Horticulture Innovation Australia

Final Report

Statistical review and re-design of the National Bee Pest Surveillance Program

Plant Health Australia Limited

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Summary

The European honey bee (*Apis mellifera*) plays an important role in pollination of horticultural and agricultural crops, with \$4-6 billion per annum in agricultural production per annum estimated to be responsive to honey bee pollination (Department of Agriculture, 2011 and Keogh *et al.* 2010).

Australia's honey bee industry and pollination reliant industries maintain a production advantage over many other countries, as Australia is currently free of many bee pests and pest bees that cause significant issues overseas. As a result, exotic bee pests and pest bees pose a serious biosecurity risk, and the Honey Bee Industry Biosecurity Plan (2013) (Plant Health Australia Ltd., 2013) has identified 12 pests and diseases that have been ranked as the highest priority biosecurity threats. Of these 12 pests, *Varroa* mites (*Varroa destructor* and *Varroa jacobsoni*) are considered the most significant, and it is predicted an incursion of *Varroa* could cost as much as \$1.3 billion to manage over a period of 30 years (ABARES 2012; Hafi *et al.* 2012; Cook *et al.* 2007).

A key component of biosecurity preparedness for the honey bee and pollinator-reliant industries is surveillance that contributes to early detection of high priority pest threats, as rapid detection of an incursion of a new pest will increase the likelihood that eradication or containment will be successful. The honey bee and pollinator-reliant industries and the regional economies they support will therefore benefit significantly from investment in a National Bee Pest Surveillance Program (NBPSP), that enables early detection of high priority pests.

The current National Bee Pest Surveillance Program has been a leading example of a successful industry government partnership that has benefited from a nationwide approach to surveillance. Prior to a new investment in this activity, it has been timely to review the program and to ensure resources are being utilised effectively and efficiently to maximise outcomes for early detection of pests and maintain bee health and pollination services in Australia. Based on the review undertaken in this project and consideration of what an optimal design would look like, a redesign of the NBPSP has been proposed and costed. The work undertaken in this project also provided an opportunity to enhance our statistical understanding of surveillance methods to determine if further improvements could be made in the design and operation of the NBPSP.

Within this project, there was strong emphasis on reviewing the sentinel hive component of the NBPSP, due to its significant central role for the detection of high priority mites that also vector viruses of serious concern. The sentinel hive component can be used in conjunction with other surveillance activities such as sugar shake, alcohol washing and drone uncapping, which are highly sensitive for detecting mites such as Varroa. As the sentinel hive component is a core component of surveillance and is also one of the most costly components of the NBPSP, it was vital to establish whether the current hive arrangements were adequate for the intended detection capabilities, and were operating at the most efficient and effective arrangement.

To achieve this, a *Varroa* Spread Model was developed to evaluate the optimal surveillance design associated with the sentinel hive component operating at Australian ports. The model estimated broadly that the optimal arrangement of sentinel hives for detection of *Varroa* mites at high risk ports, is an array of 6 hives at 2 km spacings, inspected and checked every 6 weeks. This is largely consistent with the current resourcing of the NBPSP. Further refinement of the *Varroa* Spread Model was used to identify surveillance components that would be required to achieve the highest likelihood of detection within infested areas of 100 km², 150 km² and 200 km². Results indicated that an inclusion of 4 sentinel hives at 2 km spacings, inspected every 6 weeks, deployed at lower risk rated ports (along with the optimal 6 hives at high and medium risk ports) would confer an overall increase in the confidence of detection from the sentinel hive program to 72%.

In addition to the sentinel hive component, this project also assessed other key surveillance components that make up the NBPSP. These other surveillance activities are critical and sensitive to the high priority pest (including pest bees and viruses) that can be detected. It was observed that despite a lack of analytical information to statistically place a figure on the additional surveillance activities, it is known that these along with an optimal sentinel hive arrangement creates a strong and sustained program that cover surveillance for the 14 high priority pests and diseases of bees.

Through review of the NBPSP, it was noted that a significant increase in resources is required simply to maintain the existing program (Table 1). As the existing program has expanded past that originally contracted, the costings currently do not reflect the activities undertaken, thus an analysis of the input and costs was needed. This analysis was used as the basis for understanding the differences in cost vs. activities across contracted, as well as current activities and further proposed options.

To redesign and scope the NBPSP, the sentinel hive analysis indicated by the *Varroa* Spread Model described a minimum of 4 hives for lower risk sites and an optimum of 6 hives for higher risk sites. This arrangement was used to appropriately cost three proposed programs, along with additional surveillance activities, to develop a strong and nationally appropriate revised NBPSP for the future. The source of difference between the three proposed options are in the number of ports and numbers of sentinel hives. The additional surveillance activities are either maintained, increased or enhanced (such as inclusion of surveillance for the high priority biosecurity threats Asian honey bees, Asian hornets and exotic viruses). These data suggest an array of sentinel hives across high, medium, low and unknown (unanalysed) ports will be supported appropriately annually, and additional surveillance will be included as key components for detection of many exotic pests. The proposed programs summarised in Table 1 also provide estimated costing related to initial one-off costs for investigations into new surveillance enhancements, as these are vital for the success of the proposed NBPSP and the early detection of exotic pests.

The costs of the proposed program are then further extrapolated to 30 years to express the investment in the NBPSP in terms of the cost of managing for a pest (such as Varroa) for the same time period (Table 2).

Given the complex nature of activities, funding streams and the need for coordinated data collection and capture and efficiencies to be gained in undertaking some functions at a national level, ongoing facilitation and maintenance of the program must be incorporated into the future program. The NBPSP has been a leading example of an industry government partnership and through this review and redesign project, it is considered that the program could be significantly enhanced by implementing the recommendations of this report. **Table 1.** Summary of annual costings comparing the 'actual' cost of the current contracted program, the activities currently contracted and non-contracted, and three proposed options. The table is split into costs annually for sentinel hives, additional surveillance activities, and the surveillance enhancements for the future. Total annual costs for these separate programs is provided, as well as the total annual costs for the term of a 5-year program including a one off cost for investigations in establishment of new surveillance enhancements (\$185,000).

	'Actual' cost of current contracted program	Currently contracted + non- contracted program	Proposal #1 All ports with 6 hives/port	Proposal #2 All medium and high risk ports with 6 hives/port	Proposal #3 All high and medium ports 6 with 6 hives/port and 4 hives/port at low and unknown risk port
Sentinel hive arrangement	\$284,000	\$377,500	\$525,000	\$300,000	\$450,000
Additional surveillance activities	\$254,500	\$254,500	\$254,500	\$254,500	\$254,500
Surveillance enhancements	\$28,000	\$28,000	\$28,000	\$28,000	\$28,000
Annual cost	\$566,500	\$660,000	\$807,500	\$582,500	\$732,500
Total costs over a five-year period (incl. one off enhancements at \$185,000)	\$3,017,500	\$3,485,000	\$4,222,500	\$3,097,500	\$3,847,500

Table 2. Investment in the NBPSP for 30 years expressed in terms of the cost of managing for a pest (such as Varroa) for the same period.

	Current contracted program	Currently contracted + non- contracted program	Proposal #1 All ports with 6 hives/port	Proposal #2 All medium and high risk ports with 6 hives/port	Proposal #3 All high and medium ports 6 with 6 hives/port and 4 hives/port at low and unknown risk port
Total costs for the NBPSP over a 30- year period (incl. one off enhancements at \$185,000)	\$17,013,500	\$19,985,000	\$24,410,000	\$17,660,000	\$22,160,000
Cost of Investment in an NBPSP for 30 years expressed as a % of the cost of managing for a pest for the same period (\$1.3					<i>\$22,100,000</i>
billion)	1.3%	1.5%	1.9%	1.4%	1.7%

Keywords

Varroa mites, bee biosecurity, surveillance, sentinel hives, pollination

1 Introduction

1.1 Biosecurity risks to the bee industry

Exotic bee pests and pest bees pose a serious biosecurity risk to the honey bee industry and to industries reliant on honey bees for pollination in Australia. High priority pests and diseases identified in the Honey Bee Industry Biosecurity Plan (2013) (Plant Health Australia Ltd, 2013), include *Varroa* mites, Tropilaelaps mites, Tracheal mite, Giant Honey Bee, Asian Honey Bee, Red Dwarf Honey Bee, exotic strains of *Apis mellifera*, Asian hornets and three exotic viruses.

Of these, one of the most serious is *Varroa* mite (comprising the two species *Varroa destructor* and *V. jacobsoni*). It is accepted that, given its spread and colonisation in other countries, there is a very high likelihood *Varroa* mite might enter and become established in Australia (Keogh, Robinson & Mullins 2010) and, since 1995, at least 13 border interceptions of *Varroa* have occurred in Australia (Appendix 2). While eradication of *Varroa* has not been successful in other countries where incursions have occurred, to have any possibility of eradicating or containing an incursion of this pest in Australia, populations would need to be detected before they are able to spread and establish widely. Surveillance for early detection of new incursions of *Varroa* alone, the cost of management should it establish in Australia could be expected to range from \$630 million–1.3 billion over 30 years depending on the port of entry (Hafi *et al.* 2012).

Further information on the key bee biosecurity threats and common surveillance methods to detect them is provided in Appendices 2 and 3.

1.2 Pollination - reliant plant industries

In addition to providing honey and other products, the European honey bee (*Apis mellifera*) also plays an important role in pollination of horticultural and agricultural crops. There are currently approximately 12,000 registered beekeepers in Australia operating a total of 520,000 hives (Crooks 2008). Of these 102,000 hives are used for paid pollination. The majority of beekeepers are located in NSW and Victoria (Table 3).

State	Number of beekeepers	% of beekeepers	Number of hives	% of hives	Beekeepers with ≥50 hives
NSW	3,461	29	214,296	41	489
QLD	3,098	26	103,539	20	305
WA	999	8	28,204	5	106
SA	1,030	8	61,322	12	171
TAS	174	1	16,212	3	42
VIC	3,389	28	97,508	19	224
Total	12,151	100	521,081	100	1337

Table 3 Number of beekeepers and hives, by state, 2013-14¹.

¹ Cooks 2008, and BeeAware website (<u>http://beeaware.org.au/industry/</u>)

Honey bees provides a major benefit to agriculture and the broader economy through the provision of pollination services to a range of agricultural and horticultural industries (Hafi *et al.* 2012; Gordon and Davis 2003). Of Australia's \$30 billion agricultural production per annum, approximately \$1.8 billion is estimated to be responsive to honey bee pollination (Keogh *et al.* 2010). These benefits are related particularly to 35 of the most pollination-responsive crops. When all agriculture is included, estimates run as high as \$4-6 billion per annum (Department of Agriculture, 2011). The broad range of estimates reflects differences in how much crop yield the reports apportion to honey bee pollination (versus pollination by other insects) and how much crop yield is apportioned to other inputs (irrigation, nutrient and pest management) on crop production.

Plant species differ in their responsiveness to pollination by honey bees. Crops such as almonds are 100% reliant on pollination by insects for an almond crop to be produced, of which managed honey bees are the principal contributor. Other crops such as strawberries are self-fertile, however strawberries benefit from the provision of managed honey bees for pollination, through an increased fruit set and a reduction in small or misshapen fruit. Specialist pollination services are also essential to the breeding of new strains of many crop, pasture and horticultural species.

