produced. If Varroa numbers are over the treatment thresholds, it may be necessary to use chemical control.

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Appendix 12
Pod cast for the bee collective.
Dr Mary Whitehouse
6.00pm AEST
Tuesday 30 May. 1 hour max
Acknowledgment to Country
Question 1: Let's start by talking about your area of research. How did you find yourself in this field of work?
Question 2: Can you explain what Integrated Pest Management is?
Question 3: Why is IPM important to beekeepers?
Question 4: Part of your research has uncovered the pseudo scorpion that predates on varroa mite - can you tell us a little more about that?
Questions 5: The varroa mite incursion in NSW has alarmed the agricultural sector all over Australia. Can you tell us if there is any research happening in the impacted zones?
Question 6: What are your thoughts on the effectiveness of the DPI Emergency response and the current poisoning program?
Question 7: What are some of the learnings from other countries?
Question 8: Do you think there are alternatives to the current response?
Question 9: 5 questions we ask all our guests:
• Best advice for happy bees?
• Best advice for new beekeepers?
• Best bee-friendly plant?
• Your favourite honey?
• Favourite beekeeping hack?
Question 10: Finally, if people want to contact you, what is the best way for them to reach you?
Thank you

Appendix 13
SBS world news interview.

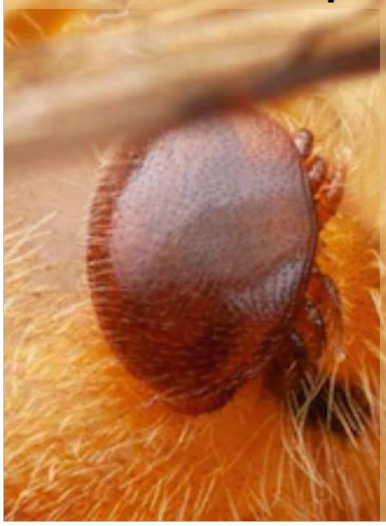
Varroa mites are a devastating pest of honeybees. For over a year Australia has been trying to eradicate an incursion of this pest, but earlier this month the decision was made to stop eradication and move to management of the pest. Dr Mary Whitehouse (ABS) leads a project identifying new and emerging non-chemical methods to monitor and manage this pest that could be applicable in Australia. She was interviewed by SBS in a segment aired on SBS world news at 6:30 on September 21st. In the segment, she noted that management of varroa mites would require a multi-pronged approach, and that an overreliance on pesticides could lead to resistance by the mites.

Australia gives up fight to eradicate bee-killing mite https://www.sbs.com.au/news/video/australia-gives-up-fight-to-eradicate-bee-killing-mite/xwsv7wure?cid=newsapp:socialshare:copylink (https://www.sbs.com.au/news/video/australia-gives-up-fight-to-eradicate-bee-killing-mite/xwsv7wure?cid=newsapp:socialshare:copylink)

Appendix 14

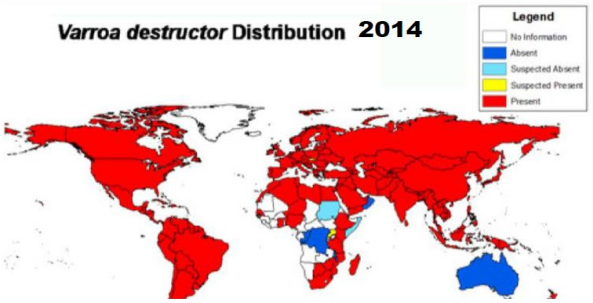
NSW Apiarists Association Conference 2023. Talk given by Dr Mary Whitehouse.

Aim of this presentation:

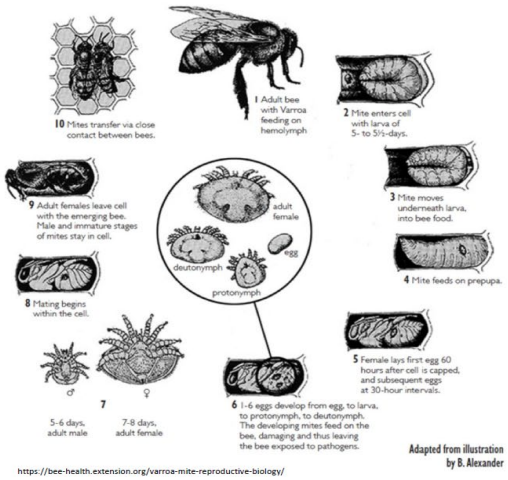


- The Varroa Mite & current control methods
- Introducing Integrated Pest Management
- Successful IPM – Australian Cotton
- Challenges of IPM
- Requirements for IPM
- How we can assist
- How you could help

The Varroa mite threat



- Varroa mite devastating
- Able to develop resistance to insecticides rapidly
- Aim to keep Australia free of varroa mite



Control Methods

Synthetic Acaricides
- Organophosphates (eg Coumaphos)
- Pyrethroids (eg Fluvalnate, Flumethrin)
- Formamindines (eg Amitraz)
- (abandoned acaricides: eg Cymiazole, bromopuropylate, Fenpyroximate)

Organic Acaricides
- Formic acid – kills Phoretic and reproductive mites in brood – toxic to bees at high temperatures
- Oxalic acid – resistance in NZ?? – contact acaricide – doesn't affect mites in broods
- Essential oils – (monoterpenes) eg Thymol – toxic at high temperatures, garlic oil
- Hop Beta Acids – (lupulones) results mixed

Biopesticides
- Entomaopathogenic fungi – *Metarhizium*, *Beauveria* – contact toxicity, may effect bees
- Mite diseases – Bt – 2 strains killed mites but not bees – lab based so far.
- RNAi sprays to disrupt the ability of mites to activate key genes causing them to die.

Biocontrol agents
- Parasitoids - ?
- Predators – *Stratiolaelaps* (a mite) – will attack mites in the lab, but not in the hive – may attack brood. *Chelifer* (a puesdoscorpion) will attack mites in the colony, has little effect on brood in colony, but efficacy unclear

Mechanical control
- Screen bottom boards – mites fall out of hive
- Drone brood trapping – mites prefer to breed in drone cells, which can be removed –effective but time consuming
- Heat treatments – mites can't tolerate above 40°C – eg mite zapper that heats drone combs to 43°C -efficacy unclear
- Modified entrances that knock mites off as they enter the hive
- Adding icing sugar to encourage grooming.

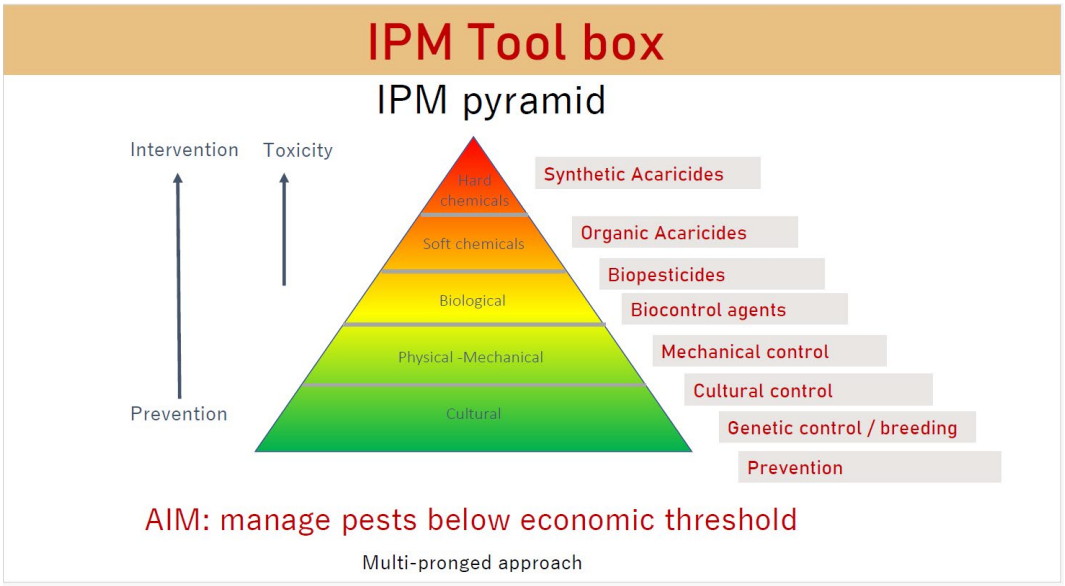
Cultural control
- Queen caging (24 days) breaking the brood cycle- forcing varroa onto adult bees

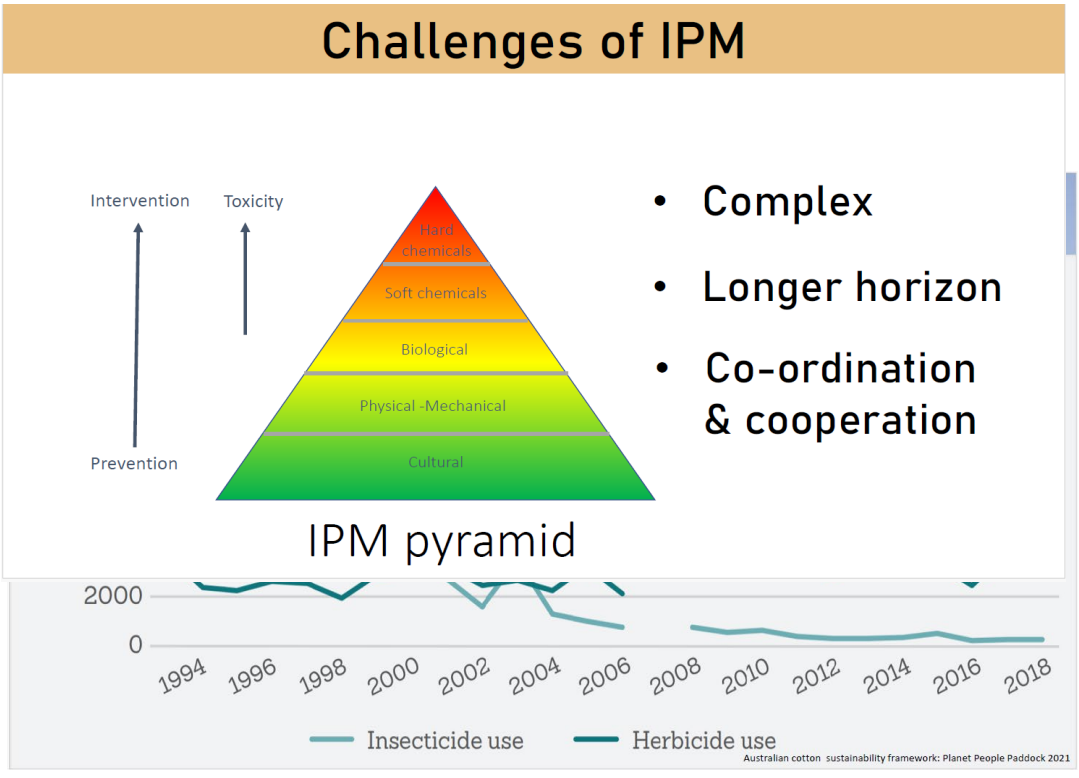
Genetic control / breeding
- Selecting bees more likely to groom.
- Hygenic behaviours: varroa-sensitive gene - better at detecting infected brood which are uncapped and removed

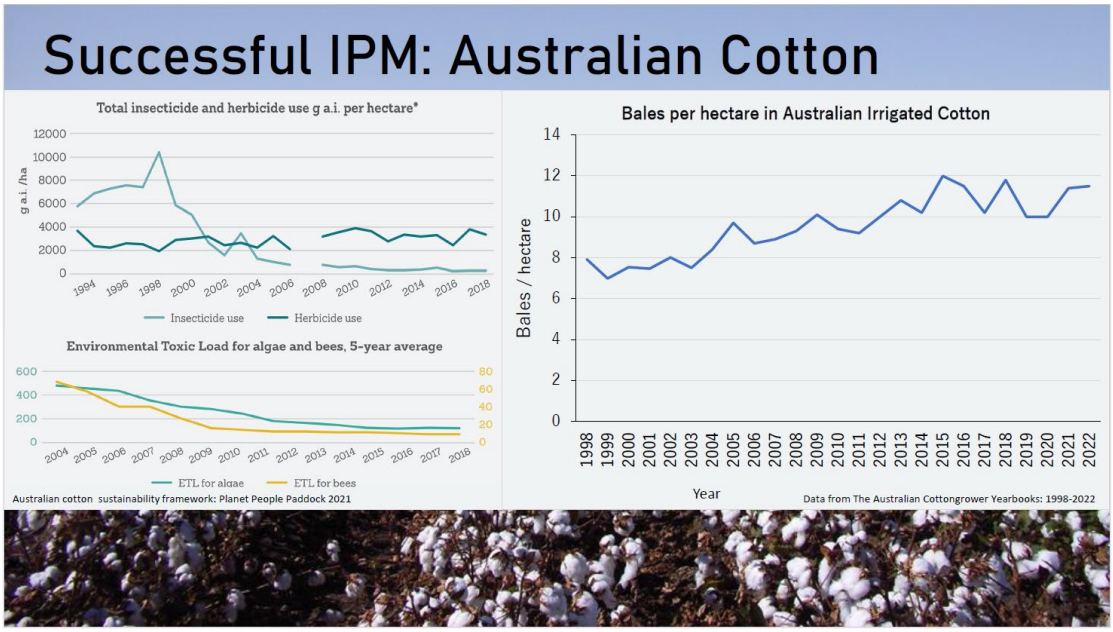
Prevention
- Reducing drifting and robbing within apiaries
- Effective swarm control
- Regulating the movement of hives

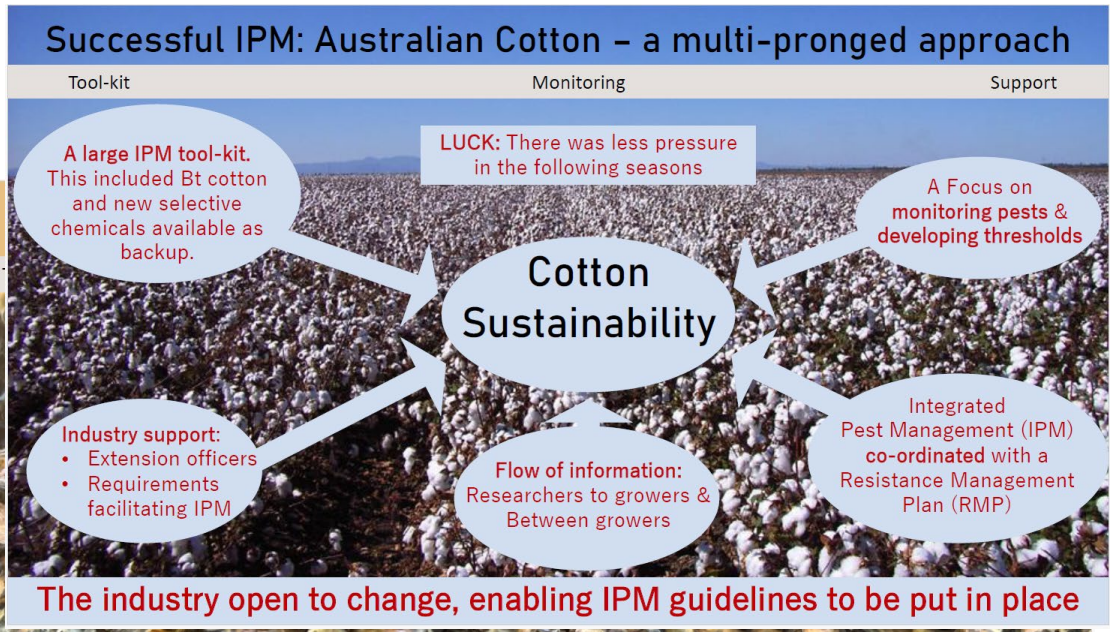
AIM: manage pests below economic threshold

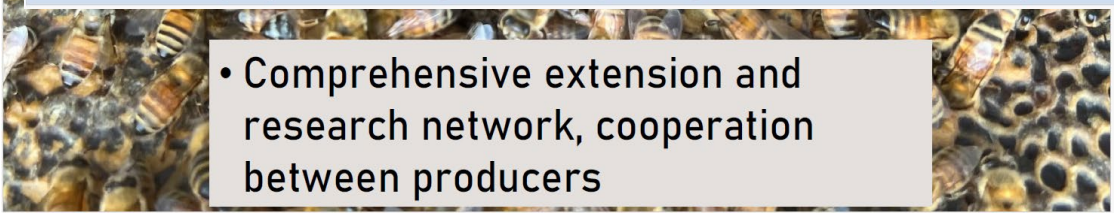
Multi-pronged approach


IPM Tool box
IPM pyramid
Intervention
Toxicity
Prevention
Hard chemicals
Soft chemicals
Biological
Physical -Mechanical
Cultural
Synthetic Acaricides
Organic Acaricides
Biopesticides
Biocontrol agents
Mechanical control
Cultural control
Genetic control / breeding
Prevention
AIM: manage pests below economic threshold
Multi-pronged approach


Challenges of IPM
Intervention
Toxicity
Prevention
Hard chemicals
Soft chemicals
Biological
Physical -Mechanical
Cultural
• Complex
• Longer horizon
• Co-ordination & cooperation
IPM pyramid
2000
0
1994
1996
1998
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
Insecticide use
Herbicide use
Australian cotton sustainability framework: Planet People Paddock 2021


Successful IPM: Australian Cotton
Total insecticide and herbicide use g a.i. per hectare*
g a.i. /ha
Insecticide use
Herbicide use
Environmental Toxic Load for algae and bees, 5-year average
ETL for algae
ETL for bees
Australian cotton sustainability framework: Planet People Paddock 2021
Bales per hectare in Australian Irrigated Cotton
Bales / hectare
Year
Data from The Australian Cottongrower Yearbooks: 1998-2022


Successful IPM: Australian Cotton – a multi-pronged approach
Tool-kit
Monitoring
Support
A large IPM tool-kit. This included Bt cotton and new selective chemicals available as backup.
LUCK: There was less pressure in the following seasons
A Focus on monitoring pests & developing thresholds
Cotton Sustainability
Industry support:
• Extension officers
• Requirements facilitating IPM
Flow of information: Researchers to growers & Between growers
Integrated Pest Management (IPM) co-ordinated with a Resistance Management Plan (RMP)
The industry open to change, enabling IPM guidelines to be put in place


• Comprehensive extension and research network, cooperation between producers

How we can assist? – Develop tool kit

How could you assist?