For a high priority bee pest such as *Varroa* mite, the potential present value of losses estimated to producers and consumers from an unhindered *Varroa* mite outbreak could be expected to range from \$21.3–50.5 million per year or \$630 million–1.3 billion over 30 years depending on the port of entry (ABARES 2012; Hafi *et al.* 2012; Cook *et al.* 2007). However, if the spread of *Varroa* mite could be slowed through containment, it is estimated that the losses would range from \$630 million–0.93 billion over 30 years, depending on the port of entry.

Pollination-reliant industries that are represented by Horticulture Innovation Australia Ltd (HIA) include almonds, apple and pear, avocado, canned fruits, blueberry, cherry, lychee, macadamia, mango, melon, onion (for seed), papaya, passionfruit, prune, rubus, strawberry, summerfruit and vegetables (for seed). Given the wide ranging impacts predicted for an incursion of a high priority pest in the honey bee industry, and the predicted flow on effects for both pollination services and the unmanaged bee populations undertaking pollination, these industries should be considered as key beneficiaries of a bee surveillance program.

It is important to understand that in addition to the pollination services provided by managed hives there are numerous colonies of unmanaged honey bee colonies established in most parts of Australia (Keogh *et al.* 2010). The impact of *Varroa* on these bees will have significant impact on industries that rely on these currently free pollination services alone.

Whilst this report is targeted to the benefits of the NBPSP for the Australian Honey Bee and pollinator reliant horticultural industries it is important to acknowledge that the Grain Producers of Australia also invest in the NBPSP in recognition of the value that bees bring to pollination for the grains industry.

1.3 The National Bee Pest Surveillance Program (NBPSP)

The NBPSP is one of the leading surveillance programs for bee pests in the world, and within Australia is an exemplar model for government and industry partnerships with wide ranging benefits. The NBPSP (2013–2016) is a key biosecurity preparedness program comprising several surveillance activities (Table 4), to protect the honey bee and pollination-reliant industries of Australia. It is also an important tool in providing the evidence of absence information from bee pests and pest bees, which assists maintaining market access for trade in bee queens. Since the establishment of the NBPSP, the program has essentially identified 73 Asian honey bee, 49 Giant honey bee and 12 Red dwarf honey bee detections between 1995 and 2015 (Appendix 2), with *Varroa* mites detected 13 times within this period.

The NBPSP (2013–2016) comprises a range of surveillance activities to cover the 14 high priority pests, and components of the NBPSP provided data captured from 151 sentinel hives, 171 catchboxes and 19 remote catchboxes operating at 38 air and sea ports of entry in Australia. In addition, tracheal mite assessment and floral sweep netting has been conducted at 24 ports. A key part of the NBPSP is an extensive sentinel hive component, and one of the aims of this project has been development of a risk-based statistical model to analyse optimal design (hive numbers and spacings) for the early detection of exotic bee pests, such as *Varroa* mite. The information was used to provide recommendations on optimal activities for the NBPSP, and whether more efficient arrangements could be proposed to maximise outcomes of surveillance activities.

In addition to the sentinel hive component of the NBPSP, several other surveillance activities for bee pests and pest bees operate across the biosecurity continuum of pre-border, border and post-border that contribute to the biosecurity system in Australia. For example as part of the NBPSP, in Victoria, 39 bee colonies have been detected in catchboxes at ports and assessed for exotic pests. All colonies were *A. mellifera*, and no exotic pests were present, however these data indicate catchboxes can be effective at detecting bee swarms at ports. Further examples of complementary surveillance within the continuum include the capture of swarms at the ports (e.g. by the Department of Agriculture and Water Resources staff). The swarms are submitted to entomologists for identification for the presence of *Varroa* mite and other parasitic mites, such as tracheal mites. Activities pre-border such as the collection of bee and larvae samples for molecular analysis of viruses in Papua New Guinea also support the goals of the NBPSP.

Additional information on the importance and activities of the NBPSP is provided in Appendix 1. An outline of major bee pests and pest bees targeted for surveillance is provided in Appendix 2 and a description of surveillance techniques in Appendix 3.

	Qld	NSW	Vic	WA	SA	NT	Tas
Number of	8 ports,	10 ports,	5 ports,	9 ports,	8 ports,	3 ports,	4 ports,
ports	4 major	3 major	3 major	3 major	3 major	2 major	2 major
Ports with	4	10	5	8	3	2	4
sentinel hives							
Number of	24	27	32	27	24	6	23
sentinel hives							
Ports with	8/8	8/10	5/8	9/9	8/8	1/3	4/4
Swarm		-	-	-	-	-	
capture							
Number of	11	50	54	2	26 + 30 at	0	0
catchboxes					depots		
Remote	15	0	0	0	0	4	0
surveillance							
catchboxes							
Ports with	5/8	3/10	1/5	1/9	8/8	2/3	2/4
floral sweep							
netting							
Ports with	8/8	10/10	5/5	9/9	3/5	1/3	4/4
hobby		-	-	-	-	-	
beekeeper							
involvement							
Ports where	4/8	3/10	4/5	4/9	1/8	2/3	2/4
tracheal mite	-	-	-			-	
analysis is							
undertaken							

Table 4 Summary	of hee nest and	d nest hee surveillanc	e activities at air a	and sea ports in Australia.
	א טו אבב אבזו מוונ	u pest dee suiveillarit	כ מכנועונוכא מנ מוו מ	anu sea ports in Australia.

2. Methodology

2.1 Operation of the Current National Bee Pest Surveillance Program

The NBPSP operates with a program coordinator (based in Plant Health Australia) and jurisdictional officers who manage NPBSP activities within their own state/territory, to ensure that the NPBSP objectives are met. The Department of Primary Industries (or equivalent) in all states and the Northern Territory take the lead role in coordinating surveillance activities on the ground. The NBPSP is currently comprised of the following components:

- National Sentinel Hive Program conducted at key ports in Australia
- Remote catch box program pilot program conducted at 3 ports in Queensland and 4 catch boxes in Northern Territory
- Oil trapping or Apithor harbourages conducted in Tasmania, Northern Territory and parts of Western Australia for detection of Small Hive Beetle
- Floral sweep netting and swarm capture conducted in most ports

2.2 Review of the National Bee Pest Surveillance Program

The review phase of the project included the consideration of the following:

- Interception data for the 14 high priority pests for the duration of the program (a measure of success)
- Evaluation of the critical role and the suitability of each surveillance method contributing to coverage of all 14 high priority pests
- Real cost of delivery of both the contracted components and additional surveillance activities carried out as part of the NBPSP components of the NBPSP
- Acknolwedgement of the role of surveillance outside of the NBPSP also contributing to the overall confidence of detection.
- A gap analysis of surveillance across the continuum to consider areas requiring attention in developing the next phase of the NBPSP
- Model development to elicit the optimal design of the sentinel hive component of the NBPSP (described in 2.2.1 and 2.2.3)
- Calculation of the level of confidence of detection through the sentinel hive component of the NBPSP

2.2.1 Development of the Varroa Spread Model

A key component of this project was the evaluation of surveillance requirements for early detection of Varroa. To undertake this evaluation, a *Varroa* Spread Model was developed to estimate the potential hazard of an arrival of *Varroa* at a port, based on a set of external assumptions, geographic features of the port, and design components. This evaluation was initiated to assess the sentinel hive component of the NBPSP with a particular emphasis on detection of *Varroa* mites to determine if improvements could be recommended to make surveillance more cost-effective, efficient or robust.

Appendices 4 and 5 provide more detailed information on development of the *Varroa* Spread Model. Further refinement of the *Varroa* Spread Model was undertaken using a Regression Tree Model to reveal the more influential factors in predicting the hazard (Appendix 6).

The *Varroa* Spread Model consists of three main activities, namely (1) Developing a stochastic spatial spread model for *V. destructor* within and between beehives, (2) Calibrating this spread model to known incursion events of *Varroa*, and (3) Running this model forward to evaluate the surveillance effectiveness of various sentinel hive surveillance options. A summary of sentinel hive parameters is provided in Table 5.

It needs to be noted when reviewing the outputs of the model that the approach for the surveillance activities undertaken by NBPSP, and the evaluation in the *Varroa* Spread Model assumes that the most likely point of entry of *Varroa* will be in the immediate port environs (specifically up to 5km from the port) arising from either a Varroa-infested swarm or a hitch-hiking individual bee. A swarm is the reproductive stage of a colony. However, honey bee colonies only exist for relatively short periods of time as a swarm, while they search for a cavity to inhabit.

A swarm has no food reserves other than the contents of the bee's stomach and therefore has a limited life span. Although shipping times between countries immediately to the north of Australia may allow a swarm to survive to reach a northern port, it is unknown whether swarms could survive the trip to southern ports in Australia.

For these reasons a full colony inhabiting a cavity in goods entering Australian ports may carry the largest risk introducing *Varroa* if undeceted at the border. A full colony will have food stores and possibly also larvae. The current implementation of the *Varroa* Spread Model does not address the introduction of *Varroa* via undetected colonies that are transported potentially large distances away from the port environs. It does, however, accommodate the possibly of mite leakage into the port environs during the period up until a colony is detected and destroyed.

The hazard described in the *Varroa* Spread Model is outlined in terms of a set of measures, each of which is represented as a probability distribution. The median and 95% percentile of the distribution was used to predict the following respective predicted hazards for a port scenario:

- Time to first detection (months)
- Outbreak area at first detection (km²)
- Number of infested hives at first detection

Factor	Description	Levels
Number of		1 hive
sentinel hives*		2 hives
		4 hives
		6 hives
		9 hives
с. н. н.:		12 hives
Sentinel hive spacing	Distance between sentinel hives	1 km
spacing	nives	2 km
		3 km
		5 km
Inspection interval	Time between hive inspection	1 month
Interval		2 months
		4 months
Apistan® resistance	The reduction in susceptibility to Apistan® arising from	0 equating to P(Kill of phoretic mite)=0.70
resistance	genetic resistance	50% equating to P(Kill of phoretic mite)=0.35
Distance to	Distance to the nearest	1 km
hobbyists	suburbs where hobbyist bee	
T	keeping occurred	5 km Circle has besting Creiter
Incursion type	Characteristics of incursion event	Single bee hosting 6 mites
	CVCIIL	Swarm hosting 100 mites
		Swarm hosting 1,000 mites
Incursion location	The spatial location of	Random bearing from port with distance drawn
	incursion in relation to the port of arrival	from a Uniform (0,5) distribution.
Carrying capacity	Density of hives (managed and unmanaged combined)	5 km ⁻² (low density scenario)
		10-20 km ⁻²
*We also assessed	'bespoke' arrangements such as F	Brisbane with 2 sites containing 3 hives each.

Table 5 Factors, their description and levels for sentinel hive scenarios by simulation.

2.2.2 Aggregation of surveillance activities in the NBPSP

Given the NBPSP is comprised of activities other than sentinel hives and has a number of high priority bee pest targets, most high risk ports have at least 4 activities that include a range of surveillance methods for multiple targets (see Table A1.2 Appendix 1 and Appendix 3).

The contribution of these activities was assessed by the following approach:

- Specifying a desired overall power (probability of detection of the pest, given the pest has arrived at one of the ports of entry).
- Allocation of this power to the different surveillance components, based on their sensitivity (sigma), footprint (area of detection) and cost.
- Determination of the number of items required to achieve this power for each surveillance component.
- Allocation of the surveillance components to the different points of entry, based on their area of surveillance and risk (entry and establishment).

2.3 Redesign of the NBPSP

The review process above informed the redesign phase for a future NNPSP and included the following elements:

- Development and consideration of proposed options
- Costing of proposed options (including real value for delivering each option and analysis of the level of investment)
- Development of recommendations for the next phase of the NBPSP based on the review and redesign process.