- Feedback – what could work for you
- Support – your industry and institutions
- Cooperate – "all in this together"

Beekeepers survey

https://www.surveymonkey.com/r/87CHC3N

The ultimate aim is that Australia has a resilient, profitable industry

How we can assist? – Scoping monitoring Techniques

The Team

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Dr Ashley Mortensen Plant & Food Researc
Dr Michelle Taylor Plant & Food Research, N
Dist. Prof. Phil Taylor Applied BioSciences,

ology

ols

Danny Le Feuvre
Steve Fuller
Stephen Targett

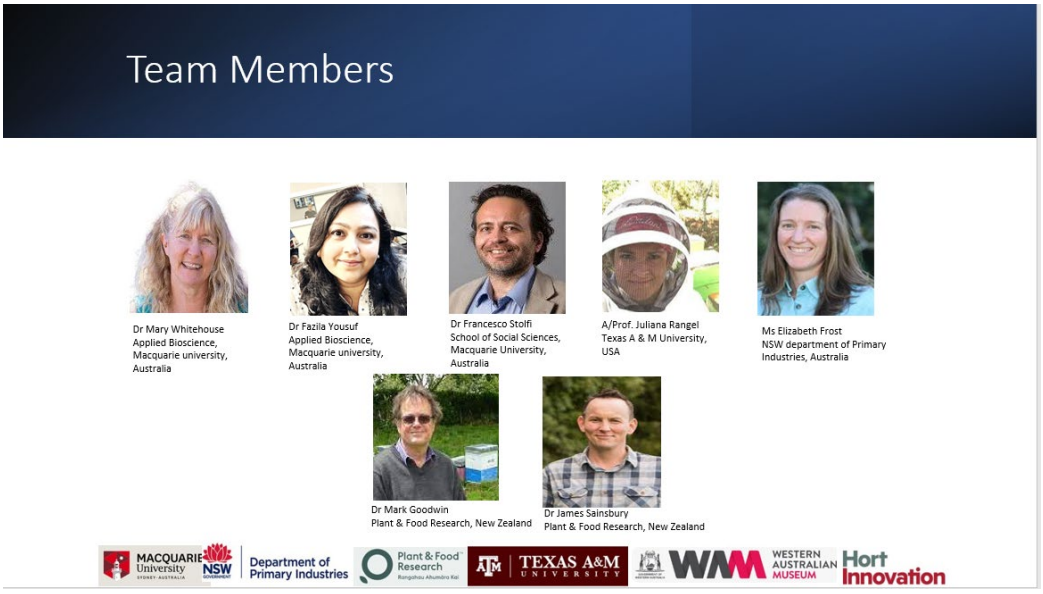
Beekeepers survey

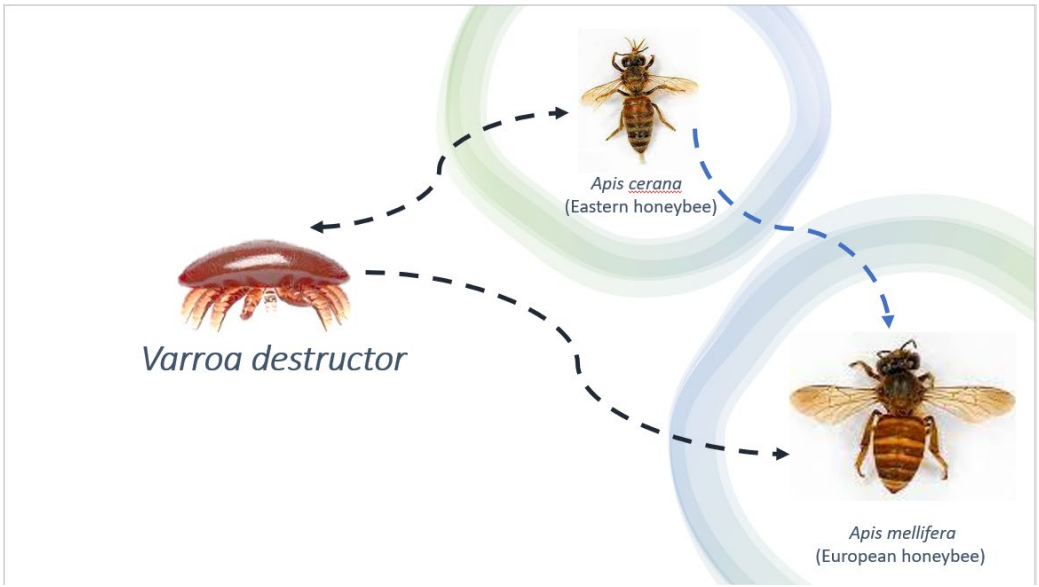
https://www.surveymonkey.com/r/87CHC3N

MACQUARIE University | NSW Government | Department of Primary Industries | UNIVERSITY | WESTERN AUSTRALIAN MUSEUM | Hort Innovation

Appendix 15

Apiculture New Zealand (4th NZ Honeybee Research Symposium) 2023 held in Rotorua, New Zealand. Talk given by Dr Fazila Yousuf.





Varroa infestation

- Wingless, eyeless, and unable to crawl between widely spaced honeybee nests.
- BUT have excellent chemosensing abilities.
- Feed on the fatty liver tissue of honeybees and reproduce in brood cells.
- Dangerously highly efficient vector of several honeybee viruses.
- Weakening honeybees and causing mortality.

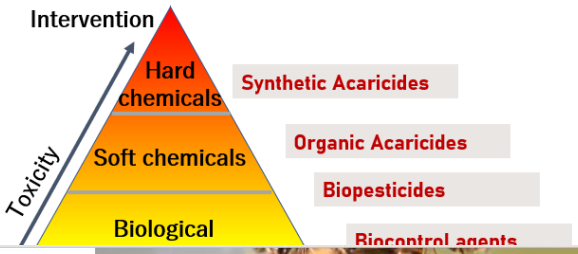
https://pdnbeekeepers.org/knowledgebase/varroa/

Cross-section of honeybee's abdomen, varroa can be seen lodged between the bee's abdominal plates (feeding on bee fat body tissue).
https://phys.org/news/2019-01-honey-bee-parasites-fatty-blood.html

Resistance to Pesticides

- Successful approach in New Zealand: Pesticides in rotation or combination.
- Rotation through pesticides, can develop resistance too.
- As observed in North America and Europe.
- Where varroa become resistance to each new chemical within 8-10 years.
- Chemical reliance not sustainable.

Integrated Pest Management (IPM)



Prevention

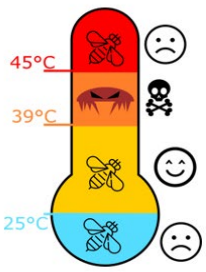
- Use cultural methods to manage pest populations before they become significant.
- Prevent varroa from invading new areas.
- For Example: Control swarming to prevent new nests from living within your colony's foraging area and serving as a source population of varroa.

Cultural control

- Make your colony environment less suitable for varroa while minimally affecting the honeybees.
- Small sized-comb
- Hygienic honeybee stock
- Brood break (slow or halt varroa reproduction)

Mechanical control

- Screened bottom boards
- Drone comb removal
- Drone removal
- Hyperthermia



- Entomopathogenic fungi (*Metarhizium anisopliae*)
- Nematodes
- Predators (Pseudoscorpion or *Stratiolaelaps* mite) and
- parasitoids

Nothing has been effective at controlling Varroa within hives so far.

Image courtesy of Sam Read, PFR

***Stratiolaelaps* mite**
https://www.abeilleduhain.be/predateurs-terricoles-du-varroa-1/

Chemical control

- **Organic**
  - Oxalic acid
  - Thymol
  - Formic acid
  - Essential oils
- **Synthetic**
  - Pyrethroids (Tau-Fluvalinate/Bayvarol)
  - Organophosphate (Coumaphos)
  - Fornamidine (Apivar/Apitraz)

Benefits of IPM

- Good for bees
- Sustainable and environmentally friendly
- Good for your wallet
- Preserve treatment efficacy of chemical treatments and ensures their effectiveness for future use.

Developed resistance" cotton became unprofitable.

- **Mid-1990s Australian cotton breeders began incorporating Bt insect resistance genes.**
- **Bt cotton" plants dispatch an insecticide from a bacteria – *Bacillus thuringiensis* (Bt) – that is toxic to the bollworm.**
- **Resistance against Bt cotton in some places**
- **Cotton growers must plant non-Bt refuges. "Refuges" provide a home for non-resistant pests to breed.**
- **Also incorporates IPM practices such as killing resistant pupae under the soil during the winter and planting their Bt crop in a short window of time.**

https://blog.csiro.au/

https://blog.csiro.au/

Our Goal

- Do horizon scan to identify emerging trends, potential future developments, and novel approaches.
- Review globally the status and availability of non-chemical control methods and how they would operate within an IPM framework within Australia.
- Identify and understand innovations in Varroa detection technology. Early detection is critical for an effective IPM approach to Varroa mite management.
- Review previous failures in technology uptake.
- Develop a shortlist of biological and cultural control methods and systems to be studied further, and identify what work would be required to improve their fit in Australia.
- The outcome of this project will be a **comprehensive list of emerging Varroa detection, biological and cultural control methods**.

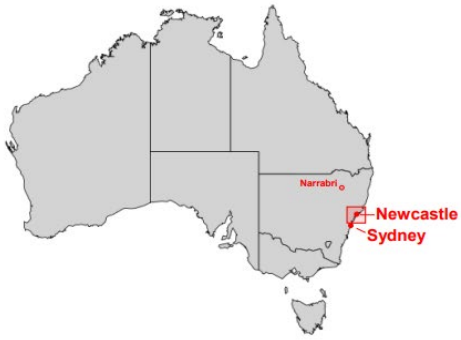
How could you assist?

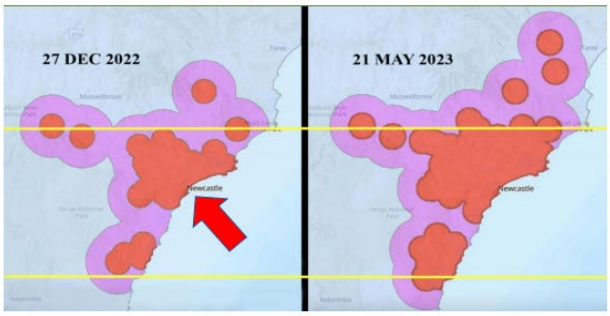
- Feedback- What could work for?
- Your thoughts on varroa IPM

Appendix 16

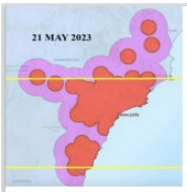
COLOSS *RNSBB* Spring Workshop 2024, Bilbao, Spain. Invited talk given by Dr Mary Whitehouse.

The spread of Varroa in Australia



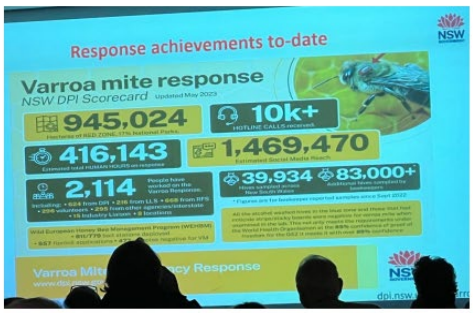


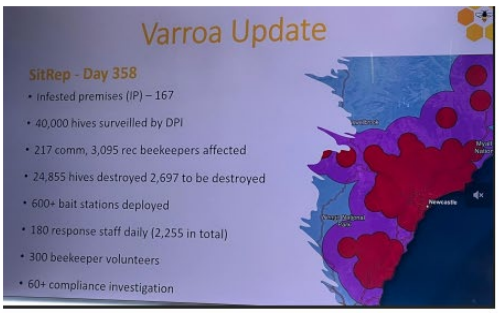
Varroa destructor was detected in Australia in sentinel hives in Newcastle in June 2022



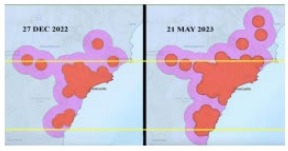
The Eradication effort

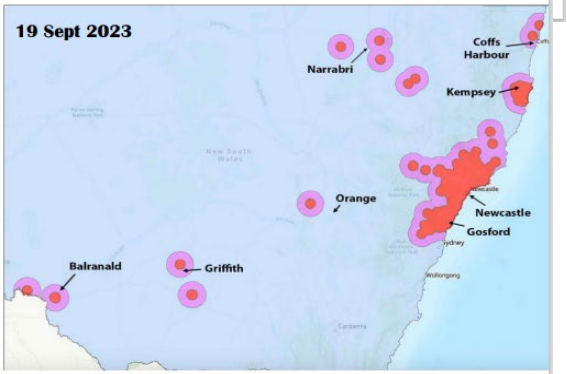
May 2023





The spread of Varroa in Australia





By15 September 2023, surveillance by the NSW Department of Primary Industries (DPI) revealed that Varroa Mites had spread to 277 locations in NSW

The Eradication effort abandoned

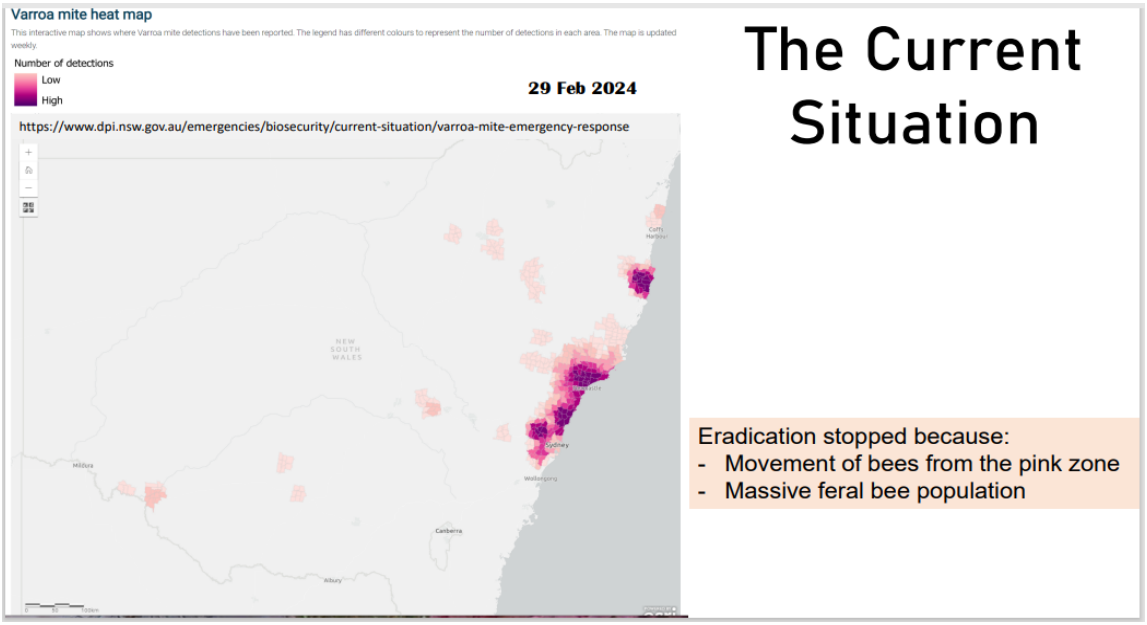
Attempts to eradicate varroa abandoned 19 September 2023

Australia abandons effort to eradicate varroa mite after 14,000 bee hives destroyed

Despite a $100m effort over 14 months to stop the invasive parasite, scientists say eradication is no longer possible

Non-compliance by some beekeepers, a recent spike in new detections and over a wider area made eradication a non-viable option, the group said.

More than 14,000 hives were euthanised in the following four months as authorities tried to halt the spread.



The Current Situation

Eradication stopped because:
- Movement of bees from the pink zone
- Massive feral bee population

The Current Situation

- **The hot spots in Australia are experiencing an Invasion or Acute Phase**
  - Official monitoring results over 3 weeks = 100 mites/hive/week – linear invasion
  - Reports of 60 mites entering sentinel hives in 2 days
  - One report of 277 mites/326 bees (83% infection)
- **Swarming bees with heavy varroa load**
  - One swam: 8L or bees, over 10,000 varroa mites
- **Bad year for small hive beetle.**
  - No reports of varroa killing hives, but
  - Hives with small hive beetle and varroa have died

Photograph by Peggy Greb, USDA.

The Current Situation – the response

- **Current insecticides:**

Active ingredient	Registered trade name
Amitraz	Apivar
Amitraz	Apitraz
Fluvalinate	Apistan
Flumethrin	Bayvarol
Formic acid	FormicPro
Thymol essential oil	Apiguard

Oxalic Acid is currently not registered in

- Australian varroa mites are not resistant to insecticides
- There is no deformed wing virus in Australia – yet.

But:

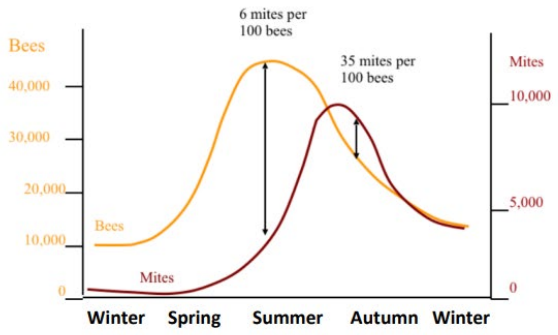
- Many locations in Australia have brood and supers all year around.

The Challenges going forward

1. Range of situations:

The Challenges going forward

Invasive or Acute Phase



Invasive phase, rate of increase dominated by mites entering hive
Chronic phase, rate of increase dominated by reproductive rate within hives

The Challenges going forward

Invasive stage - limited management options

Continuous insecticides

- Amitraz
- Flumethrin
- Fluvalinate
- Formic Acid
- Thymol essential oil

?