3. Outputs

This project assessed the effectiveness of the current NBPSP through review of the sentinel hive component and provision of recommendations for post border surveillance for bee pests and pest bees. This review will assist funding parties make decisions that will contribute to a rigorous and comprehensive surveillance program. Outcomes of this project will benefit all pollination responsive industries and the honey bee industry.

The output of this project is this a report that describe the following:

- The preferred approach to surveillance for Varroa mite and other bee pests
- The recommended methods and suggested costings for bee pest surveillance
- Suggested frequency and array of sentinel hives at key Australian ports
- Proposed organisation and management of the NBPSP
- Recommendations for further information and activities required to improve surveillance for bee pests and pest bees

To address the above Outputs, the project has undertaken the following:

- An outline of the likely cost of not detecting *Varroa* early enough (Appendix 2). Information has been summarised from previous reports on the predicted impact of an incursion of *Varroa* on pollination-reliant industries.
- A review of the NBPSP with a specific focus on statistical analysis of the sentinel hive component for detection of *Varroa* mites (see also Appendices 1, 4, 5 and 6).
- Key port locations for surveillance as indicated by previous work conducted by Caley *et al.* 2013 to assess hazard status of ports (see also Appendix 1).
- The key bee pest and pest bee threats of the honey bee and pollination-reliant industries and the preferred surveillance methods for these threats (Appendices 2 and 3).
- The required probability that a *Varroa* incursion will be detected early enough, as indicated by the estimated area of infestation before *Varroa* is detected in a sentinel hive (Section 4.4 and Appendices 4 and 5). Determination of whether eradication was possible was inferred by determining a predicted threshold for the maximum area of infestation and describing the key parameters required for surveillance.
- The recommended methods for surveillance and their costings (Sections 4.7 and Table 7 and 9, see also Appendices 1 and 7).

4. Outcomes

4.1 National Bee Pest Surveillance Program 2013–2016

From 2013–2016, the NBPSP undertook targeted surveillance for *Varroa* mites, Tropilaelaps mites, Tracheal mite, small hive beetle and pest bees such as Asian honey bee, Giant honey bee, Red dwarf honey bee and exotic strains of *A. mellifera*. Surveillance activities included sentinel hives, swarm capture, catchboxes, floral sweep netting and awareness programs with 541 surveillance activities in undertaken in 2013, 868 activities in 2014 and 939 activities in 2015. A detailed outline of activities in the NBPSP is provided in Appendix 1 and 2.

4.2 Varroa Spread Model - Sentinel hive component

The general conclusions from the evaluation of the *Varroa* Spread Model assessing the sentinel hive component of the NBPSP found that an optimal arrangement of sentinel hives was 6 hives at each port with an array spacing of 2 km and checked every 6 weeks. The expected size of a *V. destructor* incursion at first sentinel hive detection for this surveillance setup up was estimated to be in the order of 100 km² following a delay of 6 months since introduction assuming that the introduction was a swarm or unmanaged colonies (estimated to be in the 100s) that was not carried out of the port and past the surveillance zone. This is equivalent to a circular infestation area of diameter *c.* 11 km. By this stage, a considerable number of unmanaged (100s) and hobbyist (estimated to be in 10s to 100s) hives will also be infested, depending on how close suburbs are from the port. Intuitively, the reason for the high infestation of unmanaged bee colonies and/or hobbyist hives, by the time of the first detection in sentinel hives is that all hives are infested by the same method. Furthermore, it is unlikely that the infestation of sentinel hives will occur prior to infestation of unmanaged bees, particularly when the latter are assumed much more numerous.

A summary of the outcomes of the *Varroa* Spread Model is provided in Appendix 4 and a detailed examination of the results is provided in the Technical Report (Appendix 5). A description of the approach used for Regression Tree Modelling is provided in Appendix 6.

4.3 Required probability for early detection of bee pests for eradication

The *Varroa* Spread Model provided information on the likely time to first detection (in months), the size of outbreak (in kilometres) and the number of infested hives given the input parameters used in the model. While this assessment revealed broad outcomes in terms of the spread of an outbreak, a more refined analysis using a regression tree model was undertaken to determine the most influential factors impacting detection of Varroa. In these analyses it was determined that, not surprisingly, the size of the initial infestation played a large role in the time to detection, with a best case scenario of detection time of 2.3 months if a swarm with 1000 mites entered and a surveillance regime of 4 sentinel hives was deployed at a 1 km spacing. The worst case scenario in this instance was a time to detection of 8.3 months if a single bee with mites entered and a surveillance regime of less than 4 sentinel hives and greater than 2 km spacing of hives was deployed.

This analysis was also used to estimate the surveillance requirements for different predicted sizes of infestation. The following three areas were selected as targets for the analysis as these were considered reasonable for coverage of a potential eradication program:

- 100 km² or a diameter of approximately 10 km

- 150 km² or a diameter of approximately 12 km
- 200 km² or a diameter of approximately 14-15 km

For target infested area of 100 km², there was a 74% chance that a surveillance regime of more than 5 sentinel hives, spaced at 2 km would detect *Varroa* within this area. This was based on the assumption that surveillance was undertaken using sentinel hives alone and that a swarm of 1000 mites was the original source of infestation. In this scenario, the likelihood of detection increases if additional surveillance activities were deployed in conjunction with sentinel hives.

For the largest infestation area assessed of 200 $\rm km^2$, there was a 69% chance of detection if only 1 sentinel hive was used if the original infestation was a swarm with 1000 mites.

4.4 Determination of whether eradication of *Varroa* will be possible

Based on the information available, it was not possible for the *Varroa* Spread Model or Regression Tree Model to determine if eradication of a predicted *Varroa* outbreak would be possible as this is largely dependent on the size of the eradication program that is implemented. The following key parameters were identified however within workshops and consultation with experts that were anticipated to greatly improve the likelihood of eradication:

- Eradication may be more likely if first detection of *Varroa* within an area was restricted to less than approximately 200 km² (i.e. approximate diameter of a detection zone of 14 km). These figures were chosen, in part, based on natural transmission which was considered slow averaging approximately 10-15 km per year (Stevenson *et al.* 2005). It was recognised however the main source of long distance dispersal will be by human assisted movement.
- Movement of hives can be strictly controlled to limit longer distance dispersal of Varroa.
- The key finding from the work of Penrose & Caley (2011) is that eradication will be very difficult and unlikely to be successful without the use of remote poisoning. It will therefore be essential for a remote poisoning program to be implemented to control bee numbers (and hence *Varroa* populations) in the eradication areas, as location of all unmanaged (and potentially managed) hives will not be possible over larger areas.

There are also factors associated with an incursion that are likely to increase the chance of early detection. These factors include:

- The initial incursion being made up of a larger population of mites. Modelling indicates that a swarm containing 1000 mites entering Australia is likely to be detected as early as 2.3 months following entry if there are greater than 4 sentinel hives at a 1 km spacing. Conversely, it was estimated that incursion of a single bee with mites may take over 5 months to first detection under the same surveillance regime, as it will take time for populations to build to detectable levels.
- Number of bee keepers near the entry point of an incursion. If an incursion enters a port area with a large number of either hobby beekeepers or numbers of commercial beekeepers, the likelihood of dispersal through human-assisted means is increased, spreading an incursion beyond a 10 km distance more quickly.
- A suggested optimal number of 6 hives. If 6 hives cannot be achieved, a minimum number of 4 hives at high or medium risk ports is recommended.

Given resourcing constraints within the program and physical constraints relating to placement of sentinel hives at ports, there will be situations at ports where it will not be possible to deploy the optimal arrangement of hives. It is noted that ports currently have differing arrangements of hives. For example, in Western Australia, there are 2 medium risk ports with only 1 sentinel hive. Using results from the *Varroa* Spread Model, this resulted in a median (50%) incursion size at detection of 121 km² (with the 95% percentile having an area of 376 km²) compared to a slightly improved median (50%) estimation of 95.7 km² (with the 95% percentile having an area of 325 km²) for the reference 6 hives at 2 km layout.

The refined regression tree model further predicted a need to deploy greater than 5 sentinel hives at 2 km spacing to achieve a 74% chance of detection within an infested area of 100 km² (or approximately 10 km diameter).

4.5 Review of additional surveillance methods used during NBPSP 2013– 2016

A review and analysis of each of the additional surveillance activities was conducted to understand how each activity adds value to the current program, and it also provides examples of gaps where activity could be increased to improve surveillance outcomes.

Swarm Capture

Currently there are 41 ports which have recorded a capture of a swarm. The majority of these are at major ports across the jurisdictions, namely QLD, NSW and WA. It is acknowledged that the current swarm capture activity occurring in a number of ports is a part of the current contracted program, but there is also additional swarm capture activity at lower risk ports. This additional activity adds significantly to the program providing a broader geographical distribution of surveillance activity which is important given the large distances involved.

Catchboxes

Currently there are 15 ports (11 high and medium risk ports, 4 low and unknown risk ports) where 171 catchboxes are deployed. Establishment of the majority of these catchboxes are at high and medium risk ports adding value to the surveillance of *A. mellifera* and pest bees at these ports. Review of the program suggests deployment of catchboxes could be increased in WA, NT and Tas. Beekeeper involvement would add further value and sensitivity to this activity by providing assistance to improve the attractiveness of catchboxes to *Apis* species.

Remote catchboxes

Five ports (4 in QLD and 1 in NT) currently have remote catchboxes deployed. The use of remote catchboxes has been a recent addition to the NBPSP as significant research has been required to identify requirements for their successful deployment. Remote catchboxes provide additional surveillance in remote locations in high and medium risk ports, and it has been proposed in this report that a further 20 remote catchboxes be deployed across the ports, particularly where access is difficult. Analysis of this surveillance activity has identified areas of improvement including but not limited to software and hardware upgrades and website interface. These improvements will increase robustness and sensitivity of the system. Beekeeper involvement would add further value and sensitivity to this activity by providing assistance to improve the attractiveness of these to *Apis* species.

Floral sweep netting

Floral sweep netting is undertaken in a total of 19 ports. The success of this component is reliant on an understanding of the environment and key floral resources within the surveillance area, and floral maps are needed to enable sweep netting to be timed appropriately to achieve positive outcomes. This method can be beneficial in providing a better understanding of the movement of bee populations within an area (including *A. mellifera* and established *A. cerana* populations). Floral sweep netting will be an important method for incorporation into a fully scoped and implemented Asian Honey Bee Surveillance Plan in the future.

Beekeeper involvement

Beekeepers are involved in all high risk ports, undertaking in sentinel hive surveillance and additional activities such as sugar shake, alcohol wash and drone uncapping. Beekeepers are also vital in additional inspection of swarm capture, floral sweep netting and general observations of bee populations within areas. Beekeeper knowledge and skills are vital for the future improvement to aspects of the NBPSP.

Tracheal mite

Tracheal mite analysis is a key component of the NBPSP, with currently 19 ports including tracheal mite surveillance. As the sentinel hive arrangement recommended in the future NBPSP would change from the current arrangements (which will achieve an increased chance of detection and be more cost effective), tracheal mite testing would also change. Scoping for improvements to the sensitivity of tracheal mite detection including molecular diagnostics have been considered in the proposed options for the new NBPSP.

4.6 Potential costs for a revised National Bee Pest Surveillance Program

Costs for deployment of sentinel hives are based on an estimate of \$2,500 per hive per annum comprising \$1,000 for diagnostics, \$700 for maintenance of each hive and \$800 for assessment at 6 week intervals. This is likely to be an underestimation for remote ports as additional travel may be required to monitor these areas on a 6 weekly basis.