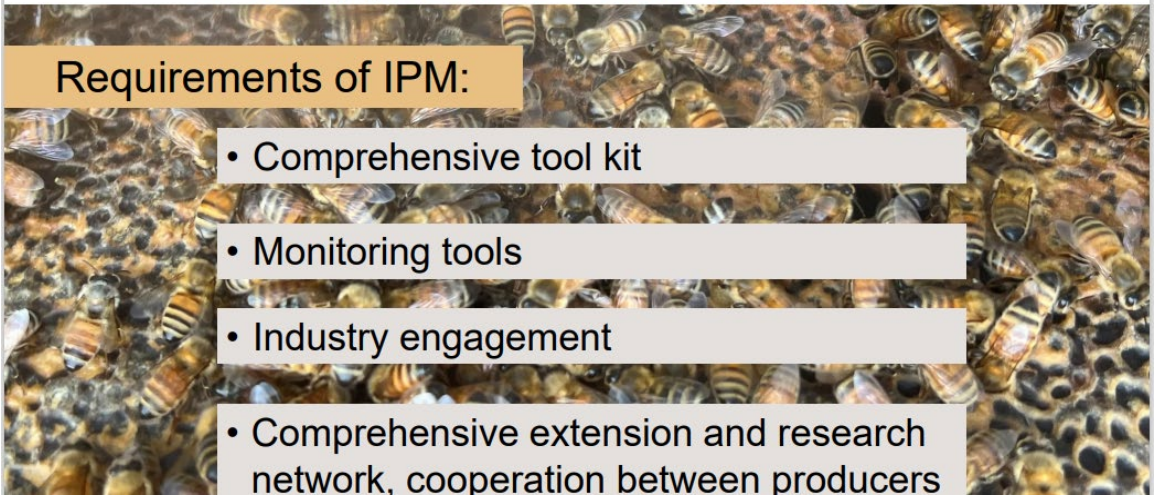
Restrict mites entering the hives

- Which bees are bringing in the mites?
- Does this change over the season / in different phases?

The Challenges going forward

The Challenges going forward

IPM approach - Chronic phase



Requirements of IPM:

- Comprehensive tool kit
- Monitoring tools
- Industry engagement
- Comprehensive extension and research network, cooperation between producers

The Challenges going forward

Chronic phase

A diverse Tool kit

Range in types of control methods

The Challenges going forward

Chronic phase

Fazila Yousuf

Workshop: Identifying what control techniques could be adopted for Australia

The Challenges going forward

Chronic phase

A diverse Tool kit
Range in types of control methods

Need an extension network to inform producers on when and how to use the different techniques

The Current Situation –

Economic threshold – adopted from the USA

Recommended treatment thresholds by colony phase

- Varroa % is the number of Varroa mites per 100 adult bees (adapted from Honey Bee Health Coalition 2022 by E. Frost)
- In brackets (or number of mites found) is the **total mites found in an alcohol wash, soapy water wash, or sugar shake of 300 worker bees or ½ cup**

Colony phase	Wait - immediate control not needed	Urgent - Control immediately
Dormant (broodless period)	Under 1% (less than 3 mites found)	Over 1% (3-5 mites found)
Population increase (typically spring)	Under 2% (5 or less mites found)	Over 2-3% (6-9 mites found)
Peak population (typically summer)	Under 2% (5 or less mites found)	Over 3% (9+ mites found)
Population decrease (typically autmn)	Under 2% (5 or less mites found)	Over 2-3% (6-9+ mites found)

https://www.dpi.nsw.gov.au/emergencies/biosecurity/current-situation/varroa-mite-emergency-response/managing-your-hives-with-varroa

The Challenges going forward

Use BEHAVE to simulate honeybee colony survival..

BEEHAVE

BEEHAVE is a computer model to simulate the development of a honeybee colony and its nectar and pollen foraging behavior in different landscapes.

The purpose of BEEHAVE is to allow multiple stressors of honeybee colonies within a hive and in the landscape to be represented, either alone or in combination, to understand their potential influence on colony development and survival: e.g. varroa mites transmitting deformed wing virus (DVW) or acute paralysis virus (APV), effects of several beekeeping practices, poor forage availability or even forage gaps in the landscape, and pesticide-induced losses of in-hive bees and foragers, in-hive bees, or brood.

Matthias Becher

Janine Powell

…in order to develop economic thresholds

Need extension support to inform producers about thresholds and the program

The Challenges going forward

Monitoring complexity

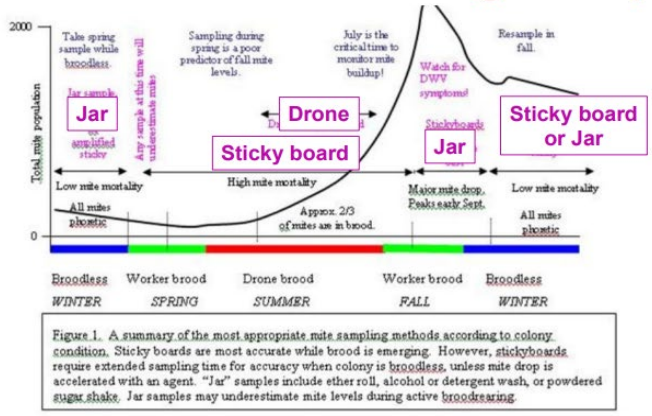


Figure 1. A summary of the most appropriate mite sampling methods according to colony condition. Sticky boards are most accurate while brood is emerging. However, stickyboards require extended sampling time for accuracy when colony is broodless, unless mite drop is accelerated with an agent. "Jar" samples include ether roll, alcohol or detergent wash, or powdered sugar shake. Jar samples may underestimate mite levels during active broodrearing.

From Randy Oliver - Scientific Beekeeping: https://scientificbeekeeping.com/fighting-varroa-reconnaissance-mite-sampling/

Need extension support to assist producers with monitoring techniques

The Challenges going forward

Chronic phase

The Challenges going forward

Extension – the industry

Francesco Stolfi

- Commercial
- Sideliners
- Hobbyists

The beekeeping industry: A very skewed size distribution

NSW		QLD		TAS	
< 50	91%	< 50	95%	< 50	97%
> 1000	?	> 1000	0.25%	> 1000	0.5%

NZ		US (FL)	
< 50	89%	< 100	77.8%
> 1000	14%	> 1000	3.3%

Sources:
NSW: Clarke, Michael and Danny Le Feuvre (2021)
QLD: Queensland Department of Agriculture and Fisheries (2023)
TAS: Department of Natural Resources and Environment Tasmania (2023)
NZ: New Zealand Ministry for Primary Industries (2022)
FL: Court et al (2022)

Many don't contribute to the centralized organization
Need information presented in different ways

Francesco Stolfi

The Challenges going forward

Extension – the industry

- Commercial
- Sideliners
- Hobbyists

The importance of size

INFORMATION ← REACHABILITY *(increases with size)*

↓

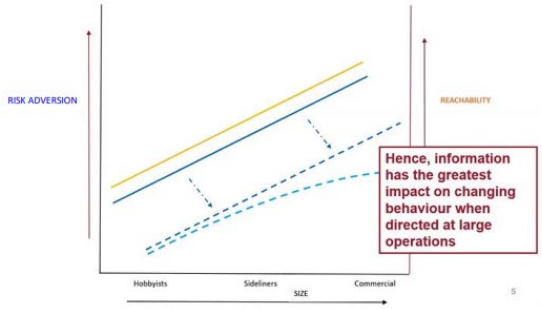
ACCURATE ASSESSMENT OF COST/BENEFITS

↓ −

RISK AVERSION TO IPM ADOPTION ← PROFIT MOTIVE *(increases with size)*

Size increases both the importance of the profit motive and the reachability of beekeepers, hence it both positively and negatively affects risk aversion

Large operations are the most risk averse, but also the easiest to reach



Hence, information has the greatest impact on changing behaviour when directed at large operations

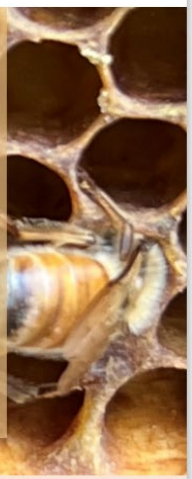
The Challenges going forward

Extension – Commercial beekeepers

- Smaller gross margins than other agricultural sectors
- Older men

Concluding remarks

- Australia is in the Invasion Phase of Varroa mite
- How Varroa enter hives –does it change?
- Develop an easy-to-use hive survival model for Australia
  - Incorporating economic factors to develop **Responsive Thresholds**
  - Improve knowledge of malfeasant-varroa interactions
  - Incorporate new management techniques
- Extension
  - Trusted source
  - Long term relationships



The ultimate aim is that Australia has a resilient, profitable industry

Charts of Varroa Mite Monitoring & Detection Methods

Compiled by Fazila Yousuf

as part of the Project PH22002
"Exploration of advanced control and detection methods for Varroa mite"
Lead by Mary Whitehouse
With contributions from additional collaborators
James Sainsbury, Juliana Rangel, Francesco Stolfi, Mark Harvey, Maciej Maselko, Mark Goodwin.

Funded by the Hort Frontiers strategic partnership initiative
Developed by Hort Innovation, with co-investment from Macquarie University, and contributions from the Australian Government.

These charts contain summarised information distilled from 2000 references on currently used and researched control methods (excluding synthetic insecticides) of Varroa mites.

Contact: mary.whitehouse@mq.edu.au; fazila.Yousuf@mq.edu.au

Category	No.	Label	Varroa life stage	Bee life stage	Mode of application / Action	% varroa	Cost	Environ Regions	Season	Bee-keeper type
Bee inspection	**M1**	Visual inspection (bees)	On bees	A	NA	100	Nil	All	All	S R
Brood inspection	**M1.1**	Visual inspection (brood)	In Brood	A	NA	3.7	Nil	All	Sp, S	S R
	M1.2	Visual inspection (brood)	In Brood	A	uncapping tool (Cappings scratcher, wide-blade shearing comb mounted on a handle)	11.2	Nil	All	Sp, S	All
Removing mites from worker bees	**M2.1**	Alcohol wash	On bees	A	Beaker/Jar, mesh, alcohol+water mix (25%) or methylated spirit (25%)	79.2	Low	All	All	All
	M2.2	Alcohol wash	On bees	A	Beaker/Jar, mesh, alcohol+water mix (25%) or methylated spirit (25%)	92.1	Low	All	All	All
	M2.3	Alcohol wash	On bees	A	Beaker/Jar, mesh, alcohol+water mix (25%) or methylated spirit (25%)	97.5	Low	All	All	All
	M3.1	Soapy water wash	On bees	A	Beaker/Jar, mesh, soap+water mix (1Tsp in 1000ml of water-not very foamy)	79.5	Low	All	All	All
	M3.2	Soapy water wash	On bees	A	Beaker/Jar, mesh, soap+water mix (1Tsp in 1000ml of water-not very foamy)	92.4	Low	All	All	All
	M3.3	Soapy water wash	On bees	A	Beaker/Jar, mesh, soap+water mix (1Tsp in 1000ml of water-not very foamy)	97.2	Low	All	All	All
	M4.1	Sugar shake	On bees	A	Beaker/jar, mesh, white tray, icing sugar	78.6	Low	All except wet	Non-rainy	All
	M4.2	Sugar shake	On bees	A	Beaker/jar, mesh, white tray, icing sugar	95.1	Low	All except wet	Non-rainy	All
	M4.3	Sugar shake	On bees	A	Beaker/jar, mesh, white tray, icing sugar	96.9	Low	All except wet	Non-rainy	All
	M5.1	Apistan® strip in jar	On bees	A	Black mason jar, wire mesh lid, index card, staple pins (to staple Apistan strip)	42.2	Mod	All	Sp	All
	M5.2	Apistan® strip in jar	On bees	A	Strip in black mason jar	81.6	Mod	All	Sp	All
	M5.3	Apistan® strip in jar	On bees	A	Strip in black mason jar	93.8	Mod	All	Sp	All
	M6.1	Ether roll	On bees	A	Mason jar, spray can, 2mm wire mesh	42.4	Low	All	Sp, Au	All
	M6.2	Ether roll	On bees	A	Mason Jar+Tray, white card	66	Low	All	Sp, Au	All
Bottom board mite counts	**M7**	Natural mite fall	In hives	A	Tray/board/sticky board under a mesh floorboard or plastic floorboard	Variable	Mod	All	W, Sp	S R
	M8	Miticide + Sticky board	On bees In hives	A	Apistan /Bayvarol strips, sticky boards	95	High	All	Sp, Au	All

A= Adult; L= Low, M= Moderate, H= High; C= Commercial, S= Sideliner, R= Recreational; Au= Autumn, Sp= Spring, S= Summer, W= Winter

No.	Restrictions and Limitations	Advantages	Time	Repeat	Lit support	Additional Comments	References
M1	Time consuming	Sensitive	2 min/ bee	No	No	Difficult to see mites if hidden underneath the sclerites of honey bees.	Taylor and Goodwin 2021
M1.1	Destructive sampling (kills larvae, pupae). Does not give a quantitative determination of how many mites are present in the hive. Not sensitive, unreliable		5min	No	No	Drone broods are not present most of the year.	Taylor and Goodwin 2021
M1.2	Destructive sampling (kills larvae, pupae), Does not give a quantitative determination of how many mites are present in the hive. Unreliable		5min	No	No	Drone broods are not present most of the year.	Taylor and Goodwin 2021
M2.1	Destructive sampling (kill adult bees). Not sensitive, unreliable		30sec	1 rinse	No		Flores et al 2015; Taylor and Goodwin 2021
M2.2	Destructive sampling (kill adult bees). Not sensitive, unreliable		45sec	2 rinses	No		Flores et al 2015; Taylor and Goodwin 2021
M2.3	Destructive sampling (kill adult bees).	Sensitive, reliable	60sec	3 rinses	Yes		Flores et al 2015; Taylor and Goodwin 2021
M3.1	Destructive sampling (kill adult bees). Not sensitive, unreliable		30sec	1 rinse	No		Taylor and Goodwin 2021
M3.2	Destructive sampling (kill adult bees). Not sensitive, unreliable		45sec	2 rinses	No		Taylor and Goodwin 2021
M3.3	Destructive sampling (kill adult bees).	Sensitive, reliable	60sec	3 rinses	Yes	Reports from Germany indicate need to shake for 45 mins	Taylor and Goodwin 2021 COLOSS 2024 workshop
M4.1	Won't work in wet weather (high humidity) or during a honey flow. Not sensitive, unreliable	Less destructive	15sec	Shake 1	No	Non-lethal	Flores et al 2015; Taylor and Goodwin 2021
M4.2	Wont work in wet weather (high humidity) or during a honey flow.	Less destructive, Quick, sensitive, reliable	30sec	Shake 2	Yes	Non-lethal	Flores et al 2015; Taylor and Goodwin 2021
M4.3	Wont work in wet weather (high humidity) or during a honey flow. Third shake not necessary	Less destructive, Quick, sensitive, reliable	45sec	Shake 3	No	Non-lethal	Flores et al 2015; Taylor and Goodwin 2021
M5.1	Time consuming, won't work if varroa resistant to fluvalinate. Jars must be left to stand for 10min. Not sensitive, unreliable		15sec	+10 min	No		Taylor and Goodwin 2021
M5.2	Time consuming, won't work if varroa resistant to fluvalinate. Jars must be left to stand for 20min	Can be reliable	15sec	+20 min	Yes		Taylor and Goodwin 2021
M5.3	Time consuming, won't work if varroa resistant to fluvalinate. Jars must be left to stand for 30min	Can be reliable	15sec	+30 min	Yes		Taylor and Goodwin 2021
M6.1	Destructive method, mites stick to jar or bee but tray count might give better count. Not sensitive, unreliable		10sec	Yes	No	Environmentally unfriendly and dangerous because of the highly flammable nature of ether.	Ellis et al 1988; Taylor and Goodwin 2021
M6.2	Destructive method, mites stick to jar or bee. Not sensitive, unreliable		20sec	Yes	No	Environmentally unfriendly and dangerous because of the highly flammable nature of ether.	Taylor and Goodwin 2021
M7	Large variation between colonies and time of year. Time consuming. With other information, this can be reliable and accurate. But it requires 2 trips to the apiary.	Can provide efficient results if the mite population is high.	5min	24h	Yes	An effective method in determining whole colony mite populations. Non-invasive and non-destructive method. It depends on the colony population. Other insects such as ants might remove mites from the sticky board (if the board is not sticky).	Taylor and Goodwin 2021
M8	High cost, only detects avg. 15% mites on bees during full brood rearing, two trip are required. Resistant varroa might not respond. Unreliable		15sec	24h	Yes		Taylor and Goodwin 2021

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Monitoring and Control Methods 2 (Researched)

Category	No.	Label	Varroa life stage	Bee life stage	Mode of application / Action	% varroa	Cost	Environ Regions	Season	Bee- keeper type
Remove mites from workers	**M9**	Tobacco smoke & extract	On bees In hives	A	Extracts from tobacco leaves are either sprayed directly in the hives or the leaves are burned in a smoker and then introduced into the hive. Nicotine, a potent alkaloid, present in Tabacco is thought to have acaricidal properties that could potentially disorient or kill the mites. Mites fall onto sticky mat.	13.6	Mod	All	Sp, Au	All
	M10	Crosote or Grapefruit smoke	On bees In hives	A	Crosote bush (Larrea tridentata) or Grapefruit leaves are burned in a smoker and then introduced into the hive. The smoke disrupt the Varroa mites' ability to detect, attach to, or reproduce on the bees. Mites fall onto sticky mat.	Variable	Low	All	Sp, Au	All
Chemical Sensors	**M11**	Olfactory (Gas sensors)	In hives	A	Samples hive air for chemical signs of Varroa mites using technologies like infrared analysers, FT-IR spectrometers, gas chromatography with FID, mass spectrometry, or electronic nose sensors. Detects changes in chemicals/volatiles inside the beehive if varroa infestation is present.	Variable	High	All	All	C
Multi- sensors: Chemical, weight, vibrations	**M12**	Soft-sensor system - "SmartComb"	In hives In brood	A L P	Uses machine learning and advanced analytics to assess environmental and hive conditions for indirect signs of infestation. Requires: metal-oxide gas sensors from hive air+ temperature+ relative humidity+ honey weight+ hive sound.	Unknown	Mod High	All	All	C
Vision - cameras	**M13**	Computer vision system	On bees At entrance	A	Uses computer vision and spectral sensors to monitor bees for Varroa mites as they enter and exit the hive. Cameras and LED lights record the bees, while AI processes the images to detect infestations. One example is sentinel purple hive.	Variable (max 70% av)	High	All	All Incl high brood	C
	M14	Edge-Cloud hybrid computing	On bees At entrance In hives	A	It detects Varroa mites on bees entering hives by using edge devices with a convolutional neural network (CNN) algorithm and long-term tracking on cloud servers.	70%	High	All	All	C
	M15	Laser beam & Camera	On bees At entrance	A	The system scans bees with a laser as they enter the hive, allowing a camera to capture images of Varroa mites for real-time monitoring and management. Analysis is done via image processing algorithms, requiring equipment like a camera sensor, webcam, and precision laser.	Bee count:97% , Mite count:91%	High	All	All	C