Of the total 44 ports listed in Table 6, (Appendix 1; Table A1.2); 25 ports are assessed as high or medium risk, of which 20 ports already have sentinel hives deployed. If these 20 ports were to have a recommended array of 6 hives at 2 km spacing, the cost to the program (in terms of diagnostics, consumables and maintenance) is estimated to be approximately \$300,000 per annum. In addition to this 6 hive array, if 4 sentinel hives were deployed at each lower risk port (low and unknown rated), that currently have sentinel hive activity (16 ports), then the total estimated cost would be \$450,000 per annum. This estimated total cost (6 and 4 sentinel hives deployed at all risk level ports) provides a more enhanced and efficient (cost-effective) surveillance system incorporating high and medium risk ports with the optimal sentinel hive array for detection success, and raises the lower risk ports to a standard that will provides adequate, efficient and worthwhile surveillance outcomes. This proposed system also allows the incorporation of an additional lower rated port used for sentinel hive surveillance for NT (which other proposed programs would exclude). The addition of 4 sentinel hives at lower rate ports provides strength and vigour to the NBPSP, nationally.

These figures do not take into account additional surveillance components such as catchboxes, floral sweep netting and swarm capture, resources for diagnosis for key pests, resources for program coordination or surveillance activities for new targets such as viruses and Asian hornets and an expanded Asian honey bee program.

Therefore, nationally, a revised NBPSP which includes the optimal surveillance activity across all jurisdictions, is estimated to be \$732,500 annually and is proposed in Table 1 and Table 7 (plus an additional one off cost of \$185,000 for recommended enhancements). It is recognised within this proposed program that the cost-effectiveness of the NBPSP has to be balanced between an effective sentinel hive component for early detection of *Varroa* and the inclusion of other vital activities that undertake surveillance for a range of key targets, as well as the coordination and data capture for the program. As a result, it is anticipated that while this program is considerably larger than the existing NBPSP, there is some movement within the proposed plan to discuss with each jurisdiction regarding the physical constraints at each port, and the deployment of surveillance activities that limit deployment at some ports.

It should be noted that this analysis has identified that the existing program operating from 2013–2016 has been considerably under-funded, with significant in-kind commitment having been provided by all agencies (see Appendix 7 for costing analysis).

5. Evaluation and Discussion

5.1 Contribution of the Sentinel hive component to the NBPSP

The results from the *Varroa* Spread Model incorporate considerably more realism than previous treatments of the problem, both in terms of model detail and the underlying data (see Appendices 4 and 5 for detailed description of the methods and results from this Model). That said, uncertainty remains in the parameterisation of the spread of *Varroa*, arising in part from irreducible uncertainty in the details of the New Zealand incursions used for model calibration. Our analysis has included this uncertainty through to the outputs. The following conclusions have been obtained from this modelling approach:

- All else being equal, a spacing of 2 km between sentinel hives seems optimal, however a 1 km spacing will improve the likelihood of detection.
- Surveillance performance starts to deteriorate noticeably once the interval between hive inspections exceeds two months.
- Fewer hives at more locations is better than multiple hives at fewer locations. An optimal number of hives at each port is considered to be 6 hives, however a minimum of 4 hives is estimated to be required for greatest effectiveness.
- At the time of first detection of *Varroa* mite in sentinel hives, the number of infested unmanaged colonies will be in the 100s.
- Depending on how close domestic beekeeping occurs to the port environment, the number of infested domestic hives ranges from 10's to 100s. This clearly represents of risk of generating satellite foci of infestation through hive movement.
- To successfully eradicate such a number of infested unmanaged colonies will undoubtedly require the use of toxins (e.g. Fiprinol) in combination with effective management of hobbyist hives. Effective movement control would be critical.

Further refinements of the *Varroa* Spread Model using regression tree modelling (Appendix 6) assessed potential target areas for detection of 100 km², 150 km² and 200 km² in an attempt to assess the requirements for surveillance components that may increase the likelihood of detecting *Varroa* within these areas of infestation. From this work, to achieve an area of infestation of 100 km² (i.e. an approximate diameter of 10 km), there was a 74% chance of detecting *Varroa* with 5 or more sentinel hives at a 2 km spacing. This assumed a larger swarm incursion (1000 mites) had entered, compared with only a 26% chance of detection if a single bee or swarm with only 100 mites entered.

It should be noted that in addition to their main purpose as surveillance tool, sentinel hives also play a role in maintaining awareness of the importance of bee biosecurity at ports, and it is not known whether reducing hives at ports that currently have higher sentinel hive numbers such as Geelong and Melbourne, would have a negative impact on this awareness component in those locations.

The *Varroa* Spread Model showed that the number of unmanaged colonies and/or hobbyist hives has a major impact on detection statistics. The greater the number of hives, the faster the rate of spread, the later the first detection in sentinel hives, and the larger the infestation area at first sentinel hive detection. Consideration of suppression of unmanaged colonies around ports may be warranted, along with inspection regimes for hobbyist hives close to high risk ports. The exact implications of commercial beekeeper movement of hives are difficult to quantify without explicitly incorporating such operations into the simulation model, with all the complexity of integrating over yard sizes, location in relation to the port of interest and timing of movements. However, some robust generalisations can be made. First, given the recognition that a considerable number of unmanaged colonies and hobbyist hives are likely to be infested at the time of first sentinel hive detection, it stands to reason that a commercial apiary would also be infested at this time. The key issue then relates to the timing of movement of commercial hives for pollination.

The worst case scenario is that the commercial hives have been present from the initial incursion, and are infested immediately. This being the case, there is still about a 30% chance the beekeeper involved is one of those that does not move his bees, in which case no export of *Varroa* has occurred. If not, the chance of the hives being shifted and exporting *Varroa* in the expected 6-month delay before sentinel detection (and hive movement controls) is about 90% based on the movement rate. The distances involved are likely in the order of 100s of kilometres (see Gordon *et al.* 2014), and the possibility of contamination en-route high due to the infrequent use of netting to prevent losing bees.

5.2 Improvements to the NBPSP (2016–2021)

Sentinel hive component

The *Varroa* Spread Model demonstrated there was a small to moderate impact on the surveillance sensitivity assuming a 50% reduction in toxin-induced mortality arising from chemical resistance in *Varroa* to the current miticide strips used within sentinel hives. New permits have been obtained for the use of formic acid and given the relatively minor cost component in the context of the NBPSP, it is recommended that additional chemical acaricide components are included in sentinel hives.

Most ports considered to be high risk are currently serviced by an optimal of 6 sentinel hives, with 5 ports (Port Botany, Fremantle, Bell Bay, Geelong and Melbourne) currently operating with greater than 6 hives (which under the proposed option will be brought to an efficient array of sentinel hives) (Table 5). Exceptions of high and medium risk ports with fewer hives are the ports of: Weipa, Mackay, Mourilyan, Port Alma, Portland, Esperance, Albany (port), Geraldton, Bunbury and Darwin; with either zero or only 1 hive (which will be supported in the costs to establish the optimum number of 6 sentinel hives). In Table 6, current lower risked ports with sentinel hives will be supported to bring the number to a minimum of 4 sentinel hives for effective surveillance activities.

Improvements may be possible to the optimal spacing of hives at all ports, given the prediction that improved effectiveness will occur if hives are set up at an array spacing of 1 or 2 km. All ports will need to be assessed to determine the feasibility of 6 hives at a 2 km array and, if not practical given geographic or other constraints, provide reasons for the deviation from an optimum array recorded within the program.

Increased additional surveillance activities such as sweep netting, catch boxes, beekeeper involvement and tracheal mite analysis are recommended at all ports, especially encouraged at the minimum number of sentinel hives/port (lower risk ports). As noted in Table 7 the deployment of additional remote catchboxes is proposed and will be available, in addition, to the optimal and minimum sentinel hive array per port. The deployment of remote catchboxes will be considered for jurisdiction which reduced surveillance due to area size and remoteness. These remote catchboxes will add value to the surveillance outcomes per jurisdiction. Table 6 Comparison of port risk rating and current sentinel hive deployed vs. option #3 proposed NBPSP. The proposed program brings all jurisdictions into a minimal (4 hive array) and optimum (6 hive array) design, this results in an efficient cost:time:successfulness surveillance program.

Surveillance activity	Port hazard rating ²	Sentinel Hives ³	Proposed NBPSP #3			
Queensland Ports ⁴		_				
Brisbane	Н	6	6			
Cairns	М	6	6			
Gladstone	Н	6	6			
Townsville	М	6	6			
Weipa	М	0	04			
Mackay	М	0	04			
Mourilyan	Н	0	04			
Port Alma	М	0	04			
NSW Ports						
Port Botany/Kurnell	н	8	6			
Newcastle	н	6	6			
Port Kembla/ Wollongong	н	6	6			
Richmond	U	1	4			
Goodward Island	U	1	4			
Chifley	U	1	4			
Jervis Bay	U	1	4			
Parma	U	1	4			
Eden	U	1	4			
Victorian Ports						
Geelong	М	11	6			
Melbourne	н	11	6			
Portland	М	2	6			
Westernport	U	6	4			

² Hazard rating for incursion of *A. mellifera* derived from Caley *et al.* 2013. H = High hazard (first quartile); M = Medium hazard (second quartile); L = Low (third or fourth quartile); U = unknown (not assessed) 3 Targets *Varroa* & Tropilaelaps mite. Sentinel hives are monitored every 6 weeks.

⁴ It is suggested 6 sentinel hives could be deployed in addition to current activities at H or M risk ports at an additional cost of \$90,000 (6 hives added to 2 ports in Qld, 1 port in Tas., 1 port in NT). Table 6: Proposed Option #3 and the sentinel hive number at ports was calculated/modified from the current sentinel hive locations and their numbers. Therefore; if sentinel hives are going to be maintained at high/medium risk ports then there needs to be 6 hives, and for low/unknown risk ports if jurisdictions have already deployed hives then they need to have at least 4 hives (this is required to meet the statistical sensitivity in detection of bee pest/pest bee). Currently Qld has hives only deployed at their contracted sites, however we have recommended that a further 6 hives could be deployed at 2 additional high/medium risk ports. This is similar for Tas. (1 port) and NT (1 port) (please locate footnote 4 in text). This deployment across 3 jurisdictions (24 hives) would be at an additional \$90,000.

Tullamarine airport	U	2	4				
Western Australian Ports	Western Australian Ports						
Fremantle	Н	7	6				
Kwinana	U	1	4				
Perth airport	U	6	4				
Perth	U	3	4				
Esperance	М	1	6				
Albany (port)	М	1	6				
Dampier	L	0	0				
Geraldton	М	2	6				
Bunbury	Н	1	6				
South Australian Ports							
Port Adelaide	Н	6	6				
Whyalla	L	0	0				
Port Pirie	L	6	4				
Wallaroo	L	6	4				
Adelaide airport	U	0	0				
Northern Territory Ports ⁴							
Darwin	М	3	6				
Berrimah Farm	U	3	4				
Airport	U	0	0				
Groote Eylandt	М	0	0				
Tasmanian Ports ^₄	Tasmanian Ports⁴						
Hobart	Н	8	6				
Devonport	М	4	6				
Burnie	L	4	4				
Bell Bay	Н	7	6				
Total		151	180				

Links to other bee biosecurity programs

Formal links should be created between the NBPSP and the newly established National Bee Biosecurity Program, coordinated by Plant Health Australia and anticipated to operate in South Australia, Victoria, New South Wales, Queensland, Western Australia and Tasmania. The Bee Biosecurity Program component operating in each state is likely to provide additional facility for bee sample collection from commercial and hobby beekeepers. Links to both the Bee Biosecurity Program and BeeAware may be able to assist target areas that may be deemed as having insufficient coverage in the current port program based on findings of the *Varroa* Spread Model and regression tree modelling.