A= Adult, L= Larvae, P= Pupae; M Moderate; C= Commercial; Sp= Spring, Au= Autumn

No.	Restrictions and Limitations	Advantages	Time	Repeat	Additional Comments	References
M9	Harmful to the bees in large amounts or if used for longer duration. Unreliable	Natural method.Tobacco is widely vaialble.	20sec	No	-	Ruijter and Eijnde 1984; Abdol-Ahad et al, 2008; Taylor and Goodwin 2021
M10	Prolonged exposure of this smoke kills the bees. Creosote leaves a thick tarry resin. Grapefruit smoke may cause eye irritating effect in humans. Not sensitive, unreliable	Natural method.	1.5 min	No	-	Nguyen 2021
M11	Expensive, time-consuming, and requires skilled personnel. Equipment is bulky, heavy, and energy-intensive.	Enable early detection, non-invasive, may help to determine colony health.		No	Differening reports on effectiveness. One report stated that they are not designed for qualitative and quantitative analysis of complex gaseous mixtures, limiting their usefulness for detailed monitoring. Another that it can detect changes in chemicals/volatiles inside the beehive if varroa infestation is present. Compensates for the air changes within beehive at least diurnally.	Szczurek et al 2020
M12	Positioned in brood chambers. One time sensor purchase, can be expensive if used in all hives -false detections.	Non-destructive, non-invasive. Sensitive, Reliable. Allows beekeepers to manage mite levels proactively without direct hive inspection. May help determine colony health.	1min/6 sam	No	Aims to identify when threshold is reached through remote monitoring.	König 2022
M13	In some versions video is only used at hive entrance because of light availability. Expensive technique. Reliability dependant on how the bees are videoed and scored. Invasive, beehive modification is required. Camera inside brood box is required in some cases. Cloud subscription for data storage is required. AI detection tools, some can work in remote area without internet connections. Variable reliability	Non-destructive, non-intrusive, real-time bee monitoring using cameras.		No	Estimating the % of bees infested is challenging. Purple Hive a key player. Xailient (Australian Tech company uses solar powered computer vision).	Bjerge et al 2019; Schurischuster et al 2018; Earney 2022; Voudiotis et al 2022
M14	Limited to bees entering and leaving hives. Camera image resolution is critical (5Mpx is minimum requirement), requires a cloud subscription. Unreliable. Early detection of mites is limited to detecting varroa on bees at entrance.	Non-destructive, non-invasive,Sensitive, Real-time bee monitoring using cameras. Can notify beekeepers directly, no be-hive modification required.The setup optimizes computational resources.	104-275 secs/ bee image	No	275 secs/ bee image online, 104- 125 secs when offline. Several video based technologies with different modifications. Battery operated, requires minimum of one detection per hour/ once per day. Difficult to match images to bee mites at the measurement distance of 700mm or more. Deep learning models are used.	Mrozek et al 2021; Lee et al 2023; Voudiotis et al 2022
M15	limited to bees entering hives. At least 320x240 resolution is required. Expensive technique.	Sensitive, accurate detection of Varroa on honey bees. Non-destructive, Reliable.	0.477s/ image+	No	0.477s/image, also need time to shake off bees into reading structure. In trial phase, perform well on detecting single bees on the beehive door openings or white background but fail significantly on detecting bees inside the frames (where the mites reside) due to the vast concentrations of bees on each frame.	Chazette et al 2016

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Monitoring and Control Methods 3 (Researched)

Category	No.	Label	Varroa life stage	Bee life stage	Mode of application / Action	% varroa	Cost	Environ Regions	Season	Bee keeper type
Computer analysis of visual images	**M16**	Visual Object detector	On bees At entrance In hives	A	Uses neural networks, YOLOv5 and SSD, to identify Varroa in real-time. Uses high-resolution images.	70%	Mod	All	All	C
	M17	Nvidia Jetson Nano detector	On bees At entrance	A	Machine learning using in-hive cameras to spot Varroa mites on bees entering hives. Uses a CNN model to process images and an IoT module alerts beekeepers immediately upon detection.	Un-known	Mod	All	All	C
	M18	ADAM optimizer technique	On bees At entrance	A	Detects Varroa on bees entering hives using MobileNet and ADAM optimizer. MobileNet is designed for mobile and embedded vision applications. The ADAM optimizer is an optimization algorithm used in training deep learning models. Requires AI, computer vision, and IoT. Uses mobile phones for images.	95%	Mod	All	All	C
Combining sound & image	**M19**	Acoustic & video imaging	On bees In hives	A	Combined acoustic and video imaging with deep machine learning. Camera recordings, data storage and analysis, used Tensor Processing Unit (TPU) and machine learning (ML) models (AI tools),Object detection algorithms YOLOv8, YOLOv7, YOLOv5 and SSD were compared. Audio analysis used Mel spectrograms and mel-freuency spectral coefficients.	49%	High	All	All	C
Vibration	**M20**	Vibration	On bees In hives In brood	A	The method uses accelerometers in beehives to detect unique vibration patterns caused by bee activity and Varroa mite infestations. By applying signal processing and machine learning, it non-intrusively identifies mites, offering beekeepers crucial insights for managing hives.	Un-known	High	All	All	C
Biochemical	**M21**	Fluorescence Spectroscopy + Other Electrochemical techniques	On bees In hives In brood	A	The method measures Varroa mite infestation by analysing honey's biochemical changes, detectable by fluorescence markers with a spectrofluorometer. Parallel factor analysis (PARAFAC) is used to assess infestation levels.	-	High	All	All	S C
Molecular	**M22**	Data analysis using LAMP detection	On bees In hives In brood	A L P	Varroa mite detection is performed using specialized primers targeting the COI locus via LAMP, a rapid and efficient single-tube DNA amplification technique. This method requires mite samples from bee colonies and can be conducted directly in the field.	>99%	Mod	All	All	S C
	M23	Environ DNA (eDNA)	In hives	NA	Parts of bees, mite fragments, bee faeces, or other materials shed from the bees and mites are collected and DNA is extracted by using specific primers and identified using PCR or (qPCR) assays.	-	Mod	All	All	S C

A= Adult, L= Larvae, P= Pupae; M Moderate; C= Commercial, S= Sideliner

No.	Restrictions and Limitations	Advantages	Time	Repeat	Additional Comments	References
M16	Online measurements requiring powerful hardware for deep learning. Conficiting reports of reliability	Provides instant, remote alerts for beekeepers.High resolution images not important for accurate detection.		No	Computer programs: YOLO (You only look once) and SSD (Single shot detector).	Bilik et al 2021
M17	Limited to bees entering hives, Cloud storage and a good quality camera are required.	Sensitive, Reliable. Minimize human interference in beehives.Can be used to monitor mites on a regional scale.		No	-	Wachowicz et al 2022
M18	Limited to bees entering hives The image quality is critical. Give false positive (not sensitive enough to distinguish bee pupa eyes with varroa mites).	Uses three validation methods to monitor beehives. Will also detect hive beetles, ant problems and missing queens.		No	Deep learning models are used.	Divason et al 2023; Torky et al 2023
M19	Cloud subscription. Audio only aimed to distinguish between strong and week hives - had a max 0.998 accuracy at predicting hive health. Not sensitive. Unclear if AI training would be needed with new hives.	Non-destructive and non-invasive, can provide early detection of varroa mites.		No	Ability to detect varroa visually =0.5, accuracy of those detected = 0.974;	Mahajan et al 2023
M20	Research is undergoing to improve accuracy. Requires mites to move. Detecting mites inside brood is not sensitive and unreliable. Variable reliability	Non-destructive and non-invasive. Maybe relatively cheap	3-30 secs	No	BeeHero is researching using sound to measure hive health. Could be a promising and accurate tool. More accuracy is required. Other players in this space include Beeright (Was purple hive, is also using sound to measure hive health) and Y-Trace via the tool Apis Prime$^{(TM)}$ ..	Qandour et al 2014; Hall 2022; Hall et al 2023
M21	Microorganisms can also catalase honey. May give a false positive for varroa mites if honey is not sterile. Variable reliability. Sensitivity depends on many factors, specialised person required, expensive use to equipment, time consuming. Expensive technique.	Can measure the infestation levels.		No	Algorithm based. Catalase is the key marker. Based on determining several parameters of honey quality and composition, eg pollen counts, honey dew elements i.e. algae, fungal spores and hyphae, pollen of nectar less plants. The ratio of protein and phenolic components obtained from the honey emission spectra may be a useful indicator for the level of infestation to which the honey bees were exposed. Other Electrochemical techniques under development for other pests could be used to detect varroa.	Stankovic et al 2023. For more information on other electrochemical techniques please contact Soo Jean Park at soojean.park@mq.edu.au
M22	Only used to confirm Varroa Identification. Sampling from hives required (adult mites from sticky boards or directly from infested bees). Requires varroa sample for ID. Expensive technique.	Rapid ID of varroa in field. Sensitive, Reliable	12-17 min	No	-	Rako et al 2023
M23	Sampling from hives required (through swabs mainly). Chances of contamination. Needs calibration. Only detects presence /absence.	Sensitive, Reliable. Rapid detection of varroa mites. It will be through swabs.		Yes	This technology is developing fast, while in the past it was limited to presence /absence, it is now possible to quantify mite numbers, but this needs calibration.	Dr Roberts, Dr Trujillo Gonzalez (P.Communications) Online search

A= Adult, L= Larvae, P= Pupae; M Moderate; C= Commercial, S= Sideliner

Charts of Varroa Mite Control Methods

Compiled by Fazila Yousuf

as part of the Project PH22002
"Exploration of advanced control and detection methods for Varroa mite"
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With contributions from additional collaborators
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These charts contain summarised information distilled from 2000 references on currently used and researched control methods (excluding synthetic insecticides) of Varroa mites.

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Physical Control 1 (Practised)

Category	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Manage-ment success	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Brood Interup-tion	P1	Queen caging	In brood	Q	L-H	40-90%	LF, W, Esp, Sp	R, C	Placing queen in cage	Varroa can't reproduce because there is no brood. Often used in conjunction with a miticide that targets free-living mites on bees	No
	P2	Queen Trapping comb	In brood	Q	-	-	LF, W,E Sp	C	Confining the queen into trapping comb	Varroa can't reproduce because there is no brood. Often used in conjunction with a miticide that targets free-living mites on bees	No
	P3	Queen ringing	In brood	Q	-	-	LF, W,E Sp	C	placing a ring around the Queens abdomen so she cannot lay	Varroa can't reproduce because there is no brood.	Yes
	P4	Old Queen replacement	In brood	Q	-	-	Sp, S, Au	R, C	A royal cell or a fertilized queen is inserted into the formed nucleus. Alternatively, the honeybees are allowed to raise a queen from the present brood.	The brood cycle is temporarily interrupted. This means that there are fewer, or no, new brood cells available for Varroa to infest and reproduce in.	Yes
Total Brood Removal	P5	Killing capped brood cells	In brood	A,P,L	M-H	50-93%	Sp, ES	R, C	Splitting a hive into two parts one with the brood combs and nurse bees, the other with the foragers.	Removing all the combs containing brood	No
Drone Brood Removal	P6	Capped drone brood cell removal	In brood	P,L	M-H	-	Sp, ES	R, C	Removing capped drone cells	Removing drone brood from a hive by cutting out or removing capped drone cells.	No
	P7	Killing capped brood cells	In brood	P,L	M-H	-	Sp	R, C	Placing drone foundation (trapping comb) into the brood-rearing area of the colony and removing it.	Trapping comb	No
Worker brood removal	P8	Targeted worker brood	In brood	P,L	-	-	Sp, ES	R, C	Causing mites to enter particular worker cells	Trapping comb	Yes
Screen bottom boards	P9	Wire netting bottom boards	On bee In hive	None	L	11-14%	All	C	Board under the hive	Fallen varroa are dropped through screen bottom boards onto a sticky board or tray underneath.	No
Encourage grooming	P10	Powdered sugar	On bee	A	H	>86%	Sp, ES	C	Sprinkling or applying powdered sugar on bees	Dislodges varroa mites from adult bees.	No
	P11	Inert dust	On bee	A	-	-	Sp, ES	R	Sprinkling or applying inert dust on bees such as Talcum powder, Wheat Flour, Baking soda, Corn starch, cinnamon etc.	Dislodges varroa mites from adult bees.	No

A= Adult, P= Pupae, L= larvae, Q= Queen; L= Low, M= Moderate, H= High; C= Commercial, S= Sideliner, R= Recreational; Au= Autumn, Esp= Early spring, Sp= Spring, ES= Early summer, S= Summer, W= Winter, LW= Late winter

Physical Control 1 (Practised)

No.	Restrictions and limitations	Advantages	Additional Comments	References
P1	Handling queens without causing any harm. Possible Queen mortality if not handled carefully. If used in conjunction with miticides, Queen must be removed from the hive before applying the miticide.	Can increase the efficacy of most of the anti-varroa treatments	In combination with oxalic acid or other products would be effective. Cause break in bee brood rearing cycle which can disrupt varroa mating biology. Queens need to be kept in cage for at least 25 days. In short season climates it may affect honey production. Research indicates that caging queens during population decrease can negatively impact hive survival. This technique can be combined with 'total brood removal'.	Wagnitz and Ellis 2010, Gregorc et al. 2017; Buchler et al 2020 (Buchler suggested summer brood interruption)
P2	Handling queen without causing any harm. Possible Queen mortality if not handled carefully.	Can increase the efficacy of most of the anti-varroa treatments	In combination with oxalic acid or other products	Toomemaa and Kaart 2021; Buchler et al 2020
P3	Requires some practise. Possible Queen mortality if not handled carefully. Not recommended for summer brood interruption as queen cells may arise.	Enables the Queen to move with the colony in the hive	Technique needs to be practised on drones to master the technique- similar level of difficulty as marking queens.	Uzunov and Chen 2023
P4	Handling queen without causing harm. Possible Queen mortality if not handled carefully.	Less labour-intensive than total brood removal.	The first step is the orphanage of the colony so there is lack of brood and presence of a new queen meeting certain parameters. A royal cell is inserted and left for 24-25 days so all previous brood merges and have varroa in phoretic phase. At this stage queen fertility is checked and oxalic acid treatment is applied.	Vercelli et al 2023
P5	Need to trap and remove capped brood in timely manner before adult emerges. Applicable when broods are present. Labour intensive, experienced person needed, sacrifice of many broods.	Prevent colonies from continuously rearing brood with critical mite levels. No effect on colony size or honey production.	In combination with oxalic acid or other products. This technique can be combined with 'Queen caging'.	Calis et al 1999, Wilkinson and Smith 2002, Charriere et al 2003, Calderone 2005, Wantuch and Tarpy 2009; Giacomelli et al 2017; FAO. 2020
P6	Need to remove capped brood in timely manner before adult drones emerge. Applicable when drones are present. Labour intensive, experienced person needed, sacrifice of many drones. If drones with varroa not killed it might spike the varroa population.	Prevent colonies from continuously drone rearing brood with critical mite levels. No effect on colony size or honey production. Inexpensive	In combination with oxalic acid or other products. The frame with capped drone cells are removed and frozen to kill drone and mites and placing them back into the hive. The bees can then detect and remove diseased drones.	Calis et al 1999; Wilkinson and Smith 2002; Calderone 2005; Wantuch and Tarpy 2009; FAO. 2020; Bava et al 2023
P7	Need to trap and remove capped brood in timely manner before adult emerges. Applicable when broods are present. Labour intensive, experienced person.	Prevent colonies from continuously rearing brood with critical mite levels. No effect on colony size or honey production.	In combination with oxalic acid or other products (e.g. thymol)	FAO. 2020
P8	Would need to remove attractive brood in a timely manner.	Targets mites in brood chambers without relying on drone production	Use when there are no drones or very high varroa numbers. Can be combined with oxalic acid treatment.	Gregorc et al 2017 For more information contact: Juliana.RangelPosada@ag.tamu.edu; Ph: . 979 845 1074 - paper in prep
P9	Remove during very cold season; May attract scavengers beneath hive; may reduce brood rearing in lowest box during Population Increase (early spring) and bees may be hesitant to go downward into lowest brood box to rear brood. Minimal to little control; may need to close hive bottom when fumigant control chemicals are used; may inhibit brood rearing in lower frames in spring with cool temperatures. Not a stand-alone technique, requires other methods for effective control.	Low-tech and inexpensive; may be used with hive debris sticky board (the sticky board can be used as a monitoring method for Varroa infestation). Varroa fall out of a hive rather than landing on the solid bottom board and returning to the hive on bees entering the hive		Ellis et al. 2001a; Rinderer et al. 2003; Harbo and Harris 2004; Delaplane et al. 2005
P10	Wet or humid weather. Removes free living varroa only	Non-destructive to bees. Removes free living varroa only	Long term, comprehensive field studies have not shown any promising results (e.g. Berry et al. 2012)	Fakhimzadeh 2001; Asha and Sharma 2009; Ellis et al. 2009b; Berry et al. 2012; Stevanovic et al. 2012
P11	Wet or humid weather. Removes free living varroa only.	Non-destructive to bees. Removes free living varroa only	Long term, comprehensive field studies have not shown any promising results (e.g. Berry et al. 2012)	Berry et al. 2012: Macedo and Ellis 2002

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Physical Control 2 (Researched)

Category	No.	Label	Varroa life stage target	Bee life stage	Efficacy	Season	Bee keeper type	Mode of application	Mode of Action	Further Research required
Heating hives Thermo-therapy/ Hyperthermia	P12	Thermovar, Varroa Terminator, Vatorex, The Victor, Mighty Mite Killer, Silent Future Tec, Varroa Kill II	On bee In hive In brood	A, Young bee	>90%	All	C	Electronically heating brood chamber.	The brood combs are heated either from outside or inside.	Yes
	P13	Thermosolar Hive	On bee In hive In brood	A, P, L	80-90%	All	C	Modified hive with Thermosolar Hive that heats the colony periodically.	The bee colony and combs are heated gradually.	Yes
	P14	Mite-Zapper, Drone brood trapping + hyperthermia	In brood	P, L	Up to 100%	Sp, ES	C	Heating brood cells.	Heated trapping comb.	Yes
	P15	Sodium Acetate Trihydrate (SAT)	On bee In hive In brood	A, P, L	-	All	C	An active phase change material (PCM) pack is placed to the brood box.	Heat is distributed within the hive.	Yes
Acoustic disturb-ances	P16	Frequency control	On bee In hive In brood	A, Young bee	-	All	C	Noises/ Ultrasound/ ultrasonic/ square/ sine waves with frequencies (14000-16000 Hz with a decibel level of 80-100 dB).	The sound is applied for 20-40 days. The sound acts on the central nervous system of the varroa mite, so that the old mites die within 10-20 days. Affects varroa mites orientation/communication.	Yes
Electro-magnetic/ Electrostatic forces	P17	Magnetic field	In brood	P	-	All, peak varroa	R	Scanning device, magnets, laser beam are used.	The radio wave blocks the development of Varroa and its larvae and ultimately killing.	Yes
Humidity/ water	P18	Water	On bee In hive	A	-	Sp, ES	R	Swarms are completely plunged for 5 min; Hive relative humidity is increased to 79-85%.	Varroa dislodges from the bees; Impacts varroa reproduction.	Yes
Varroa pheromone Traps	P19	Temperature control pheromones traps	In brood	P	-	All	C	Vapouriser is used to evenly distribute pheromones and temperature for pheromone stability near/inside brood cells.	The sex pheromones disrupt male varroa's ability to copulate with suitable females. Also affects the number of spermatozoa. Other pheromones in the pheromone mix can affect the mite's searching ability of nurse bees.	Yes
Varroa lure trap	P20	Varroa Frame trap	On bees	A	-	All	All	A varroa trapping frame Varroa are lured in by drone pheromones.	The frame contains small entrances that allow varroa to enter but not leave.	Yes
Varroa restriction traps	P21	Modified frame with grid	In hive	P, L	-	All	All	There are two grids in the wooden frames, an upper grid of mesh size of 2mm and a lower grid of 0.2mm.	The upper brood box contains capped brood and varroa. The queen is restricted to the lower brood box where she lay eggs. When Varroa mites from the upper box emerge, they attempt to reach the lower box to infest new brood. However, unable to enter through the lower grid, they ultimately starve and die.	Yes
Varroa Blocker	P22	Varroa Removal Plate (VRP)	On bees	A	23-36 varroa/ day	All, specifically Au, Peak varroa	All	A varroa barrier is placed at the hive entrance with a mesh covered oil tray underneath.	Varroa dislodges from the bees while entering the hive and drops onto the oil tray covered with mesh, catching and removing varroa.	Yes
Small cell foundation combs	P23	Reduced cell form	In brood	P, L	-	Sp, ES	C	~4.9 mm wide cells.	Shorter developmental times of bee pupae, impacting varroa reproduction.	Yes
Propolis	P24	Propolis/ resin	On bee In hive In brood	A	20-100%	All	All	Different methods (Heat treated propolis strip, propolis extract, powder, raw propolis, volatile) are placed within hives or exposed to mites.	The exact mechanisms and modes of action are not yet fully elucidated. But propolis has low narcoleptic (chronic neurological disorder) effect.	Yes

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No.	Restrictions and limitations	Advantages	Additional Comments	References
P12	Requires 360-480min of time per treatment on average. Can be laborious and expensive. Not many commercially available products.	Short exposure of high temperature ≥40°C does not harm bees but lethal to varroa. Environmentally friendly.	Potential to kill mites in capped brood cells and opperate with honey supers.	Athanasakis 2006; Goras et al 2018; Kablau et al 2020; Porporato et al 2022; https://www.vatorex.com/products; SabliĆ and Tolj 2018 (https://patents.google.com/patent/WO2018215806A1/en)
P13	Varroa mites attached to adult bees outside the hive or at the bottom of the hive may not be affected by the initial treatment, necessitating subsequent treatments within 7 to 14 days.	Can cause varroa mortality of 100% within capped brood. Can also protect hives from severe and long winters. Can also suppresses presence of Nosema disease caused by Nosema parasites (Not currently in Australia).	The hive uses solar energy.	https://www.thermosolarhive.com/en/; https://modernagriculture.ca/technology/thermotherapy-varroa-mite/
P14	Might not be effective in the long run. If temperature is not controlled can affect bees. The battery requires replacement.	Efficient in killing varroa mites. Not labour intensive.	It's a modified drone comb with 12- volt battery. The battery heats the comb for 1-5min reaching to 43°C.	Huang 2001; Berry et al 2012; Kablau et al 2020
P15	External ambient temperature has a considerable impact on the performance of the PCM pack.	Environmentally friendly, no need for chemicals, bee losses will be reduced.	More in field research is required.	Brito 2022 (PhD Thesis, University of Nottingham, UK)
P16	Can be expensive to use.	No effect on bees behaviour in any manner. Environmentally friendly. No chemical treatment is required.	The noise/ultrasound is unpleasant and stressful to mites and affects mites feeding. Varroa die after 10-20 days. Long-term field trials are required.	Rosenkranz et al 2010; Rainer 2017; Bary et al 2018; https://www.beesuppliesireland.ie/bee-shop/p/varroa-killer-sound-ireland; Gleich 2017 (https://patents.google.com/patent/DE102016119694B3/en)
P17	Hive modification might be required. Electricity required.	Can also affect other bee parasites in addition to Varroa mites. Does not affect the viability of the bees.	Further research is required to test this technique and suitability of using with other management options such as pesticide (e.g. see Lupi et al 2021). This technique has good potential.	https://patents.google.com/patent/US5162014A/en; https://patents.google.com/patent/RU2383133C1/en; https://patents.google.com/patent/DE102014000968A1/en; Pain et al 2022 (https://patents.google.com/patent/WO2023174982A1/en) Nußer and Wagner 2014 (https://patents.google.com/patent/DE102014000968A1/en)
P18	May impact bees.	Chemical free and environmentally friendly.	This technique under controlled conditions ineffective (Berg, pers. comm. cited from Rosenkranz et al 2010) but require more research. Varroa losses fecundity at absolute humidities of 4.3 kPa (approx. 30 gm−3) and above (Mitchell 2019).	Kraus and Velthuis 1997; Rosenkranz et al 2010; (also see Mitchell 2019)
P19	Reaching inside combs and on honeybees could be challenging. Might cause interference with bee pheromonal communication.	Induces male mites to have sexual activity at an inopportune period in the reproductive cycle of females. Sexually active females have reduced sexual activity	Pheromone-Based Robotic Varroa Trap would be the ultimate design that would attract varroa and then kill them using electric current (Meister et al 2022). Could be combined with other techniques such as varroa restriction traps, thermal devices, predators etc.	Meister et al 2022
P20	Need to add and remove the frames. At concept stage only. Not sure what affect the pheromones would have on the bees - would it stop them producing drones themselves? Would they try to clog up the frames?	Could potentially set and leave for a few weeks.	It could be used with chelifers on the frame to scavenge trapped varroa.	Jermone Favand Personal communication
P21	Some bee larvae are sacrificed. Only effective when bees have brood. Requires varroa to be attracted to brood - no clear evidence of this. Egnores that varroa move around the hive on nursery bees.	Chemical-free method.	This is a concept for varroa restriction within hives by using some modifications in the frames, called Muller Brett. No clear evidence that it works.	Schmid 2020; http://www.imkerpate.de/mullerbrett-erfahrungen/
P22	Cleaning oil tray and mesh.	No harm to the bees; no loss to pollen.	This is a prototype Varroa Removal Device. Could replace oil trap with a sticky mat or even scavenging Chelifers. Could be modified to count mites entering the hive.	Ronald Van Toor Personal communication (rfvt53@gmail.com)
P23	Need special combs.	No harm to the bees.	Standard cells are ~5.3 mm. More studies required. No measurable impact on mite population in several studies (Taylor et al 2008, Ellis et al 2009, Berry et al 2010, Coffey et al 2010, Seeley and Griffin 2011).	Ellis et al 2009; Underwood and Lopez-Uribe 2022 (https://extension.psu.edu/methods-to-control-varroa-mites-an-integrated-pest-management-approach)
P24	Could be an expensive method. Time and labour required.	No effect on bees. Propolis have antimicrobial properties and may help to block virus transmission.	This technique has potential and can be explored for Varroa treatment in Australia.	Garedew et al 2003; Damiani et al. 2010; Pusceddu et al 2018; Habbi-Cherifi et al 2021; Laercio et al 2023 (review on this topic)

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Biological Control 1 (Researched)

Category	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Predator	**B1**	Pseudoscorpion (*Nesochernes gracilis*)	In hives	N/A	?	-	S,C	Augmentative release	varroa predator. Venom to paralyse and kill mites.	Yes
	B2	Pseudoscorpion (*Chelifer cancroides*)	In hives	N/A	~25 %	All	H	Augmentative release	varroa predator. Venom to paralyse and kill mites.	-
	B3	Mite (*Stratiolaelaps scimitus*)	In hives	N/A	-	No brood period	S, C	Augmentative release	varroa predator	No
Secondary metabolites	**B4**	Purified destruxin (DTX) (=mycotoxins), fractions A, B, CE and D - derived from *M. anisopliae*.	On bees, In hives	A,P,L	-	All	All	Crude or fractionated Dtx, dissolved with water, ethanol or acetone were sprayed with a small volume perfume sprayer or vaporised with a compressor nebulizer (on mites/bees).	The mode of Action is not fully understood.	Yes
	B5	*Lactobacillus johnsonii*	On bees	A	-	All	All	Metabolites are synthesized by the bacteria in cell-free supernatant (CFS). The CFS was then supplied to the freshly emerged bees in the lab setup.	Pathogens cause infections in Varroa. Mode of action is unclear.	Yes
	B6	Platynecine, (Alkaloid produced by different bacteria (Bt, *Bifidobacterium asteroides*)	on bees	A	Variable	All	All	Solution spray	Miticidal activity. Mode of action is unclear.	Yes
	B7	Venoms (*Chelifer cancroides*)	On bees	A	-	All	All	Venom applied as bioactive compounds.	Causes inhibition of a voltage-gated insect potassium channel (Shaker IR) and modulates the inactivation process of voltage-gated sodium channels from varroa	Yes

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No.	Restrictions and limitations	Advantages	Additional Comments	References
B1	Lab based, only few field trials. Will consume varroa mites. NZ species - not sure if in Australia. Breed in temperatures much cooler than hives	Can be mass-reared, making it easy to consider them a long-term solution against varroa mite.	Another pseudoscorpion, *Heterochernes novaeealandiae* , was also found in NZ hives and consumed varroa, but could not be bred in captivity and preferred cooler temperatures.	Donovan and Paul 2005; Read et al 2014
B2	Not found in Mainland Australia, only known from Tasmania. Field tests with 50 Chelifers /hive did not reduce mite numbers. The juvenile chelifers disappeared from the hives - not sure if eaten or moved.	Can be mass-reared, making it easy to consider them a long-term solution against varroa mites. Breeds at 36 degrees (prefers 30 degrees). Doesn't attack bee larvae or eggs and will go into brood cells. Can breed at brood temperatures and is tolerant to pyrethroids and thymol miticides. Structures developed enabling them to live in hives. They can be phoretic on honeybees, thus presenting the possibility of natural spread to new beehives.	Could be useful if it attacks other pests as well, such as small hive beetles. Has been used with a device to knock mites off bees, which are then consumed by the chelifers. A recent study shows the potential of using venom from *C. cancroides* to control varroa mites (Krämer et al 2021). See below.	Donovan and Paul 2005, van Toor et al 2015; Krämer et al 2021 For more information contact Ronald.vanToor@plantandfood.co.nz / RonvT@gmail.com; +64 (27) 285 2720, Additional papers in prep
B3	Only feed on non-sclerotized parts of varroa. The hive environment is not suitable (lack of firm substrate and high temperature). Prefer honeybee eggs to adult mites (unprotected brood stages of the honeybee itself). Early and late fall introductions did not lead to a decrease in mite pressure in hives. Sensitive to organophosphate insecticide (chlorpyrifos), however, pyrethroid insecticide lambdacyhalothrin is slightly harmful.	Several pesticides (such as spinetoram, abamectin, azadirachtin, azoxystrobin, difenoconazole, iprodione, and thiamethoxam) were harmless. No negative effect of *Metarhizium brunneum* and *Beauveria bassiana* (Lin et al. 2017; Sun et al. 2018) or the entomopathogenic *nematode Steinernema feltiae* (Saito and Brownbridge 2016)	Endemic to the rainforests of Australia. At 25–33 °C mites moved rapidly while 14 °C or below they are inactive.	Rangel et al 2018; Rondeau et al 2018; Rondeau et al 2019
B4	High dose can also cause high mortality in honeybees (particularly brood). Need more research.	Extraction of destruxin is quick and inexpensive. Soluble in water making spray application the easiest to treat the hive. Once extracted, they remain stable for a month when stored at temperatures between 4 and 8 °C. It should also be noted that the action of destruxin is not affected by the microenvironmental characteristics of the hive. Dtx activity is restricted to host-pathogen, thereby not posing a risk to human health by contamination of the environment or by entering the food chain.	Dtx C and E showed the most promising results against Varroa. Dtx B produced a high Varroa mortality, but also caused a significantly higher mortality in bees when used with the same concentration that was effective on the mites. Further field research is required to investigate fractions, doses, solvents, and methods of administration, which may contribute to the control of Varroa populations without harming bees.	Lodesani et al 2017
B5	-	No toxic effect on bees. Field trial showed an increase of colonies population over time. Nosema ceranae development also affected.	Future studies should be performed to increase our knowledge of the physiological effects of bacterial metabolites on the health of bee colonies.	Audisio et al 2015; Piano et al 2020
B6	-	No toxic effect on bees.	More work is required.	Manici et al 2020
B7	-	-	A recent study shows the potential of using venom from *C. cancroides* to control varroa mites.	Krämer et al 2019; Krämer et al 2021

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Biological Control 2 (Researched)

Category	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Bacteria	B8	*Snodgrassella alvi*	On bees	A	-	All	All	Bee symbiotic bacterium caused varroa mortality when varroa fed on bees.	Symbiotic bacterium from honeybees' gut. S. alvi repeatedly producing dsRNA against essential genes for the acari and were successfully fed to the bees.	Yes
	B9	*Bacillus thuringiensis*	On bees, In hive	A	80-93%	All	All	Contact; Spray (ingestion by varroa); agar disc onto the top bars of the frames of comb in the hive (1/hive box).	Produces toxins that damage the gut lining of the mite. Varroa shook, regurgitated, suffered intestinal inflammation, and died. Causes intestinal inflammation (dysentery) in varroa.	Yes
	B10	*Bacillus asteroides*	On bees, In hive	A	46%	All	All	Sprayed/ Immersion, Contact.	Pathogens cause lethal infections in Varroa.	Yes
	B11	*Bacillus mycoides*	On bees, In hive	A	62%	All	All	Sprayed/ Immersion, Contact.	Pathogens cause lethal infections in Varroa.	Yes
	B12	*Lactobacillus johnsonii*	On bees, In hive	A, P, L	72%	All	All	Sprayed/ Immersion, Contact.	Pathogens cause lethal infections in Varroa. The mode of action is not known yet.	Yes
	B13	*Lactobacillus salivarius*	On bees, In hive	A	70%	All	All	Sprayed/ Immersion (mixed in sugar syrup), Contact.	Pathogens cause lethal infections in Varroa. The mode of action is not known yet.	Yes
	B14	*Lactobacillus kunkeei*	On bees, In hive	A, P, L	95-100%	All	All	Sprayed/ Immersion, Contact.	Causes mortality within 3 days. Miticidal effect of unidentified mode of action.	Yes
	B15	*Lysinibacillus fusiformis*	On bees, In hive	A, P, L	95%	All	All	Sprayed/ Immersion, Contact.	Pathogens cause lethal infections on mites.	Yes
	B16	*Lysinibacillus macroides*	On bees, In hive	A, P, L	90%	All	All	Sprayed/ Immersion, Contact.	Causes mortality within 3 days.	Yes
	B17	*Lysinibacillus varians*	On bees, In hive	A, P, L	83%	All	All	Sprayed/ Immersion, Contact.	Pathogens cause lethal infections on mites.	Yes
	B18	*Bifidobacterium asteroides*	On bees, In hive	A, P, L	-	All	All	Sprayed/ Immersion, Contact.	Pathogens cause lethal infections on mites. Miticidal effect of unidentified mode of action.	Yes
	B19	*Pantoea dispersa*	On bees, In hive	A, P, L	53%	All	All	Sprayed/ Immersion, Contact.	Pathogens cause lethal infections on mites.	Yes
	B20	*Enterobacter cloacae*	On bees, In hive	A, P, L	70-89%	All	All	Sprayed/ Immersion, Contact.	Cause lethal infections in varroa (bursting of membranes between dorsal and metapodal shields.	Yes

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No.	Restrictions and limitations	Advantages	Additional Comments	References
B8	Lab trial only	Ecto-parasites fed from bees nourished with the engineered bacteria died faster than mites fed upon control bees.	Bees' gut bacteria can contribute to the better survival of parasitized honeybees.	Leonard et al 2020
B9	Generally, no lethal effect on honeybee adults and larvae in the short term with low dosage was observed. Dosage is critical for causing toxicity to bees. Bt toxins are very specific. Requires 24h to kill Varroa in most studies.	Some Bt strains showed no negative affect on adult bee and larvae. Can be naturally present in honey samples.	Different strains of Bt produce 100s of proteins each of which is toxic to specific invertebrate groups. These proteins rupture the intestinal wall of the targeted insect,which dies of septicemia. Not enough field studies to support Bt use for varroa control. Bt is present on Varroa corpses. Bt-derived products constitute 95% of the world's biopesticide market.	Alquisira-Ramírez et al 2012, 2014, 2017; Manici et al 2020; Sacca and Lodesani 2020; Kadhim et al 2021; Usta 2021
B10	-	-	-	Manici et al 2020
B11	-	Can be naturally present in honey samples. Also found from Varroa.	Present within varroa and have insecticidal role and can potentially be used against varroa. More work is required.	Usta 2021
B12	-	Potential for colony health improvement, enhance bee survival and increase bee proteins. Reduce the infestation levels of both Nosema spp. and Varroa.	Indirect approach. Feeding honeybees with probiotics can enhance bees defence against Varroa. Known for immune system stimulation in bees.	Audisio et al 2015; Paino et al 2017; Hubert et al 2017; Sabaté et al 2012; Usta 2021
B13	-	Reduce the infestation levels of both Nosema spp. and Varroa. Found in bee intestine. Promotes a high honey yield (Novicov et al 2017).	Reduced the levels of in situ varroosis (the disease caused by Varroa mites) by 50-80%	Tejerina et al 2020
B14	Safety and long-term effects need assessment, efficacy in varied environmental conditions and impact on bee health.	Natural antagonist to Varroa	Honeybee's cuticle microbiota, where bacteria already fit the micro-environment of the hive, the isolated strains able to induce (95-100%) Varroa mortality within 3 days after spraying (Lab results). Field studies are required to understand its efficacy and mechanism.	Manici et al 2020; Sacca and Lodesani 2020
B15	Safety and long-term effects need assessment	Natural antagonist to Varroa. Also found from Varroa.	More work is required. Part of a study isolating bacteria from Varroa.	Sabaté et al 2012; Audisio et al 2017; Usta 2021
B16	-	Also found from Varroa.	Present within varroa mites and have insecticidal role and can potentially be used against varroa. More work is required.	Usta 2021
B17	-	Also found from Varroa.	Present within varroa mites and have insecticidal role and can potentially be used against varroa. More work is required.	Usta 2021
B18	-	Can be naturally present in honey samples.	honeybee's cuticle microbiota, where bacteria already fit the micro-environment of the hive, they isolated strains able to induce V. destructor's death within 3 days after spraying. Also play a role in inhibiting bee pathogens, in particular (*Paenibacillus larvae*, *Melissococcus plutonius* and *Ascosphaera apis*)	Manici et al 2020; Sacca and Lodesani 2020
B19	-	Also found from Varroa.	Present within varroa mites and has an insecticidal role and can potentially be used against varroa. More work is required. Isolated from the gut of *A. cerana* , benefit the bees by improving immunity (Disayathanoowat et al 2012).	Usta 2021
B20	-	May also cause fungal hyphal lysis and cytoplasmic leakage and inhibit chalkbrood fungal (*Ascosphaera apis*) disease in bees (khan et al 2020). *Enterobacter cloacae* has optimum growth temperature between 30-37 °C making it suitable for hive conduction.	Other species of Enterobacter has potential to be tested as biocontrol agent against varroa. More work is required.	Hrabak 2003; Nazzi et al 2020