Links to these programs will also provide broader coverage to improve awareness of bee biosecurity and surveillance for new pests post-border. This is of particular importance, as while there is a recognition that ports are a high risk for entry of bee pests and pest bees, they are not the only means of entry of new pests and there is a recognition that human-assisted dispersal can move pests from a point of entry.

Funding for the NBPSP

One of the strengths of the current National Bee Pest Surveillance Program is its national coverage and engagement with multiple stakeholders. The current system of co-investment highlights the shared responsibility and high levels of engagement with stakeholders that contribute to a national surveillance dataset for bee biosecurity.

The funding model with multiple stakeholders poses a risk however as it is dependent on several funding sources to be effective and requires resources to manage multiple contracts and ensure engagement occurs across stakeholders. Coordination across the research components that comprise the program requires resources in its own right. It should also be noted that significant levels of in-kind resources are being contributed by all agencies providing the activities listed in the current NBPSP, and discussion with all parties has indicated that this is not sustainable into the future. To ensure continuity in maintaining the resource base, a 5-year program is recommended, with annual reporting and a review component after 3 years to assess effectiveness of the arrangements.

While this project has indicated that efficiencies may be possible as a result of a potential reduction of sentinel hives at five ports, additional rigour would bolster the system by increasing surveillance activities where less than 6 sentinel hives can be achieved.

To increase surveillance activities and coordination for key areas, the following is recommended for the new NBPSP:

- Expansion of the program to ensure all high and medium risk ports have surveillance activities. Within the current program there is 1 high port with no sentinel hives (Mourilyan in Queensland) and several high and medium risk ports with less than 6 hives (Table 5). Where possible, an optimum of 6 (minimum of 4) sentinel hives should be deployed at all high and medium risk ports, however for ports where deployment of sentinel hives is too difficult (e.g. Darwin), an increase in other surveillance activities such as catchboxes or sweep netting should be considered.
- An up grading of smart phone technology, software and hardware, and the web-interface is required to improve the utility of the Remote Catchboxes. It is recommended that these improvements are made, and that a further 20 Remote Catchboxes be deployed in areas where access is difficult and remoteness limits surveillance coverage.
- Further to the development of an Asian honey bee surveillance plan, surveillance activities for Asian honey bee should be expanded to monitor the spread of the existing populations in Queensland and to provide early detection for new populations of this pest. Annual or biannual surveys (floral sweep netting and rainbow bee eater pellet analyses in combination with public awareness) are required to map the extension of range of existing Asian honey bee populations and confirm no new populations have established. In addition, proof of absence data should be scoped for other states in order to meet possible future market access requirements.

- Consideration of a component for bee virus diagnostics to provide surveillance for 5 high priority virus threats. It is anticipated collection of bee samples for virus surveys would occur through the NBPSP activities (e.g. sweep netting and swarm capture), as well as through engagement with commercial and hobby beekeepers as part of the National Bee Biosecurity Program. Virus surveys will assist with area freedom for export markets. Honey and bee product exports from Australia are currently estimated at \$17.5 million (as of 2014) compared to relatively low costs of surveillance of \$20,000 per year.
- A new component for surveillance for Asian Hornet. This pest will require specific surveillance techniques, including investigation of a trap and lure for deployment at high risk sites such as Brisbane, Melbourne and Port Botany. The Asian Hornet (*Vespa velutina*) is considered a significant problem for beekeepers due to its aggressive and effective predation of the European honey bees and wild bee populations. The Asian Hornet is also potentially deadly to allergic people. For these reasons an Asian Hornet trap trial would be an important addition to the next phase of the NBPSP.
- An ongoing assessment of the effectiveness of the surveillance methods is recommended with subsequent updating of the Operations manual this could be achieved through literature review, survey of state apiarist, and OSS staff, workshops and further modelling activities.
- An improved method of data collation and reporting would assist to deliver outcomes of the NBPSP. The development of web-based tools for data capture and collection should be investigated as part of the NBPSP. A possible mechanism for data capture is through use of the recently developed web-based system, AusPestCheck. Development of Automatic Programing Interfaces (APIs) will be required, but once established, data capture mechanisms for automatic upload will ensure increased efficiencies through real-time data upload and reporting occurs for the NBPSP.
- Consideration could be given to expanding the use of hobby beekeepers hives both around ports and beyond port areas to reduce the cost of managing surveillance colonies. Given the learnings of the BeeForce project, strong links to the National Bee Biosecurity Program and/or similar resources would still be required for expanded hobby beekeeper involvement, as ongoing support is needed to maintain engagement using this approach.
- Additional coordination for the NBPSP. Given the multiple components that comprise the NBPSP and the requirement for facilitation between stakeholders and between research components, specific resources are required to manage contracts, manage data collection and ensure efficiencies are maintained.

NBPSP proposals

There are three proposals suggested for a future NBPSP, with all proposals taking into account the optimal 6 sentinel hive array for high and medium ports, and minimum 4 sentinel hives for low and unknown rated ports. All proposals include additional surveillance activities and also provide costings of enhancements to the NBPSP where appropriate, as well as one-off costs which are needed to allow for the enhancements to take effect in the future NBPSP.

The significant difference between these three proposals are related to the number of ports where sentinel hives are to be inspected. It should be noted that all new proposals take into account where sentinel hives are currently deployed. Where high and medium ports have less than 6 hives; additional sentinel hives are to be established, while at locations where there are more than 6 sentinel hives it is recommended that numbers be decreased to 6. This is suggested due to the statistical analysis showing that adding more than 6 does not significantly increase chances of detection rates enough to

warrant the increase in expenditure.

Further to these sentinel hive arrangements, for ports with low and unknown (not analysed) risk ratings with less than 4 sentinel hives, it is recommended a minimum number of 4 sentinel hives is deployed (as statistically supported).

Therefore:

- Any ports > 6 sentinel hives, reduce to 6 sentinel hives
- Any ports with 6 sentinel hives, remain at 6 sentinel hives
- Any high and medium ports < 6 sentinel hives, increase to 6 sentinel hives
- Any low and unknown ports < 4 sentinel hives, increase to 4 sentinel hives
- Any low and unknown ports > 4 sentinel hives, decrease to 4 sentinel hives

A further 6 high and medium risk ports could be added to the program at an additional cost of \$90,000 (6 sentinel hives added to 2 ports in Qld, 1 port in Tas, 1 port in NT).

Table 7 below provides a summary output comparing the current programs against the three proposed options. All three sentinel hive arrangement costed proposals can be found in greater detail in Appendix 7.

Proposal #1: An optimum level of 6 sentinel hives are deployed at all risk rated ports. Additional surveillance activities (as broken down in Appendix 7, and Table 8) is undertaken at all ports to ensure maximum coverage for all high priority bee pests and pest bees.

Proposal #2: Provides sentinel hive activity across only high and medium ports.

- 6 sentinel hives established at all high and medium risk ports.
- Additional surveillance activities for all high priority bee pests and pest bees.

Proposal #3: Provides sentinel hive activity across all ports at the following levels.

- 6 sentinel hives established at the high and medium risk ports.
- 4 sentinel hives deployed at lower and unknown risk ports.
- Additional surveillance activities for all high priority bee pests and pest bees.

This proposal takes into account what is currently happening and adds value to these activities. It ensures what currently is taking place is brought up to an effective and efficient standard for providing adequate surveillance for what the desired outcomes of the program. Comparing this proposal to the current NBPSP (contracted and non-contracted program), the proposed program is being brought up to a level that is more sustainable for the program's partners and holds strength and rigour in its activities. This proposal now provides adequate funding to support these activities, and provides confidence to the industry in surveillance.

Table 7. Summary of annual costings comparing the current contracted program, the activities currently contracted and non-contracted, and three proposed options. The table is split into costs annually for sentinel hives, additional surveillance activities, and the surveillance enhancements for the future. Total annual costs for these separate programs is provided, as well as the total annual costs including a one off cost for investigations in establishment of new surveillance enhancements (\$185,000).

	Current contracted program	Currently contracted + non- contracted program	Proposal #1 All ports with 6 hives/port	Proposal #2 All medium and high risk ports with 6 hives/port	Proposal #3 All high and medium ports 6 with 6 hives/port and 4 hives/port at low and unknown risk port
Sentinel hive arrangement	\$284,000	\$377,500	\$525,000	\$300,000	\$450,000
Additional surveillance activities	\$254,500	\$254,500	\$254,500	\$254,500	\$254,500
Surveillance enhancements	\$28,000	\$28,000	\$28,000	\$28,000	\$28,000
Annual cost	\$566,500	\$660,000	\$807,500	\$582,500	\$732,500
Total costs over a five-year period (incl. one off enhancements at \$185,000)	\$2,017,500	\$3,485,000	\$4,222,500	\$3,097,500	\$3,847,500

An indication of the budget implications for an expanded program are provided in Table 8. Table 8 utilises Proposal #3 (6 sentinel hives at high and medium risk ports and 4 sentinel hives at low and unknown risk ports). The figures are proposed figures per annum for each agency/activity component of the NBPSP. It should be noted that this still represents a considerable in-kind contribution for all surveillance activities each jurisdiction undertakes. An estimate of the proposed figures for a sentinel hive component alone, is presented in Appendix 7, Table A7.1.

Table 8 Proposed costings per annum for the NBPSP 2016-2021 for proposed option #3. Costings are broken down for understanding. Proposed option #3 is compared to the 'actual' costs of the current contracted activities (especially regarding the sentinel hive component).