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Biological Control 3 (Researched)

Category	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Fungi	B21	*Lecanicillium lecanii*	On bee In hive	A	Variable	All	All	Contact/spray	Pathogens cause lethal infections on mites	No
	B22	*Metarhizium anisopliae*	On bee In hive In brood	A, L	Variable (50-100%)	All	All	Coated on strips placed between frames; sprinkling as dust in the hive; as a liquid (spray between frames), solid (sporulating fungus +media); using auto-applicator device; mixed with wax powder; protein patty.	Repellent affect. Nurse bees carrying spores repel Varroa. Pathogenic effect on mites. Spores infect Varroa by forming conidia and penetration via appressoria followed by haemocoel invasion causing death.	Yes
	B23	*Metarhizium bruneum*	On bee In hive	A	90%	All	All	Contact/spray; Agar disc onto the top bars of the frames of comb in the hive (1/hive box).	Pathogens cause lethal infections on mites.	-
	B24	*Beauveria bassiana*	On bee In hive In brood	A	90-100%	All	All	Coated on strips placed between frames; sprinkling as dust in the hive; as a liquid (spray between frames), solid (sporulating fungus +media); using auto-applicator device; mixed with wax powder; protein patty.	Pathogens cause lethal infections on mites.	Yes
	B25	*Hirsutella sp.: H. thompsonii, H. gigantea, H.citriformis, H. kirchneri, H. necatrix*	On bee In hive	A	50-97%	All	All	Contact/spray	Penetrate mites through legs, later forming hyphal bodies in chains in the hemolymph.	Yes
	B26	*Verticillium lecanii*	On bee In hive In brood	A, P, L	59-100%	All	All	Contact/spray	Pathogens cause lethal infections on mites.	Yes
	B27	*Paecilomyces ssp.: P. farinosus, P. fumosoroseus*	On bee In hive In brood	A, P, L	Up to 100%	All	All	Contact/spray	Pathogens cause lethal infections on mites.	Yes
	B28	*Tolypocladium spp.: T.inflatum, T.niveum*	On bee In hive In brood	A, P, L	Up to 100%	All	All	Contact/spray	Pathogens cause lethal infections on mites.	Yes
	B29	*Clonostachys rosae*	On bee In hive In brood	A	60%	All	All	Solution spray	Pathogens cause infections in Varroa. Mode of action is unclear.	No
	B30	*Trichoderma harzianum*	On bee In hive	A	>70%	All	All	Contact/spray	Pathogens cause lethal infections on mites.	Yes
	B31	*Apergillus sp.*	On bee In hive	A	-	All	All	Contact/spray	Pathogens cause infections in Varroa. Required 1-3 days to kill Varroa. Mode of action is unclear.	Yes

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No.	Restrictions and limitations	Advantages	Additional Comments	References
B21	Less pathogenicity to Varroa. Not efficient in killing Varroa. Sensitive to hive conditions.	Little effect on the bees (limited data available). Need more research.	More work is required. Better application method to be developed.	Gerritsen and Cornelissen 2006
B22	Adaptation to hive conditions (e.g. temperature); limited by cost and availability. Require 2-13 days on average to kill varroa depending on hive temperature and humidity. May also affect honeybee brood inside capped cells/adult bees. Dosage dependent, more research is required. Mites might develop resistance against EPF.	Can stay up to 42 days after first treatment. No need for repeated treatments. Conidia carrying nurse bees may also repel varroa. No impact on colony strength and development.	Possible alternative to chemicals.Combining oxalic acid with Metarhizium increases efficacy. Variant maybe important. The var. BIPESCO 5 was effective. A commercial version Bioranza was promising. No effect on any stages of bees at this stage. Compatible with biochemicals such as vegetable oils.	Kanga et al. 2002; Shaw et al. 2002; Kanga et al 2003; Lodesani et al. 2004; James et al 2006; James and Hayes 2007; Hamiduzzaman et al 2012; Ahmed and Abd-Elhady 2013; Pirali-Kheirabadi et al. 2013; Goswami et al 2016; Reinbacher et al 2018; Steenberg et al 2018; Sinia et al 2018; Araya et al. 2019; Bava et al 2022; Wathah 2023. Field: Gerritsen and Cornelissen 2006; Kanga et al 2006; Rodríguez et al 2009; Kanga et al 2010; Ferrari et al 2020; Han et al 2021; Bava et al 2022
B23	High temperature in hive is also a problem for fungal growth.	-	This has potential in Australia if we could use as a preliminary control strategy (or alternative to chemicals). Though mites can also develop resistance against Entomopathogenic fungi (EPF). The combination of oxalic acid with *Metarhizium* increases treatment effectiveness.	Yetis 2019; Han et al 2021
B24	May affect honeybee brood inside capped cells. Dosage dependent. Mites can develop resistance against Entomopathogenic fungi (EPF).	When spores of B. bassiana were sprayed inside hives, adult bee mortality did not differ from control treatments. Naturally present in hives and in brood cells.	Possible alternative to chemicals. Isolated from varroa in Russia, France, Spain, Denmark, and Costa Rica. Multiple applications increase efficacy and cost. A commercial version of Biovar was promising. No effect on any stages of bees.	Kanga et al. 2002; Shaw et al. 2002; Meikle et al 2006; Meikle et al 2007; Meikle et al 2008; García-Fernández et al 2008; Meikle et al 2009; Steenberg et al 2010; Sinia et al 2018; Steenberg et al 2018; Hamiduzzaman et al 2012; Meikle et al 2012; Araya et al. 2019; Leite et al 2022. Field: Meikle et al. 2008; Ahmed and Abd-Elhady 2013; Sewify et al. 2015
B25	Short shelf life that can be for a few weeks and is highly sensitive to the environmental conditions.	Safe for bees. No toxicity reported.	Also used against other mites (e.g., Red spider mites and coconut eriophyid mites). Not all species of *Hirsutella* gave high Varroa mortality, e.g. *H. kirchneri* was very low only 15%.	Peng et al 2002; Shaw et al. 2002; Kanga et al. 2002; Goswami et al 2016; Reddy et al. 2020
B26	High temperature in hive is also a problem for fungal growth.	Low mortality to bees. More research is required.	Efficacy varies with relative humidity and temperature. Further research is required. Compatible with biochemicals such as vegetable oils (Wathah 2023).	Shaw et al. 2002; Meikle et al 2012; Goswami et al 2016; Wathah 2023
B27	-	-	Efficacy varies with relative humidity and temperature. Further research is required.	Shaw et al 2002
B28	-	-	Efficacy varies with relative humidity and temperature. Further research is required.	Shaw et al 2002
B29	Limited pathogenicity to varroa in laboratory trials.		More work is required. Better application method to be developed.	Hamiduzzaman et al 2012; Sun et al 2020
B30	-	Repellent effect against varroa mites	More work is required. Better application method to be developed.	Sammons and Johnson 2022
B31			More work is required.	Chernov 1981

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Genetic Control 1 (Researched)

Category	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Hygienic Behaviour	G1	The Minnesota Hygienic Bees	In brood	A,P,L	25-75%	-	C	Queen bees	Bees remove varroa-infested broods	No
	G2	Russian Honeybees	On bee In hive In brood	A,P,L	25-75%	-	C	Queen bees	Bees remove varroa-infested parasitized larvae. inhibit mite reproduction. Bees groom off varroa.	No
	G3	POL-line Hygienic	On bee In hive In brood	A,P,L	0-24%	-	C	Queen bees	Bees remove varroa-infested broods. Bees groom off varroa mites.	Yes
	G4	Indiana "mite-biter"	On bee In hive In brood	A,P,L	25-75%	-	C	Queen bees	Bees groom off varroa and they bite their legs off.	Yes
	G5	Saskatraz	On bee In hive In brood	A,P,L	~68%	-	C	Queen bees	Bees remove varroa-infested sick or dead broods	Yes
	G6	Primorsky A. mellifera	On bee In hive In brood	A,P,L	-	-	C	Queen bees	Bees remove varroa-infested sick or dead broods	Yes
	G7	Varroa Sensitive Hygiene (VSH)	On bee In hive In brood	A,P,L	25-75%	-	C	Queen bees	Bees remove varroa-infested broods	No

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No.	Restrictions and limitations	Advantages	Additional Comments	References
G1	Maintaining these traits in populations over time and ensuring these traits don't reduce other beneficial bee behaviours or honey production. Bee selection is through assessing the removal of freeze-dried dead broods. Labor intensive.	Removes varroa infested broods.	These are bred from Italian stock (Apis mellifera. ligustica). Can remove 66% of varroa-infested pupae.	Spivak and Gilliam 1998; Spivak and Reuter 2001; Ibrahim and Spivak 2006
G2	Maintaining these traits in populations over time and ensuring these traits don't reduce other beneficial bee behaviours or honey production. High frequency of queen loss when managed commercially (Danka et al 2012).	Has high heritability of hygienic behaviour. Russian bees have lower percent brood infestation and fewer multiply-infested cells, and bees inoculated with the mite-vectored deformed wing virus exhibit significantly less viral replication.	More resistant to varroa and tracheal mites (Acarapis woodii) than other A. mellifera stock.	Rinderer et al 2001a &b; de Guzman et al 2005, Tarpy et al 2007; Ward et al 2008; Danka et al 2012 & 2013; Rinderer et al 2014; Kirrane et al 2018; Underwood and López-Uribe 2022
G3	Maintaining these traits in populations over time and ensuring these traits don't reduce other beneficial bee behaviours or honey production. Low pesticide tolerance in brood, Sensitive to deformed wing virus and Israeli Acute Paralysis virus infections.	Can reduce mite populations when compared to VSH stock	Lack of enough literature to proof its efficiency. This breed is a result of outcrossing VSH queens to U.S. commercial stocks (Italian) and then selecting for low mite infestations.	Bhatia et al 2021; Danka et al 2016; Khongphinitbunjong et al 2016; Milone et al 2020
G4	Maintaining these traits in populations over time and ensuring these traits don't reduce other beneficial bee behaviours or honey production.	Can reduce mite populations when compared to non-selected stocks	Lack of enough literature to proof its efficiency. Also see Ankle biters/leg chewers trait honeybees (Underwood and López-Uribe 2022).	Hunt et al 2016; Morfin et al 2020; Smith et al 2021; Underwood and López-Uribe 2022
G5	Maintaining these traits in populations over time and ensuring these traits don't reduce other beneficial bee behaviours or honey production.	Survive longer, gentle behaviour, and produce more honey than non-resistant stock. Have good overwintering abilities.	Lack of enough literature to proof its efficiency. Result of cross among different races (*A. melifrea* carnica, ligustica, mellifera) with Russian bees in an isolated apiary in Canada.	Robertson et al 2014; Robertson et al 2020
G6	Maintaining these traits in populations over time and ensuring these traits don't reduce other beneficial bee behaviours or honey production. Population survivability is unclear.	Have good overwintering abilities. Heightened aggression toward small hive beetles and exhibit resistance to tracheal mites.	The resistance level to varroa is unclear. Original stock bees from Tunisia has lower mortality and mite infestation rates but in introduced areas their success is unclear.	de Guzman et al 2007; 2019; Kefuss et al 2004; Tarpy et al 2007; Kefuss et al 2004
G7	Maintaining these traits in populations over time and ensuring these traits don't reduce other beneficial bee behaviours or honey production. Finding an effective and reasonably priced way of genotyping the queens in Australia has been difficult to obtain.	A sustainable approach that uses natural bee behaviours. Good performance in crop pollination. Lower free living (phoretic) mites in colonies.	There is an adenine/guanine (A/G) Single Nucleotide Polymorphism (SNP) located on chromosome 9 at the nucleotide position 9224292 of the honeybee genome (assembly Amel 4.0). The G allele of SNP 9-9224292 is associated with VSH behaviour. Bees detect and remove infested pupae with reproducing varroa. More hygienic than the Minnesota hygienic stock of bees. Can remove 85% of infested pupae. This trait is recognized by testing.	Harbo and Harris 2005; Ward et al 2008; Underwood and López-Uribe 2022

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Genetic Control 2 (Researched)

Category	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Suppressed Mite Reproduc-tion (SMR)	**G8**	Foundress mites reproduction	In brood	P,L	-	-	C	Modifying the genes of Adult workers, and/or brood.	Foundress mites either: 1- Produce no male, 2- Produce no eggs, 3- Delayed egg laying.	Yes
Marker Assisted Selection (MAS)	**G9**	Genotyped Queens	On bee In hive	A	~28-44%	-	C	Queen bees	Bees remove varroa-infested broods.	Yes
Supressed mite reproduc-tion Genetic Maipula-tion - RNAi	**G10**	RNA Interference (RNAi)	On bee In hive In brood	A,P,L	Variabl e	A-S	C	Soaking by spraying inside the hives. Bees consume double-stranded RNA (dsRNA) which is then transferred from the bees to the mites (feeding on fat bodies).	The dsRNA leads to gene silencing in the mites, impacting their reproduction.	Yes
	G10.1	RNA Interference (RNAi)	On bee In hive	A	40%	-	C	worker bees fed sugar water, mites pick up the RNAi from them.	Targets the Deformed wing virus.	Yes
	G10.2	RNA Interference (RNAi)	In brood	A	-	-	-	Nurse bees fed RNAi in sugar water pass the RNAi to the larvae.	Uses a virus to stop production of a protein in mites necessary for reproduction.	Yes
Gene manipula-tion - CRISPR	**G11**	CRISPR-Cas9	On bees	A,P,L	Can be 100%	-	C	Genes are edited in honeybees' embryonic stage.	The adult bees have resistance to varroa characters (detecting varroa and removing, chewing varroa, affecting varroa reproduction within cells).	Yes
Releasing Modified Mites	**G12**	Targeted gene editing	On bee In hive In brood	A,P,L	NA	-	C	Releasing Modified Mites to Control Population.	Genes interfere with varroa reproduction.	Yes
Microbe RNA techno-logy	**G13**	Bees with modified gut microbe	On bee In hive In brood	A,P,L	70%	-	C	Genetically engineered bacteria are increased through feeding or injection into adult bees. These bacteria becomes part of bees and act as a living vaccine.	Honeybee gut microbe(s) produce dsRNA which circulates in the bees making them resistant to mites and their associated viruses. The dsRNA affects the mites by dismantling some of their genes (reproductive).	Yes

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No.	Restrictions and limitations	Advantages	Additional Comments	References
G8	Scoring/detecting SMR is a tedious process. Difficult to assess during low brood season. Environment also impact (such as influx of mites from neighbouring colonies).	Lower mite reproduction. SMR can be transmitted by queens to their progeny and expressed in colonies even if the founding females are mated with unselected drones. SMR has a strong dominant genetic component that can be passed across generations by males.	Not enough studies in this area. The exact mechanisms through which the brood/adult may impair varroa reproduction are still unclear. Season also impacts mites' reproduction. SMR can be increased by prolonged queen caging or the application of trapping combs. SMR bees remove more varroa-infested pupae than bees that had been selectively bred for hygienic removal of the freeze-killed brood. Bees with SMR traits are also called VSH.	Beaurepaire et al 2019; Mondet et al 2020
G9	Maintaining these traits in populations over time and ensuring these traits don't reduce other beneficial bee behaviours or honey production. In breeding, can affect MAS.	Affects free living (phoretic mites).	It is VSH genotype. If these genes are expressed in Queens, it might have higher VSH behaviour. The MAS tools help improving breeding stock at a large scale.	Sainsbury et al 2022
G10	Delivering the dsRNA to mites without harming bees (i.e. high impact on non-targets). Potential development of resistance by the mites. Successful gene knockout or knock-in could be a problem. This is reversible and the duration of the effect is temporary, so the efficiency is never 100%. It requires repeated treatments (Labour intensive). Long-term and potential risk of mutations or off-target effects are still unclear and largely debated.	It doesn't require special equipment like other CRISPR technologies. Safe for the bees, indicating no off-target effect. Can be applied in any season but preferably in autumn and summer.	The usual delivery system = horizontal transfer via the host bees. Can use electroporation (using a high voltage pulse to overcome a cell membrane) or inject bees. GreenLight Bioscience has trialed the use of RNAi to target mites. Https://www.sciencelearn.org.nz/resources/3251-using-rnai-to-control-varroa-mites. The long-term impact on bees is still unclear.	Campbell et al 2010; Garbian et al 2012; Mani et al 2022; Muntaabski et al 2022; Nganso et al 2022
G10.1	Currently patented by "Green Biosciences". Problems in field trials, possibly non-target effects. Bayer had trouble stabilizing the RNAi under field conditions, they found it wasn't effective in the field.	-	Developed by Bee-o-logics, originally to control Israeli Paralysis Virus. Bought out by Monsanto, who were bought out by Bayer. Patent has now been bought by "Green Biosciences".	S.Safer, J.Rangel Personal Communication
G10.2	How do the mites pick up the RNAi fed to the larvae - is it deposited in the fat bodies? Can worker bees attacked by mites also pass on the RNAi? How much is necessary to feed nurse bees under field conditions	Attacks mites in brood cells	Greenlight Biosciences hold the patent for the product. Most recient studies in The States indicate that underfield conditions in Autumn it an keep mite numbers stable. (J.Cameron, pers comm.)	https://www.beeculture.com/rnai-varroa-control/. Contact Prof. Phil Lester for more information: <phil.lester@vuw.ac.nz> Mobile +64 21 243 5096
G11	Inbreeding along with haplodiploidy in varroa reduces the likelihood of gene drive spreading effectively. Potential unintended consequences, and long-term impacts on bee populations. This is irreversible, knockout or knock-in gene, may be propagated to future generations. It requires expensive equipment and highly skilled personnel. Will change the honeybee genome.	Precise modifications can be made to target the mites specifically. Low impact on non-targets. This technology irreversibly affects gene expression.	This is an RNA-guided nuclease technology. Only a few studies are available. CRISPR-Cas9 system works at the DNA level in the nucleus.	Nganso et al 2022
G12	Potential ecological implications. Requires continuous release of large numbers of modified mites.	-	Using techniques similar to the Sterile Insect Technique (SIT) where modified mites are released to mate with wild mites, but no viable offspring are produced. This research is in the infant stage.	Faber et al 2021
G13	Delivering gut microbe to bees. Microbe might not be easy to contain, raising concerns about using this approach in the wild. May need approval from Australian regulatory bodies.	Can be transferred to the next generation. No harmful impact on bees. Does not alter bees' genetic.	This research is in the infant stage. https://www.science.org/content/article/mite-destroying-gut-bacterium-might-help-save-vulnerable-honey-bees	Pennisi 2020