Component	'Actual' cost of the current contracted 2015/17 (\$)	Numbers of ports and risk status	Proposal #3 2016/17 (\$)	Comments
Sentinel hive array component	t			
Queensland	60,000	4 High	60,0004*	
New South Wales	55,000	3 High, 6 Unknown	105,000*	
Western Australia	55,000	5 High/Medium, 3 Low	105,000*	
Victoria	70,000	3 High/Medium, 2 Unknown	65,000*	
Tasmania	14,000	3 High/Medium, 1 Low	55,000 ⁴ *	
South Australia	15,000	1 Medium, 2 Low	35,000*	
Northern Territory	15,000	1 Medium	25,0004*	
Total cost of sentinel hive component	\$284,000 122 hives	20 High/Medium, 14 Low/Unknown	\$450,000 180 hives	Prosed at 6 hives at H and M risk ports, and 4 hives at L and U risk ports. A total of 180 hives proposed
Current additional surveillance	e activities in place	2	1	
Swarm capture	InKind		InKind	
Catchboxes	34,200		34,200	
Remote catchboxes	7,600		7,600	
Floral sweep netting	45,000		45,000	
Beekeeper involvement	29,600		29,600	
Tracheal mite analysis	22,500		30,000*	

⁴ A further 6 H or M risk ports could be added to the program at an additional cost of \$90,000 (6 hives added to 2 ports in Qld, 1 port in Tas, 1 port in NT)

(Bugs4Bugs)					
Honey testing	2,600	2,600			
AusVet	12,900	12,900			
Chemicals/sticky mats ⁵	6,000	10,000	For all chemical and sticky mat costs (inc. formic acid)		
Miscellaneous operations	700	700			
Vehicle hire and accommodation	34,100	34,100			
Program management	800	800,			
PHA facilitation/coordination	40,000	40,000			
NAQS	5,000	5,000	Significant in-kind provided by DAWR in the NAQS component.		
Australian Capital Territory	2,000	2,000	Hobby beekeeper involvement in surveillance has jus commenced with 2 hives at Bruce, and 4 hives at Jerrabomberra Wetlands.		
Total additional Surveillance			\$254,500		
Surveillance enhancements vita	to the new NBPSP				
Diagnostics (SIBO (Virusos)		20,000	Additional activities to undertake diagnostics for a		
Diagnostics – CSIRO (Viruses)	-	20,000	survey of a minimum of 5 viruses each year		
Diagnostics – CSIRO (Viruses) Asian honey bee	-	20,000 n/avail	survey of a minimum of 5 viruses each yearThe annual cost of these two new components is		
	-		survey of a minimum of 5 viruses each year		
Asian honey bee	- - -	n/avail	survey of a minimum of 5 viruses each year The annual cost of these two new components is currently n/a, however these are key components of the surveillance scoping project, which will aim to get		
Asian honey bee Asian hornets	- - - 40,000	n/avail n/avail	survey of a minimum of 5 viruses each yearThe annual cost of these two new components is currently n/a, however these are key components of the surveillance scoping project, which will aim to ge an annual costingFurther 20 RCB deployed across jurisdictions to provide		

⁵ Note that chemical and sticky mat costs associated with the different sentinel hive options have not been factored into this costing

Yearly cost of proposed NBPSP One off surveillance enhancements vital to success of the proposed N		\$732,500 Providing optimal sentinel hive array per jurisdiction, incorporating and enhancing on existing "other" surveillance activities on these, and implementing new surveillance components. The proposed NBPSP is costed and reflected to provide the adequate surveillance needed for Australia, and provide the support needed to each jurisdiction to carry out the required surveillance activities effectively and successfully. Note: this proposed annual cost does not include annual Asian honey be and Asian hornet surveillance		
Asian honey bee surveillance and implementation	-		40,000	Develop and implement an Asian honey bee surveillance plan to monitor the established populations. Scope collection of presence/absence data for other states.
Asian hornet trap trial			20,000	Funding required for deployment of traps and lures at key ports. Research project required initially to test traps.
Updating and deploying of the RCB system			30,000	Deploy 20 previously funded remote catchboxes (\$0), update smartphone, software and hardware technology, and web interface of remote catchboxes and develop maintenance schedule.
Data collection and management	12,500		70,000	Includes one off establishment and then ongoing maintenance (at a reduced budget) of new systems such as AusPestCheck for automatic upload of data from jurisdictions.
Operations manual review and update	-		25,000	This will be required to incorporate a review and update on the current activities, as well as incorporate the new surveillance enhancements aligning to the proposed NBPSP

	\$185,000
Total cost of one off enhancements	These one off enhancements area vital requirements to the implementation and success of the revised NBPSP. These recommended enhancements are proposed as a cofunded project over a 2-year period, collaborated between DAWR and HIA.

*An increase in all components of the NBPSP is required to deliver existing surveillance, diagnostics and coordination functions. Note that for state agencies, figures comprise costs of jurisdictions managing sentinel hives. Figures do not provide full cost recovery for all activities within the proposed surveillance program for all high and medium risk ports and there is estimated to be a minimum of twice to four times the amount for these figures for in-kind contributions from state and territory jurisdictions to undertake all components.

Savings for sentinel hives may be possible through use of hobby beekeepers to maintain sentinel hives, however ongoing resources are still required for this level of engagement.

Table 9 (a repeat of Table 2 at the start of this report, Summary) below provides an extrapolation of data from Table 7 to describe the costings of the NBPSP over a 3-year period. This was undertaken to compare the costs of the program in terms of the costs of managing for a pest such as *Varroa* for a similar time period. The benefits of investment in the NBPSP become clear with the range of investment for all options extends from 1.3–1.9% of the cost of what it would be to manage for a pest such as *Varroa* for that same period.

	'Actual' cost of current contracted program	Currently contracted + non- contracted program	Proposal #1 All ports with 6 hives/port	Proposal #2 All medium and high risk ports with 6 hives/port	Proposal #3 All high and medium ports 6 with 6 hives/port and 4 hives/port at low and unknown risk port
Total costs for the NBPSP over a 30-year period (incl. one off enhancements at \$185,000)	\$17,013,500	\$19,985,000	\$24,410,000	\$17,660,000	\$22,160,000
Cost of Investment in an NBPSP for 30 years expressed as a % of the cost of managing for a pest for the same period					
(\$1.3 billion)	1.3%	1.5%	1.9%	1.4%	1.7%

6. Recommendations

Recommendation 1	Increase in funding for the NBPSP
	Within the current NBPSP, considerable in-kind contribution is occurring for all surveillance activities each agency undertakes and this model is not sustainable. A significant increase to investment is required in order to maintain and enhance components of the program.
Recommendation 2	Funding model to be determined
	A contribution model encompassing all major beneficiaries (Commonwealth, state and territory jurisdictions, the honey bee industry and all pollination-reliant industries is required).
	A sustainable 5-year funding model to be developed with a review component after 3 years to evaluate continuation of the program.
Recommendation 3	Expansion of the program to ensure surveillance is undertaken at all high and medium risk ports
	Where possible, 6 sentinel hives should be placed at all high and medium risk ports. Four sentinel hives to be deployed at low and unknown risk ports. Other surveillance activities carried out at each of these ports will support the NBPSP.
Recommendation 4	Improvements to the sentinel hive component
	For the sentinel hive component of the NBPSP, an optimum arrangement of 6 sentinel hives, at an array spacing of 2 km apart, inspected every 6 weeks should be deployed at all high and medium risk ports. Where deployment of 6 hives is not possible because of port characteristics, a minimum of 4 sentinel hives including a combination of additional surveillance components should be undertaken.
	Additional control methods such as Formic acid to be used within sentinel hives to improve detection of miticide resistant populations of Varroa.
Recommendation 5	Increase in surveillance activities other than sentinel hives
	An increase in activities such as sweep netting and installation of more remote surveillance catchboxes for ports where sentinel hives are not deployed. To deploy a further 20 RCB across ports, and for the maintenance and sensitivity in using this type of surveillance components and upgrade of technology and website interface is required. This is upgrading is recommended as a cofunded project over a 2-year period, collaborated between DAWR and HIA.
	Statistical evaluation of the cost:benefit trade offs between different types of surveillance activities is required. This could include assessment of an increase in the number of activities conducted by hobby or commercial bee keepers.
Recommendation 6	Expansion of surveillance for Asian honey bee
	An increase in surveillance activity for Asian honey bee in the eastern states of Australia to monitor the spread of the existing populations and to provide early detection for new populations of this pest. Annual or bi-annual surveys (floral sweep netting and rainbow bee eater pellet analyses in combination with public awareness) are required to map the extension of range of existing Asian honey

	bee populations and confirm no new populations have established. The
	development of an Asian honey bee surveillance plan is recommended (though currently non-costed), and once scoped and agreed be implemented (currently in process). The inclusion of evidence of absence data collection in other states should also be scoped. Initial review into the plan and implementation of an expanded Asian honey bee surveillance program is recommended as a cofunded project over a 2-year period, collaborated between DAWR and HIA.
Recommendation 7	Bee virus surveillance to be incorporated into the NBPSP
	A new component for bee virus diagnostics could be considered to provide early detection capability for high priority viruses listed in the Honey bee industry Biosecurity Plan. It is anticipated collection of bee samples for virus surveys would occur through the NBPSP activities (e.g. pooled of samples collected from sentinel hives), as well as through engagement with commercial and hobby beekeepers as part of the Bee Biosecurity Program. Virus surveys will assist with area freedom for export markets.
Recommendation 8	Surveillance for Asian Hornet to be incorporated into the NBPSP
	Surveillance for Asian Hornet is included in the NBPSP. This pest will require specific surveillance techniques, including research to investigate a trap and lure for deployment at high risk sites such as Brisbane, Melbourne and Port Botany. An initial trap trial is recommended (approval of traps for trial is currently being sought) as a cofunded project over a 2-year period, collaborated between DAWR and HIA. Overall cost and incorporation of an Asian hornet surveillance component in the annual cost of the proposed NBPSP is unknown until scoping work is completed.
Recommendation 9	Update of Operations Manual
Recommendation 9	Update of Operations Manual Evaluate surveillance methods allocating sensitivity where possible. Update and improve Operations Manual accordingly. This is a one off requirement and is vital in the implementation and success of the revised NBPSP. This recommendation is proposed as a cofunded project over a 2-year period, collaborated between DAWR and HIA.
Recommendation 9 Recommendation 10	Evaluate surveillance methods allocating sensitivity where possible. Update and improve Operations Manual accordingly. This is a one off requirement and is vital in the implementation and success of the revised NBPSP. This recommendation is proposed as a cofunded project over a 2-year period, collaborated between DAWR
	Evaluate surveillance methods allocating sensitivity where possible. Update and improve Operations Manual accordingly. This is a one off requirement and is vital in the implementation and success of the revised NBPSP. This recommendation is proposed as a cofunded project over a 2-year period, collaborated between DAWR and HIA.
	Evaluate surveillance methods allocating sensitivity where possible. Update and improve Operations Manual accordingly. This is a one off requirement and is vital in the implementation and success of the revised NBPSP. This recommendation is proposed as a cofunded project over a 2-year period, collaborated between DAWR and HIA. Improved data capture and reporting Investigation of improved data capture and collation tools to increase efficiencies and engagement amongst stakeholders within the NBPSP. A possible mechanism for data capture is the use of the recently developed web-based system, AusPestCheck. Development of Automatic Programing Interfaces (APIs) are required, but once established, data capture mechanisms for automatic upload will ensure real-time data upload and reporting occurs. One of the main purposes of collecting data is the ability to utilise this further down the chain whether for market access issues and/or understanding HPP movements. A central database is vital in easing the handling of high volume, significant data. This I recommendation is a one off requirement for further investigation into the handling of the system, it is suggested that this is a cofunded project over a 2-

	incursions	
Recommendation 12	Improved knowledge of unmanaged bee colonies around port locations	
	A research project to undertake surveys of unmanaged bee colonies within 5–10 km of high risk ports is required to improve our understanding of the numbers and locations of bee colonies, this project will also address handling methods of these unmanaged colonies, including the destruction of known unmanaged colonies within these port precincts. Information would be critical in delimiting surveillance to a bee pest outbreak and will also assist improve outputs of <i>Varroa</i> Spread Model	
Recommendation 13	Expansion of hobby beekeeper involvement to maintain sentinel hives or undertake other surveillance methods	
	Expanding the use of hobby beekeepers hives both in port vicinities and beyond port areas could reduce the cost of managing surveillance colonies. Strong links to the National Bee Biosecurity Program would be required for expanded hobby beekeeper involvement, as ongoing support is needed to maintain engagement using this approach. Hobby beekeeper involvement from the ACT to be included (2 hives in Bruce and 4 hives in Jerrabomberra Wetlands).	
Recommendation 14	Improving efficiencies across the post-border components bee surveillance	
	Formal links should be established and maintained between the NBPSP, the National Bee Biosecurity Program, BeeForce and BeeAware to provide the most effective coverage of surveillance activities for high priority pests. These linkages will form part of the coordination role within the program and will inform the activities targeted by each program.	
	Where optimal numbers of sentinel hives cannot be deployed at high risk ports as a result of resource limitations and/or port restrictions, additional activities (such as sugar shake or alcohol washing surveillance techniques) by commercial or hobby beekeepers should be targeted in these areas.	
	There is recommendation for continued complementary surveillance within the continuum including the capture of swarms at the ports (e.g. by the Department of Agriculture and Water Resources staff) as an example of at the border surveillance, and further included activities pre-border. Such as the collection of bee and larvae samples for molecular analysis of viruses in Papua New Guinea also support the goals of the NBPSP.	
Recommendation 15	Increased coordination component for the NBPSP	
	Within the NBPSP, increased support for coordination of the NBPSP is required to ensure linkages between components of the program and between related programs such as BeeAware and Bee Biosecurity Program occurs.	