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Biochemical Control 1 (Practised) (Researched)

Category	Category2	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Acids	Oxalic acid	**C1**		On bees In hives	Q, A	77-93%	Au,W	C	Strips, vapor, crystals, or as a liquid dribble (sucrose solution placed on top of brood chamber frames), Using glycerol may prevent honeybees from oral ingestion of oxalic acid. Different application methods and concentrations are available.	Acid crystalises on varroa body affecting cuticle proteolytic enzymes	No
	Formic acid	**C2**	Varromed®	On bees In hives In brood	A,L	40-92% (var)	Spr	C	Evaporator	Interferes cellular respiration	No
Botanical Canna-beaceae	Hop oil (*Humulus lupulus* L)	**C3**	HopGuard®	On bees In hives	A	50-80%	Au,W	C,H	From hop leaf extract (methanolic and ethanolic extracts), active compound is beta acids. Strips placed between frames, topical application	Asphyxiation of the varroa	Yes
Botanical Lamiaceae (mint family)	Thymol (*Thymus vulgaris L.* T. willdenowii, plus other spp.)	**C4**	Apiguard ®; Thymovar®; Apilife Var®	On bees In hives	A	Var (up to 100%)	Esp, S	C	Derived from thyme oil, contains Carvacrol and p-cymene). Fumigation, gel formulation with slow releasing vapours	Acaricidal and high repellent activity	No

Category	Category2	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Acids	Lactic acid	**C5**		On bees In hives	A		Au,W	C	Topical treatment, Spray	Impair the attachment ability of varroa to honeybees	Yes
Botanical various	Derivative of the aromatic compound allylbenzene	**C6**	Anethole oil		A,L			C	occurs widely in plants,		Yes
Botanical Amary-llidaceae	Garlic bulb *(Allium sativum)*	**C7**	Extract	On bees In hives In brood	A,L	90%-86% -		C	Fumigation		Yes
Botanical Apiaceae / Umbelli-ferae	Dill (*Anethum graveolens)*	**C8**						C	Applied with acetone topically		
	Fennel *(Foeniculum vulgare) oil*	**C9**	Fennel oil	On bees In hives In brood	A,L	66%		C	Fumigation		No
	Ferula *(Ferula assafoetida)*	**C10**						C			No
Botanical Aristol-ochiaceae	Wild ginger - Manchurian *(Asarum spp.)*	**C11**		On bees In hives In brood	A,L	24%		C	Fumigation		
Botanical Asteraceae (Daisy family)	Chilca - *Eupatorium buniifolium*	**C12**				100%		C		Winter and summer leaves, summer twigs were used to extract oil	

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No.	Restrictions and limitations	Advantages	Additional Comments	References
C1	High and sub-lethal doses can be harmful to the bees causing internal tissue damage or disruption of the proteolytic activity of the cuticle, impeding immunity. Long-term use can effect loss of brood, workers, and sometimes queen. Do not kill varroa within brood cells. Direct contact may be toxic to honeybees than vapours. Shorter efficacy (1-2days) and required repeated application.	Naturally present; Does not leave high residual concentration in wax.	No resistance reported. Varroa is unable to detect oxalic acid by olfaction. However there is a study showing that varroa microbiota (bacteria) can degrade oxalic acid for carbon sources and this may develop resistance in carrying mites.	Strachecka et al 2012; Sabahi et al 2017; Maggi et al 2017; Kulhanek et al 2022
C2	High temperatures with low ventilation in the hive can cause higher brood toxicity and lower varroa mortality. Health risk for user if used incorrectly. Size of the hive, the position of the evaporator in the hive, the humidity, and the temperature are known to affect the treatment efficacy. sub-lethal doses can cause memory impairment for bees in the short and long term.	Kills varroa within brood cells. Naturally present.	No resistance reported. Formic acid in combination with Oxalic acid increased the efficacy up to 92% but also increased honeybee mortality.	Pietropaoli et al 2018; Genath et al 2020; Steube et al 2021; Pietropaoli and Formato 2022
C3	Low toxicity to honeybees (~18-36%), do not kills varroa within brood cells	High toxicity to varroa; Hop oil may increase antioxidant activity (protein uptake and lower mortality during winter) in honeybees if given orally, Faster acting than oxalic acid. Can last for 15days	24-48h required to cause mortality in varroa. Organic beekeepers	Iglesias et al 2021; Iglesias et al 2022; Kulhanek et al 2022
C4	Effect of hive microenvironment could affect efficacy; Toxic to honeybees especially if used as vapours. Can accumulate in wax. Toxicity (low-to high) varies with *Thymus spp.*	High effectivity against varroa	Damaging to the honeybees (low larvae survival rate, delayed vitellogenin, can modify the taste of honey)	Ellis and Baxendale 1997; Mondet et al 2011; Charpentier et al 2014; Bisrat et al 2022; Alahyane et al 2022; Glavinić et al 2023

No.	Restrictions and limitations	Advantages	Additional Comments	References
C5	Dosage is critical for causing toxicity to honeybees. May be toxic to honeybees at higher concentrations	Increases natural varroa fall	24-48h required.	Vilarem et al 2023
C6		No mortality of honeybees?		Sabahi et al 2018
C7	Increases brood mortality, decreased worker honey bees walking activity; may also repel worker bees		90% (free-living); 86% (within brood cell) . Fumigation was more effective than spraying and powder dusting of the extracts.	Xavier et al 2015; Al-Kenawy et al 2021
C8	Low toxicity to honeybees			Ariana et al 2002
C9	Dosage is critical for causing toxicity to honeybees. Very low toxicity to honeybees, requires 48h to be effective	Low toxicity to honeybees	Laboratory study only. Field testing is required.	Lin et al 2020
C10	Moderately toxic to honeybees			Ghasemi et al 2011
C11	Dosage is critical for causing toxicity to honeybees		Laboratory study only. Field testing is required.	Lin et al 2020
C12		No mortality to honeybees, 48h required to see significant effect		Umpiérrez et al 2013

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Biochemical Control 2 (Researched)

Category2	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Lonchocarpus	**C13**	Rotenone	On bees In hives	A			C	derived from the root		No
Mesquite leaves *(Prosopis glandulosa)*	**C14**	Mesquite leaves (*Prosopis glandulosa*)					C			
Rosewood-Odoriferous (*Dalbergia odorifera*) oil	**C15**	Rosewood oil	On bees In hives In brood	A,L	72%		C	Fumigation		No
Lemon Grass - Cymbopogon	**C16**	Citronella oil	On bees In hives	A			C			No
Lemon Grass - Cymbopogon	**C17**	Cymbopogon oil					C			Yes
Corn *(Zea mays)*	**C18**						C	Fumigation (crushed corn cobs)		No
Lavender oil (*Lavandula maroccana,* plus other spp.)	**C19**	Lavender plant	On bees In hives	A	90%	Au,W	C	Applied with acetone topically	Acaricidal and low to moderate repellent activity	Yes
Marjoram (Majorana hortensis)	**C20**	Marjoram Extract	On bees In hives In brood	A,L	82-90%		C	Fumigation		Yes
Marjoram *(Origanum vulgare)*	**C21**						C	Applied with acetone topically		
Monoterpene kentone in various species	**C22**	*Pulegone, d-limonene*					C			No
Mint oil/Menthol (*Mentha pulegium*)	**C23**	From peppermint oil, contains pulegone	On bees In hives In brood	A,L	28%	Au,W	C	Fumigation	Interferes with their respiratory systems, acaricidal and low repellent activity, may affect the development of the cuticle.	Yes
Oregano (*Origanum elangatum*)	**C24**	Oregano= Origanum oil			97%		C	Ethanol-gelatine solution; Electric vaporizer		
Oregano (*Origanum elangatum*)	**C25**	Oregano= Origanum oil	5th instar larvae?				C	As supplementation in liquid protein diet		Yes
Patchouli *(Pogostemon spp.)*	**C26**		On bees In hives In brood	A,L	28%		C	Fumigation		Yes
Rosemary *(Rosmarinus officinalis)*	**C27**						C	Applied with acetone topically		
Rosemary (Salvia rosmarinus)	**C28**				50%		C	oil		
Simple leaf Chastetree *Vitex trifolia*	**C29**						C	Extract		Yes
Summer Savory *(Satureja hortensis)*	**C30**						C	Applied with acetone topically		
Thyme (*Thymus kotschyanus)*	**C31**						C	Fumigation		No
Thyme *(Zataria multiflora)*	**C32**						C	Applied with acetone topically		
Thyme	**C33**	Carvacrol (derived from Thyme)	On bees In hives	A	Var (up to 100%)	Au,W	C	Derived from thyme oil, (contains thymol and p-cymene) Fumigation, gel formulation with slow releasing vapours		No

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No.	Restrictions and limitations	Advantages	Additional Comments	References
C13	Toxic to adult honeybees, decreased worker honey bees walking activity; may also repel worker bees			Efrom et al 2012; Xavier et al 2015
C14				Eischen and Vergara 2004
C15	Dosage is critical for causing toxicity to honeybees. Very low toxicity to honeybees, requires 48h to be effective	Low toxicity to honeybees	Laboratory study only. Field testing is required.	Lin et al 2020
C16	Toxic to adult honeybees, decreased worker honey bees walking activity; may also repel worker bees			Xavier et al 2015
C17		No mortality of honeybees?		Sabahi et al 2018
C18	Toxic to honeybees (71%)			Eischen and Vergara 2004
C19	Toxic to honeybees			Ariana et al 2002; Alahyane et al 2022
C20	90% (Free-living); 82% (within brood cell)	No mortality to honeybees?	Fumigation was more effective than spraying and powder dusting of the extracts	Rbee and Zedan 2018
C21		No mortality to honeybees?		Ariana et al 2002
C22	Toxic to honeybees			Ellis and Baxendale 1997
C23	Toxic to honeybees. Caused 24% mortality		Menthol applied with sugar syrup may give short term affect against varroa	Ellis and Baxendale 1997; Lin et al 2020; Alahyane et al 2022
C24		No mortality of honeybees?	Direct contact may be toxic to honeybees than vapours	Sabahi et al 2017
C25		No mortality of honeybees?	In combination with Thymus satureioides worked well against varroa	Sammataro et al 2009
C26	Dosage is critical for causing toxicity to honeybees		Laboratory study only. Field testing is required.	Lin et al 2020
C27		No mortality to honeybees?		Ariana et al 2002
C28				Maggi et al 2011
C29		Toxic to varroa		Anjum et al 2015
C30		No mortality to honeybees?		Ariana et al 2002
C31	Low toxicity on honeybees			Ghasemi et al 2011
C32		No mortality to honeybees?		Ariana et al 2002
C33	Toxic to honeybees	High effectivity against varroa	In combination with borneol has given 93% varroa mortality	Ellis and Baxendale 1997; Bisrat et al 2022;

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Biochemical Control 3 (Researched)

Category	Category2	No.	Label	Varroa life stage target	Bee life stage	Effi-cacy	Sea-son	Bee keeper type	Mode of application	Mode of Action	Further Research required
Botanical Lauraceae	Camphor oil	**C34**	Camphor oil					C			
	Bay tree (Laurus nobilis)	**C35**	Essential oil (EO) and hydrolate, and 1,8-cineol	Female adult mites	Young bees	50%		C	EO /hydrolate/leaf extract/1,8-cineol (a common compound present in EO and hydrolate) is obtained from the steam distillation of crushed dried leaves in 80% ethanol which was applied at the bottom of petri dishes.	In contact exposure	Yes
	Cinnamon	**C36**	Extract	On bees In hives	A	73%	All	All	Cotton swabs soaked in extract were placed on the top part of brood combs for 7 days.		No
Botanical Meliaceae	Crabwood (*Carapa guianensis*)	**C37**	Andiroba oil	On bees In hives	A			C			No
	Neem tree (*Azadirachta indica)*	**C38**	Neem oil					C	Extract (main component is azadirachtin),	disrupts chitin synthesis, development inhibitor	Yes
	Neem tree leaves	**C39**	Neem oil	On bees In hives	A	>90 %		C	Neem tree leaves Fumigation	Impair the attachment ability of varroa to honeybees	No
Botanical Myrtaceae (myrtle family)	Clove oil (*Syzygium aromaticum* L.)	**C40**	Eogenol main constituent of clove oil	On bees In hives	A	50-92%	Spr,S ,Au	C	Ethanol-gelatine solution	Affect metabolism	No
	Eucalyptus oil	**C41**	Eucalyptol	On bees In hives	A	90%		C			No
Botanical Pinaceae	Pine needles *(Pinus cembroides)*	**C42**	*Pine needles (Pinus cembroides)*					C			
Botanical Punicaceae	*Pomegranate () peel (* Punica granatum*)*	**C43**	Pomegranate Extract	On bees In hives In brood	A,L	86-95%		C	Fumigation		Yes
Botanical Ranun-culaceae (Buttercup family)	Cumin - black . *(Nigella sativa)*	**C44**		On bees In hives	A	89%		C	Spray		Yes
Botanical Rubiaceae	Coffee beans () *(Coffea arabica)*	**C45**				52%		C	Fumigation		Yes
Botanical Rutaceae	Lemon	**C46**	Lemon oil			86%		C			
	Orange	**C47**	Orange oil					C	cardboard		No
	Citrus	**C48**	Citral					C	Citral is derived from citrus. It is applied using Fumigation		No
Botanical Solan-aceae	Tobacco (*Nicotiana tabacum*)	**C49**	Tobacco (*Nicotiana tabacum*)					C			
Botanical Zingiaceae	Cardamon (red) or Cao Guo *(Amomum tsao-ko)*	**C50**		On bees In hives In brood	A,L	24%		C	Fumigation		
Botanical Zygophy-llaceae	Creosote bush *(* Larrea tridentata*)*	**C51**				18%		C	Fumigation		No
	Harmal (Peganum harmala L.)	**C52**		On bees In hives	A	92%		C	Spray		Yes
Phero-mones	Oleic acid	**C53**		On bees In hives In brood	A,L			C	Spray, sachet, plugs	Disrupts mating ability of males.	Yes
	cy{2,2}	**C54**		On bees In hives In brood	A,L			C	Spray, sachet, plugs	Disrupts host selection, varroa pick forager bees than nurse bees, reducing the chance to find a new suitable larvae in brood cells.	Yes
Lithium salts	Lithium Chloride and Lithium citrate hydrate	**C55**	Lithium salts	On bees In hives In brood	A,L	28-100 %		C	Lithium salts were mixed in sucrose solution and fed to honeybees.	Miticidal effect on Varroa	No

A= Adult, P= Pupae, L= larvae, Q= Queen; L= Low, M= Moderate, H= High; C= Commercial, S= Sideliner, R= Recreational; Au= Autumn, Esp= Early spring, Sp= Spring, ES= Early summer, S= Summer, W= Winter, LW= Late winter

No.	Restrictions and limitations	Advantages	Additional Comments	References
C34				Maggi et al 2011
C35	Only EO was toxic to worker bees. 50% (of only leaf extract)	Hydrolate, 1,8-cineol, leaf extract are not toxic to honey bees.	The mites were exposed to 30sec to leaf extract and then in 24h the mortality of varroa was 50%. Laboratory study only. Varroa were exposed to the extract through 'in contact' exposure	Damiani et al 2014
C36				Al-Kenawy et al 2021
C37	Toxic to honeybee larvae, decreased worker honey bees walking activity; may also repel worker bees.			Xavier et al 2015
C38	High toxicity to honeybees	Toxic to varroa		Anjum et al 2015
C39	Increases brood mortality, decreased worker honey bees walking activity; may also repel worker bees	Toxic to varroa		Efrom et al 2012; Xavier et al 2015; Muhammed and Fhad2022
C40		No mortality of honeybees?		Mahmood et al 2014; Sabahi et al 2017; Li et al 2017
C41	Toxic to adult honeybees, decreased worker honey bees walking activity; may also repel worker bees			Xavier et al 2015
C42				Eischen and Vergara 2004
C43	95% (free living); 86% (within brood cell)	No mortality to honeybees?		ELRoby and Darwish 2018
C44		No mortality to honeybees?	Fumigation was more effective than spraying and powder dusting of the extracts	ELRoby and Darwish 2018
C45		No mortality of honeybees?		Eischen and Vergara 2004
C46		No mortality of honeybees?		
C47			No effect on varroa	Bakar et al. 2017
C48	Toxic to honeybees			Ellis and Baxendale 1997
C49				Eischen and Vergara 2004
C50	Dosage is critical for causing toxicity to honeybees		Laboratory study only. Field testing is required.	Lin et al 2020
C51	Toxic to honeybees		Not effective against immature varroa	Eischen and Vergara 2004
C52		No mortality to honeybees?	Fumigation was more effective than spraying and powder dusting of the extracts	
C53		Promotes hygienic behaviour in honeybees. Natural sexual pheromone of mites.	Field trials are required.	Zielgelmann et al 2013 (a and b)
C54	Application at a relevant concentration to brood cells and to honeybees is difficult.	No mortality to honeybees?	Field trials are required.	Eliash et al 2014
C55	Dosage is critical for causing highest varroa mortality. Low toxicity to honeybees (0-6%); Lithium citrate performed better than lithium chloride.	Lethal effect on Varroa mites feeding on the bees. Lithium citrate causes 46-100% whereas lithium chloride 7-100% varroa mortality (depending on the dosage). Easy to apply.	28-100% (dosage dependent) May also interfere with honeybee pheromonal communication.	Ziegelmann et al 2018; Stanimirovic et al 2021; Kolics et al 2022

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New Potential Monitoring and Control Charts

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As part of the Project PH22002
"Exploration of advanced control and detection methods for Varroa mite"
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Detection Category	No.	Label	Targeted Varroa life stage	Bee life stage	Bee keeper type	Mode of application/Action	Restrictions and limitations	Advantages	Additional Comments	Readiness for Australia: Short-term/ Long-term	R&D Required: 1:Methods 2:Efficacy 3:Economic	Qualitative/ Quantitative	Acute / Chronic phase
Chemical Detection	**M11**	Olfactory (Gas sensors)	On bees In hives	A	C	Samples hive air for chemical signs of Varroa mites using technologies like infrared analysers, FT-IR spectrometers, gas chromatography with FID, mass spectrometry, or electronic nose sensors.	Expensive, time-consuming, and requires skilled personnel. Equipment is bulky, heavy, and energy-intensive.	Enable early detection, non-invasive, may help to determine colony health.	Differing reports on effectiveness. Can compensate for the air changes within beehive at least diurnally.	Long term	1, 2, 3	Qualitative	-
Mulit-sensor: Chemical, weight, vibrations	**M12**	Soft-sensor system - "SmartComb"	In hives In brood?	A L P	C	Uses machine learning and advanced analytics to assess environmental and hive conditions for indirect signs of infestation. Requires: metal-oxide gas sensors from hive air+ temperature+ relative humidity+ honey weight+ hive sound.	Positioned in brood chambers. One time sensor purchase, can be expensive if used in all hives - false detections.	Non-destructive, non-invasive. Sensitive, Reliable. Allows beekeepers to manage mite levels proactively without direct hive inspection. May help determine colony health.	Aims to identify when threshold is reached through remote monitoring.	Long term	1, 2, 3	Qualitative/ Quantitative?	-
Vision - cameras using machine learning	**M13, M14, M15**	Computer vision systems, Edge-Cloud hybrid computing, Laser beam & Camera	On bees At entrance	A	C	These systems use computer vision and spectral sensors to detect Varroa mites on bees as they enter and exit the hive. Some (M15) scan bees with lazers, others (M13) use LED lights. Various forms of AI processes are used to detect mites, including the Edge-Cloud hybrid system (M14).	Expensive. In some versions video is only used at hive entrance because of light availability. Reliability dependant on how the bees are videoed and scored. Some are invasive requiring beehive modification. Some have Camera inside brood box. Camera image resolution critical. Cloud subscription for data storage is required. AI detection tools, some can work in remote area without internet connections. Variable reliability and sensitivity.	Non-destructive, non-intrusive real-time bee monitoring using cameras.	Estimating the % of bees infested is challenging. Purple Hive was a key player- may now be shelved. Xailient (Australian Tech company uses solar powered computer vision).	Short term/ Long term	1, 2, 3	Qualitative/ Quantitative	-
Vision-cameras using enhanced machine learning	**M16, M17, M18**	Visual Object detector, Nvidia Jetson Nano detector, ADAM optimizer technique	On bees At entrance In hives	A	C	Uses neural networks, YOLOv5 and SSD, to identify Varroa in real-time (M16) or MobileNet and ADAM optimizer (M18). Machine learning using a CNN model to process images and an IoT module to alert beekeepers (M17, M18).Uses high-resolution images (M16),or moble phone for images(M18)	Limited to bees entering hives, Online identification. Requires powerful hardware for deep learning. Unreliable - some can give false positives (M18) - confuses varroa with bee pupa	Sensitive. High resolution images not important for accurate detection (M16). Uses three validation methods to monitor beehives and will also detect hive beetles, ant problems and missing queens (M18).	Computer programs: YOLO (You only look once) and SSD (Single shot detector). Varying reports of reliability.	Short term/ Long term	1, 2, 3	Quantitative	-
Combining sound & image	**M19**	Acoustic & video imaging	On bees In hives	A	C	Combined acoustic and video imaging with deep machine learning. Camera recordings, data storage and analysis, used Tensor Processing Unit (TPU) and machine learning (ML) models (AI tools),Object detection algorithms YOLOv8, YOLOv7, YOLOv5 and SSD were compared. Audio analysis used Mel spectrograms and mel-freuency spectral coefficients.	Cloud subscription. Audio only aimed to distinguish between strong and week hives - had a max 0.998 accuracy at predicting hive health. Not sensitive. Unclear if AI training would be needed with new hives.	Non-destructive and non-invasive, can provide early detection of varroa mites.	Ability to detect varroa visually =0.5, accuracy of those detected = 0.974;	Long term	1, 2, 3	Qualitative/ Quantitative	-
Vibration	**M20**	Vibration	On bees In hives In brood	A	C	The method uses accelerometers in beehives to detect unique vibration patterns caused by bee activity and Varroa mite infestations. By applying signal processing and machine learning, it non-intrusively identifies mites, offering beekeepers crucial insights for managing hives.	Research is undergoing to improve accuracy. Requires mites to move. Detecting mites inside brood is not sensitive and unreliable. Variable reliability	Non-destructive and non-invasive. Maybe relatively cheap	3-30 secs. BeeHero is researching using sound to measure hive health. Could be a promising and accurate tool. More accuracy is required. Other players in this space include Beeright (Was purple hive, is also using sound to measure hive health) and Y-Trace via the tool Apis Prime$^{(TM)}$..	Long term /Short term?	1, 2, 3	Qualitative/ Quantitative	-
Biochemical	**M21**	Fluorescence Spectroscopy + other Electro-chemical techniques	On bees In hives In brood	A	S C	The method measures Varroa mite infestation by analysing honey's biochemical changes, detectable by fluorescence markers with a spectrofluorometer. Parallel factor analysis (PARAFAC) is used to assess infestation levels.	Microorganisms can also catalase honey. May give a false positive for varroa mites if honey is not sterile. Variable reliability. Sensitivity depends on many factors, specialised person required, expensive use to equipment, time consuming. Expensive technique.	Can measure the infestation levels.	Algorithm based. Catalase is the key marker. Based on determining several parameters of honey quality and composition, eg pollen counts, honey dew elements i.e. algae, fungal spores and hyphae, pollen of nectar less plants. The ratio of protein and phenolic components obtained from the honey emission spectra may be a useful indicator for the level of infestation to which the honey bees were exposed.	Long term	1, 2, 3	Qualitative/ Quantitative	-
Molecular	**M23**	Environ DNA (eDNA)	In hives	NA	S C	Parts of bees, mite fragments, bee faeces, or other materials shed from the bees and mites are collected and DNA is extracted by using specific primers and identified using PCR or (qPCR) assays.	Sampling from hives required (through swabs mainly). Chances of contamination. Needs calibration. Only detects presence /absence.	Sensitive, Reliable. Rapid detection of varroa mites. It will be through swabs.	This technology is developing fast, while in the past it was limited to presence /absence, it is now possible to quantify mite numbers, but this needs calibration.	Long term	1, 2, 3	Qualitative	-

Control Category	No.	Label	Targeted Varroa life stage	Bee life stage	Bee keeper type	Mode of application	Mode of Action	Restrictions and limitations	Advantages	Additional Comments	Readiness for Australia: Short-term/ Long-term	R&D Required: 1:Methods 2:Efficacy 3:Economic	Qualitative/ Quantitative	Acute / Chronic phase
Heating hives Thermo-therapy/ Hyperthermia	**P12**	Thermovar, Varroa Terminator, Vatorex, The Victor, Mighty Mite Killer, Silent Future Tec, Varroa Kill II	In brood In hive On bee	A, Young bee	C	Electronically heating brood chamber	The brood combs are heated either from outside or inside.	Requires 360-480min of time per treatment on average. Can be laborious and expensive. Not many commercially available products.	Short exposure of high temperature ≥40°C does not harm bees but lethal to varroa. Environmentally friendly.	Potential to kill mites in capped brood cells and opperate with honey supers.	Long term	1, 2, 3	-	Chronic
	P13	Thermosolar Hive	In brood In hive On bee	A, P, L	C	Modified hive with Thermosolar Hive that heats the colony periodically	The bee colony and combs are heated gradually.	Varroa mites attached to adult bees outside the hive or at the bottom of the hive may not be affected by the initial treatment, necessitating subsequent treatments within 7 to 14 days.	Can cause varroa mortality of 100% within capped brood. Can also protect hives from severe and long winters. Can also suppresses presence of Nosema disease caused by Nosema parasites (Not currently in Australia).	The hive uses solar energy.	Short term	1, 2, 3	-	Chronic
	P14	Mite-Zapper, Drone brood trapping + hyperthermia	In brood	P, L	C	Heating brood cells	Heated trapping comb	Might not be effective in the long run. If temperature is not controlled can affect bees. The battery requires replacement.	Efficient in killing varroa mites. Not labour intensive	It's a modified drone comb with 12- volt battery. The battery heats the comb for 1-5min reaching to 43°C.	Short term	1, 2, 3	-	Chronic
	P15	Sodium Acetate Trihydrate (SAT)	In brood In hive On bee	A, P, L	C	An active phase change material (PCM) pack is placed to the brood box	Heat is distributed within the hive.	External ambient temperature has a considerable impact on the performance of the PCM pack.	Reduces bee losses	More in field research is required.	Long term	1, 2, 3	-	Chronic
Acoustic disturbances	**P16**	Frequency control	In brood In hive On bee	A, Young bee	C	Noises/ Ultrasound/ ultrasonic/ square/ sine waves with frequencies (14000-16000 Hz with a decibel level of 80-100 dB).	The sound is applied for 20-40 days. The sound acts on the central nervous system of the varroa mite, so that the old mites die within 10-20 days. Affects varroa mites orientation/communication.	Can be expensive to use.	No effect on bees behaviour in any manner. Environmentally friendly. No chemical treatment is required.	The noise/ultrasound is unpleasant and stressful to mites and affects mites feeding. Varroa die after 10-20 days. Long-term field trials are required.	Long term	1, 2, 3	-	Acute Chronic
Electro-magnetic/ Electrostatic forces	**P17**	Magnetic field	In brood	P	R	Scanning device, magnets, laser beam are used.	The radio wave blocks the development of Varroa and its larvae ultimately killing it.	Hive modification might be required. Electricity required.	Can also affect other bee parasites in addition to Varroa mites. Does not affect the viability of the bees.	Further research is required to test this technique and suitability of using with other management options such as pesticide. This technique has good potential.	Long term	1, 2, 3	-	Acute / Chronic
Varroa pheromone Traps	**P19**	Temperature control pheromones traps	In brood	P	C	Vapouriser is used to evenly distribute pheromones and temperature for pheromone stability near/inside brood cells.	The sex pheromones disrupt male varroa's ability to copulate with suitable females. Also affects the number of spermatozoa. Other pheromones in the pheromone mix can affect the mite's searching ability of nurse bees.	Reaching inside combs and on honeybees could be challenging. Might cause interference with bee pheromonal communication.	Induces male mites to have sexual activity at an inopportune period in the reproductive cycle of females. Sexually active females have reduced sexual activity.	Pheromone-Based Robotic Varroa Trap would be the ultimate design that would attract varroa and then kill them using electric current (Meister et al 2022). Could be combined with other techniques such as varroa restriction traps, thermal devices, predators etc.	Long term	1, 2, 3	-	Chronic
Varroa lure trap	**P20**	Varroa Frame trap	On bees	A	All	A varroa trapping frame Varroa are lured in by drone pheromones	The frame contains small entrances that allow varroa to enter but not leave.	Need to add and remove the frames. At concept stage only. Not sure what affect the pheromones would have on the bees - would it stop them producing drones themselves? Would they try to clog up the frames?	Could potentially set and leave for a few weeks.	It could be used with chelifers on the frame to scavenge trapped varroa.	Long term?	1, 2, 3	-	Acute / Chronic
Varroa Blocker	**P22**	Varroa Removal Plate (VRP)	On bees	A	All	The varroa barrier is placed at the hive entrance with an a mesh covered oil tray underneath.	The VRP dislodges varroa from the bees while entering the hive. The varroa then drop into the oil tray and die.	Cleaning oil tray and mesh.	No harm to the bees; no loss to pollen.	This is a prototype Varroa Removal Device. Could replace oil trap with a sticky mat or even scavenging Chelifers. Could be modified to count mites entering the hive.	Short term	1, 2, 3	Quantitative	Acute / Chronic

Control Category	No.	Label	Targeted Varroa life stage	Bee life stage	Bee keeper type	Mode of application	Mode of Action	Restrictions and limitations	Advantages	Additional Comments	Readiness for Australia: Short-term/ Long-term	R&D Required: 1:Methods 2:Efficacy 3:Economic	Qualitative/ Quantitative	Acute / Chronic phase
Propolis	**P24**	Propolis/ resin	In brood In hive On bee	A	All	Different methods (Heat treated propolis strip, propolis extract, powder, raw propolis, volatile) are placed within hives or exposed to mites.	The exact mechanisms and modes of action are not yet fully elucidated. But propolis has low narcoleptic (chronic neurological disorder) effect	Could be an expensive method. Time and labour required.	No effect on bees. Propolis have antimicrobial properties and may help to block virus transmission.	This technique has potential and can be explored for Varroa treatment in Australia.	Short term	1, 2, 3	-	Chronic
Bacteria	**B9**	*Bacillus thuringiensis*	On bees, In hive	A	All	Contact; Spray (ingestion by varroa); agar disc onto the top bars of the frames of comb in the hive (1/hive box)	Produces toxins that damages the gut lining of the mite. Varroa shook, regurgitated, suffered intestinal inflammation, and died. Causes intestinal inflammation (dysentery) in varroa.	Generally, no lethal effect on honeybee adults and larvae in the short term with low dosage was observed. Dosage is critical for causing toxicity to bees. Bt toxins are very specific. Requires 24h to kill Varroa in most studies.	Some Bt strains showed no negative affect on adult bee and larvae. Can be naturally present in honey samples.	Different strains of Bt produce 100s of proteins each of which is toxic to specific invertebrate groups.These proteins rupture the intestinal wall of the targeted insect,which dies of septicemia. Not enough field studies to support Bt use for varroa control. Bt is present on Varroa corpses. Bt-derived products constitute 95% of the world's biopesticide market.	Long term	1, 2, 3	-	Acute / Chronic
Fungi	**B22**	*Metarhizium anisopliae*	In brood In hive On bee	A, L	All	Coated on strips placed between frames; sprinkling as dust in the hive; as a liquid (spray between frames), solid (sporulating fungus +media); using auto-applicator device; mixed with wax powder; protein patty	Repellent affect. Nurse bees carrying spores repel Varroa. Pathogenic effect on mites. Spores infect Varroa by forming conidia and penetration via appressoria followed by haemocoel invasion causing death.	Adaptation to hive conditions (e.g. temperature). Require 2-13 days on average to kill varroa depending on hive temperature and humidity. May also affect honeybee brood inside capped cells/adult bees. Dosage dependent. Mites can develop resistance against Entomopathogenic fungi (EPF).	Can stay up to 42 days after first treatment. No need for repeated treatments. Conidia carrying nurse bees may also repel varroa. No impact on colony strength and development.	Possible alternative to chemicals.Combining oxalic acid with *Metarhizium* increases efficacy. Variant maybe important. The var. BIPESCO 5 was effective. A commercial version Bioranza was promising. No effect on any stages of bees at this stage. Compatible with biochemicals such as vegetable oils	Short term	1, 2, 3	-	Acute / Chronic
Fungi	**B24**	*Beauveria bassiana*	In brood In hive On bee	A	All	Coated on strips placed between frames; sprinkling as dust in the hive; as a liquid (spray between frames), solid (sporulating fungus +media); using auto-applicator device; mixed with wax powder; in a bee protein patty	Pathogens cause lethal infections on mites	May affect honeybee brood inside capped cells. Dosage dependent. Mites can develop resistance against Entomopathogenic fungi (EPF).	When spores of B. bassiana were sprayed inside hives, adult bee mortality did not differ from control treatments. Naturally present in hives and in brood cells.	Possible alternative to chemicals. Isolated from varroa in Russia, France, Spain, Denmark, and Costa Rica. Multiple applications increase efficacy & cost. A commercial version Biovar was promising. No effect on any stages of bees.	Short term	1, 2, 3	-	Acute / Chronic
Supressed mite reproduction Genetic Maipulation - RNAi	**G10**	RNA Interference (RNAi)	In brood In hive On bee	A,P,L	C	Soaking by spraying inside the hives. Bees consume double-stranded RNA (dsRNA) which is then transferred from the bees to the mites (feeding on fat bodies).	The dsRNA leads to gene silencing in the mites, impacting their reproduction	Delivering the dsRNA to mites without harming bees (i.e. high impact on non-targets). Potential development of resistance by the mites. Successful gene knockout or knock-in could be a problem. Reversible and temporary so efficicacy is limited. Requires repeated treatments (Labour intensive). Long-term and potential risk of mutations or off-target effects are still unclear and largely debated.	It doesn't require special equipment like other CRISPR technologies. Safe for the bees to date. Can be applied in any season but preferably in autumn and summer.	Usual delivery system = horizontal transfer via the host bees. Can use electroporation (using a high voltage pulse to overcome a cell membrane) or could inject bees. GreenLight Bioscience has trialled the use of RNAi to target mites. Https://www.sciencelearn.org.nz/resources/3251-using-rnai-to-control-varroa-mites. The long-term impact on bees is still unclear.	Long term	1, 2, 3	-	Chronic
	G10.1	RNA Interference (RNAi)	On bees In hive	A	C	worker bees fed sugar water, mites pick up the RNAi from them	Targets the Deformed wing virus	Currently patented by "Green Biosciences". Problems in field trials, possibly non-target effects. Bayer had trouble stabilizing the RNAi under field conditions, they found it wasn't effective in the field.	-	Developed by Bee-o-logics, originally to control Israeli Paralysis Virus. Bought out by Monsanto, who were bought out by Bayer. Patent has now been bought by "Green Biosciences".	Long term	1, 2, 3	-	Chronic
	G10.2	RNA Interference (RNAi)	In brood	A	-	Nurse bees fed RNAi in sugar water pass the RNAi to the larvae	Uses a virus to stop production of a protein in mites necessary for reproduction	How do the mites pick up the RNAi fed to the larvae - is it deposited in the fat bodies? Can worker bees attacked by mites also pass on the RNAi? How much is necessary to feed nurse bees under field conditions	Attacks mites in brood cells	Greenlight Biosciences hold the patent for the product. Most recient studies in The States indicate that underfield conditions in Autumn it an keep mite numbers stable. (J.Cameron, pers comm.)	Long term	1, 2, 3	-	Chronic