Scientific Refereed Publications

Nil published during the course of this project.

Intellectual Property/Commercialisation

IP has been developed in the form of new knowledge has been generated through the development of the *Varroa* Spread Model.

No commercial IP generated

References

Bakonyi T, Grabensteiner E, Kolodziejek J, Rusvai M, Topolska G, Ritter W, Nowotny N (2002). Phylogenetic nalysis of Acute Bee Paralysis Virus strains. Applied and Enviornmental Micorbiology, 68,12, 6446-6450

Barry S, Cook D, Duthie R, Clifford D, Anderson A (2010) Future Surveillance Needs for Honeybee Biosecurity. RIRDC Publication 10/107, pp. 38.

BeeAware (accessed 2016) (http://beeaware.org.au/industry/).

Bellis GA and Profke AM (2003). Rainbow bee-eaters (Merops ornatus) as a monitoring tool for honeybees (Apis mellifera L.; Hymenoptera: Apidae). Australian Journal of Entomology 42, 266–270. Boland P (2005). A review of the National Sentienl Hive Program in Queensland, New South Wales, Victoria, Western Australia and the Northern Territory. *Biosecurity Australia*, Department of Agriculture, pp 29.

Brown-Walker PL, Martin SJ, Gunn A (1999). The transmission of Deformed Wing Virus between honeybees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni* Oud. Journal of Invertebrate Pathology, 73, 101-106.

Caley P, Heersink D, Paini D, Barry S (2013) Risk assessment of ports for bee pests and pest bees. Rural Industries Research and Development Corporation (RIRDC). *RIRDC Project No PRJ-008376.*

Chen Y, Evans J, Feldlaufer M (2006). Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera*. Journal of Invertebrate Pathology, 92, 152-159.

Clifford D, Barry S, Cook D, Duthie R, Anderson D (2011). Using simulation to evaluate time to detect incursions in honeybee biosecurity in Australia. *Risk Analysis* 31, 1961-1968.

Cook CC, Thomas MB, Cunningham SA, Anderson DL, De Barro PJ (2007). Predicting the economic impact of an invasive species on an ecosystem service. *Ecological Applications* 17,6, 1832 – 1840.

Crooks S (2008). Australian honeybee industry survery 2006-07. RIRDC Publication No. 08/170, pp. 89.

De Jong, D (2005). Mites: *Varroa* and other parasites of brood. Morse, R.A. and Flottum K. [Eds.] Honey Bee Pests, Predators, and Diseases. 3rd Edition. Root Publishing. Ohio, USA. 279-327. de Miranda, JR, Drebot, M, Tyler, S, Shen, M, Cameron, CE, Stoltz, DB, Camazine, SM, (2004). Complete nucleotide sequence of Kashmir bee virus and comparison with acute bee paralysis virus. *Journal of General Virology* 85, 2263–2270

Department of Agriculture (2011) A honey bee industry and pollination continuity strategy should *Varroa* become established in Australia. Australian Government, Department of Agriculture, ACT Canberra. http://www.agriculture.gov.au/animal-plant-health/pests-diseases-weeds/bee-pests-diseases/honey-bee-pollination-continuity-strategy

Goodwin M (2012). Pollination of Crops in Australia and New Zealand. RIRDC Publication No. 12/059

Gordon J and Davis L (2003). Valuing honeybee pollination. Rural Industries Research and Development Corporation (RIRDC). *RIRDC Publication No. 03/077*.

Gordon R, Bresolin-Schott N, East IJ (2014) Nomadic beekeeper movements create the potential for widespread disease in the honey bee industry. *Australian Veterinary Journal* (8) 92, 283 - 290

Hafi A, Millist N, Morey K, Caley P, Buetre B (2012) A benefit-cost framework for responding to an incursion of *Varroa* destructor. ABARES report to client prepared for the National Biosecurity Committee, Canberra.

Plant Health Australia Ltd (2013). Industry Biosecurity Plan for the Honey Bee Industry (Version 1.0 - 2013). Plant Health Australia, Canberra, ACT.

Keogh RC, Robinson AP and Mullins IJ (2010) The Real Value of Pollination in Australia RIRDC Publication 10/081.

Monceau K, Bonnard O, Thiéry D (2014). *Vespa velutina*: a new invasive predator of honeybees in Europe. *Journal of Pest Science* 87, 1–16. Monceau, K., Bonnard, O., & Thiéry, D. (2014).

OIE (2008). Acarapisosis of honey bees: Chapter 2.2.1. OIE Terrestrial Manual http://www.oie.int/fileadmin/Home/eng/Health standards/tahm/2.02.01 ACARAPISOSIS.pdf

Penrose L, Caley P (2011). Predicting eradication of *Varroa* incursions. (Australian Bureau of Agricultural and Resource Economics and Sciences: Canberra). PHA 2013

Stevenson MA, Benard H, Bolger P, Morris RS (2005). Spatial epidemiology of the Asian honey bee mite (*Varroa* destructor) in the North Island of New Zealand. *Preventive Veterinary Medicine*, 71, 241-252

Tan, K, Radloff, SE, Li, JJ, Hepburn, HR, Yang, MX, Zhang, LJ, Neumann, P (2007). Bee-hawking by the wasp, *Vespa velutina*, on the honeybees *Apis cerana* and *A. mellifera*. *Naturwissenschaften*, 94(6), 469-472

Villemant C, Barbet-Massin M, Perrard A, Muller F, Gargominy O, Jiguet F, Rome Q (2011). Predicting the invasion risk by the alien bee-hawking Yellow-legged hornet *Vespa velutina nigrithorax* across Europe and other continents with niche models. *Biological Conservation*, 144(9):2142-2150.

Case Study 4 Response to the Incursion, of the *Varroa* Bee Mite", page 80 (http://www.oag.govt.nz/2002/biosecurity---case---studies/docs/part4.pdf)

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Review and Redesign of the National Bee Pest Surveillance Program Appendices

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Project Number: MT14057

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Appendix 1: National Bee Pest Surveillance Program background information

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A1.1 Background to the National Bee Pest Surveillance Program (NBPSP)

In January 2012, the management of the National Sentinel Hive Program was transferred from Animal Health Australia (AHA) to Plant Health Australia (PHA). This followed the transfer in responsibilities for bees at a national level from Animal Biosecurity to Plant Biosecurity. Upon the transfer to PHA, the name of the surveillance program changed to the National Bee Pest Surveillance Program to reflect a transition to a more broadly based surveillance program for bee pests and pest bees.

On 1 July 2013, the NBPSP became a cost-shared initiative between the honey bee industry (represented by the Australian Honey Bee Industry Council), plant industries that rely on pollination (represented by Horticulture Innovation Australia⁶) and the Australian Government Department of Agriculture and Water Resources (DAWR). An overview of the NBPSP is presented in Figure A1.1.

In addition, programs to improve biosecurity awareness in the honey bee industry and hobby beekeepers provides an important role in detecting new pest issues. These programs include:

- BeeForce – surveillance for bee pests conducted by hobby bee keepers around the ports of Melbourne and Geelong

- BeeAware – while not a formal surveillance activity, this program provides awareness information to commercial apiarists and hobby beekeepers on exotic bee pests and pest bees.

Surveillance for bee pests and pest bees is conducted in each state and territory in Australia (Figure A1.2 and Table A1.2) and a description of these Surveillance activities is provided in Appendix 3. The NBPSP has a national coordinator to facilitate planning and implementation of NBPSP activities across Australia to ensure that the NBPSP objectives are met. The position is currently held by PHA, with support provided by the DAWR. The primary responsibilities of PHA are:

- Coordinate purchase and distribution of chemicals used within the sentinel hive component
- Administer agreements across the multiple parties that comprise the NBPSP
- Coordinate collection and capture of data from surveillance activities

The primary responsibilities of the DAWR include:

- Involvement and assistance in conducting surveillance activities at designated high risk ports
- Auditing of chemicals used within sentinel hives.
- Coordinating emergency response arrangements in the event of an incursion.

These surveillance activities have been a significant increase from 2013, following a report released in 2013 by CSIRO titled 'Risk assessment of ports for bee pests and pest bees'. The report analysed all Australian maritime ports for the hazard of exotic bee entry and establishment. The report determined the differences between ports, including the level of shipping traffic, voyage duration, country of origin of the vessel and the suitability of the port surroundings for bee establishment and persistence. Rankings for each port were developed for both the Asian honey bee and the European honey bee.

Results of the CSIRO port risk assessment (Caley *et al.* 2013) were formally incorporated by PHA into the NBPSP from 1 July 2014. Some of these changes included redistribution of Program funding to a biosecurity risk basis, resulting in increased surveillance at some ports, and implementation of surveillance at ports where there was previously none being conducted.

6

Previously Horticulture Australia Limited

The results of this survey have enabled PHA, as coordinators of the NBPSP, to ensure the surveillance is targeting all high risk ports effectively. It has also enabled relevant stakeholders to be informed about the risk of each port.

It should be noted that there are still some discrepancies in the highest risk ports for *A. mellifera* indicated in this report and the location of surveillance activities outlined in Table A1.2. A map of ports is provided in Figure A1.2.

The jurisdictional coordinators manage and coordinate NPBSP activities within their own state/territory to ensure that the NPBSP objectives are met. In New South Wales, the Northern Territory, Queensland, South Australia, Victoria, Tasmania and Western Australia the State/Territory Department of Primary Industries take the lead role in coordinating surveillance activities.

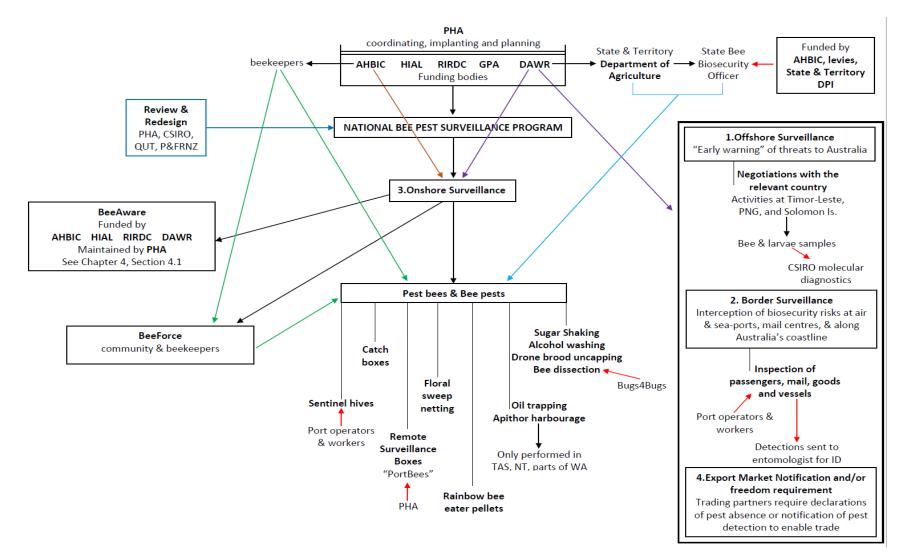


Figure A1.1 Graphical overview of the National Bee Pest Surveillance Program and the associated contributors.

A1.2 Purpose of the NBPSP

The National Bee Pest Surveillance Program (NBPSP) provides information on Australia's honey bee industry health status to support beekeeping and pollination-dependent plant industries. Technical evidence-based information is used to support Australia's pest-free status claims, assisting meet Australia's international reporting obligations and facilitating trade in honey bee industry commodities such as queen bee and packaged bee export to countries sensitive to bee pests and pest bees.

Importantly, the NBPSP provides a mechanism for early detection of new bee pests and pest bees. Early detection can increase the likelihood of successful eradication and/or reduce the cost of an eradication program if a new pest can be detected before it has a chance to establish and spread across large areas.

The NBPSP also provides information on activities with regard to pests that are already established in Australia but have a regionalised distribution.

Pollination reliant industries that are members of Horticulture Innovation Australia Ltd (HIA) include almonds, apple and pear, avocado, canned fruits, blueberry, cherry, lychee, macadamia, mango, melon, onion (for seed), papaya, passionfruit, prune, rubus, strawberry, summerfruit and vegetables (for seed). Table A1.1 demonstrates the industry's value as according to the Gross Value of Production (GVP) and the reliance of each industry on pollination and the recommended stocking rates of hives per hectare. In Table A1.1, if an industry representative body covers multiple crops, such as AUSVEG, the GVP only includes the GVP of those crops which are pollinator-reliant.

Сгор	Estimated GVP (\$)			Estimated yield losses (%) ⁸
Almonds	600 million	100	2 – 5	10 – 30
Apple and Pear ⁹	566.8 million	80	80 2-5 0	
Avocado	306 million	100	5 – 8	10 – 30
Blueberry	135 million	100	1 - 10	10 – 30
Canned fruits ¹⁰	37 million	60	2 - 4	0 - 10
Cherry	130 million	90	2 – 3	0 – 20
Citrus ¹¹	450 million	20 - 8012	2 - 3	0 - 20
Lychee	15 million	10	2 – 3	0 - 10
Macadamia	160 million	90	5 – 8	0 – 20

seedless mandarins.

¹² Depends on variety

⁷ Adapted from Barry *et al.* (2010), Keogh *et al.* (2010), Goodwin (2012) and from the BeeAware

⁽www.beeaware.org.au/pollination) and Plant Health Australia (www.phau.com.au/industries) websites

⁸ In the absence of feral Apis mellifera colonies

⁹ Excluding Nashi

¹⁰ Represents apricots, peaches, pears and plums for canning

¹¹ Includes oranges, lemons, limes and grapefruit. Mandarins are not included because growers don't want pollination for

Mango	140 million	50	2	0 - 10
Melon ¹³	150 million	100	2 – 5	0 – 20
Onion ¹⁴	10 million	100	10 – 30	10 – 30
Рарауа	25 million	25 million 20 1 - 2		0 - 10
Passionfruit	14.5 million	100	2 – 3	0 – 20
Prune	2 million	70	2 – 4	0 - 10
Rubus ¹⁵	40 million	70	1 – 3	0 – 20
Strawberry	200 million	40	2 – 4	0 - 10
Summerfruit ¹⁶	200 million	60	2 – 4	0 - 10
Vegetables ¹⁷	368.2 million ¹⁸	100	2 – 10	0 – 20

A1.3 Major activities within the NBPSP (2015–2016)

Since transferring management of the surveillance program to PHA in 2012, a large number of improvements have been made to the Program, making it one of the leading coordinated bee surveillance programs in the world. One of the improvements made is an increase in sentinel hive numbers. In 2011 there were just 26 sentinel hives established in selected ports. Hives were monitored with a sticky mat and miticide strip. By the end of 2015 the NBPSP comprised 152 sentinel hives and 141 catchboxes operating at 32 air and sea ports of entry in Australia. Tracheal mite assessment and floral sweeping netting was conducted at 19 ports (Table A1.2).

Another improvement for the NBPSP in 2015 has been the new permit issued for use in the NBPSP by the Australian Pesticides and Veterinary Medicines Authority (APVMA). This permit (PER80923), issued in September 2015, increased the use patterns for Bayvarol[®] and Apistan[®] in sentinel hives. The former permit only allowed for use for 24-48 hours every 6-8 weeks, while the new minor use permit allows for use for between 1-6 days every 6-8 weeks. Leaving miticide strips in sentinel hives for a longer period allows for much greater confidence of early detection.

Catchboxes are used to detect bee swarms in the port area and test the bees for exotic pests, such as *Varroa* mites and in Victoria, 39 colonies of bees have been collected in catchboxes from 2005–2015.

A proof of concept trial of 15 remote surveillance catchboxes was deployed, and once this trial remote surveillance hives currently placed around Australia is finalised in 2016, it is anticipated PHA will work with stakeholders to gradually replace these catchboxes with remote surveillance hives.

From 1 July 2014, a risk based surveillance program was adopted based on results from the CSIRO port risk assessment, with the highest risk ports in each jurisdiction being heavily targeted with a variety of surveillance strategies.

In June 2013, surveillance for Small hive beetle was incorporated into the Program for Tasmania and the Northern Territory, where it is currently not present, and also Western Australia, where it is currently restricted in distribution to the northern part of the state (Kununurra). Hives are tested every two months using oil traps or Apithor harbourages (containing the insecticide fipronil). This routine

¹³ Represents watermelon, rockmelon, honeydew and other melons

¹⁴ Only includes seed production of onions

¹⁵ Represents Rubus growers, including raspberries and blackberries

¹⁶ Represents apricots, peaches, plums and nectarines

¹⁷ Includes capsicums, chillies, peppers, beans, green peas, pumpkin, zucchini, cucumber, swede, marrows, squash and

vegetables for seed production, such as cauliflower, cabbage, carrot etc.

¹⁸ This figure is representative of crops which are pollinator-reliant and form part of the vegetable industry.

testing provides a means for early detection of small hive beetle as well as supporting export market access for Tasmania, the Northern Territory and parts of Western Australia through the collection of data demonstrating pest absence.

Contracts are established with each jurisdiction and partners to the program to formalise reporting and milestone arrangements.

PHA has also undertaken work with the Norfolk Island Government to implement surveillance activities on the island. This is in recognition that Norfolk Island is part of a risk region with established Asian honey bee in the region and frequent shipping between New Zealand and Australia which presents a risk for *Varroa* mite.

A summary of results from surveillance undertaken in the NBPSP is presented in Table A1.4.

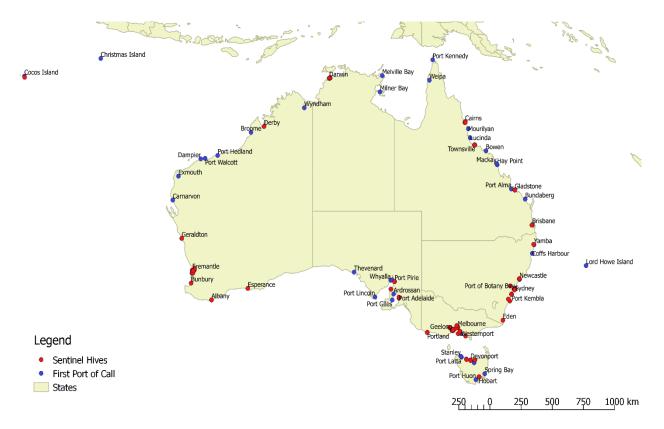


Figure A1.2 Map of sentinel hives and first ports of call in Australia.

	veniurice detrifices current		(·····	····		
Surveillance activity Queensland Ports	Port hazard rating ¹⁹	Sentinel Hives ²⁰	Swarm/feral nest capture ²¹	Catchboxes ²²	Remote surveillance catchboxes ²³	Floral sweep netting ²⁴	Hobby Beekeeper involvement ²⁵	Tracheal mite analysis ²⁶
Brisbane	н	6	Yes	6	5	Yes	Yes	Yes
Cairns	М	6	Yes	0	4	Yes	Yes	Yes
Gladstone	Н	6	Yes	0	5	Yes	Yes	Yes
Townsville	М	6	Yes	5	0	Yes	Yes	Yes
Weipa	М	0	Yes	0	1	Yes	Yes	No
Mackay	М	0	Yes	0	0	No	Yes	No
Mourilyan	Н	0	Yes	0	0	No	Yes	No
Port Alma	М	0	Yes	0	0	No	Yes	No
Total Queensland	3 High; 5 Medium	24	8	11	15	5	8	4
NSW Ports								
Port Botany/Kurnell	Н	8	Yes	19	0	Yes	Yes	Yes
Newcastle	Н	6	Yes	18	0	Yes	Yes	Yes
Port Kembla/	Н	6	Yes	13	0	Yes	Yes	Yes

Table A1.2 Surveillance activities current	y conducted (inclu	ding contracted and non-contra	acted) in each state and territo	v as at 2015/2016

¹⁹ Hazard rating for incursion of *A. mellifera* derived from Caley *et al.* 2013. H = High hazard (first quartile); M = Medium hazard (second quartile); L = Low (third or fourth quartile); U = unknown (not assessed)

²⁰ Targets *Varroa* & Tropilaelaps mite. Sentinel hives are monitored every 6-8 weeks.

²¹ Targets Asian honey bee, Giant honey bee, Red dwarf honey bee, Africanized honey bees, Cape honey bees, Braula fly & exotic mites.

 ²² Targets Africanized honey bees, Cape honey bees, Braula fly & exotic mites.
 ²³ Targets Africanized honey bees, Cape honey bees, Braula fly & exotic mites. An image is uploaded daily.

²⁴ Targets Asian honey bee. Floral sweep netting takes place every 6-8 weeks.

²⁵ Hobby beekeeper involvement takes place every 6-8 weeks and includes sugar shaking (targets Varroa), alcohol washing (Varroa) drone uncapping (Varroa, Tropilaelaps, Braula fly) & SHB surveillance.

²⁶ 1 sample of 50 bees diagnosed every 6-8 weeks

Wollongong								
Richmond	U	1	Yes	0	0	No	Yes	No
Goodward Island	U	1	Yes	0	0	No	Yes	No
Chifley	U	1	Yes	0	0	No	Yes	No
Jervis Bay	U	1	Yes	0	0	No	Yes	No
Parma	U	1	Yes	0	0	No	Yes	No
Eden	U	1	Yes	0	0	No	Yes	No
Total NSW	3 High	26	10	50	0	3	10	3
Victorian Ports			1		1			
Geelong	М	11	Yes	8	0	No	Yes	Yes
Melbourne	Н	11	Yes	22	0	Yes	Yes	Yes
Portland	М	2	Yes	6	0	No	Yes	Yes
Westernport	U	6	Yes	10	0	No	Yes	Yes
Tullamarine airport	U	2	Yes	8	0	No	Yes	No
Total Victoria	1 High; 2 Medium	32	5	54	0	1	5	4
Western Australian Po	orts		1		1		1	
Fremantle	Н	7	Yes	0	0	Yes	Yes	Yes
Kwinana	U	1	Yes	0	0	No	Yes	Yes
Perth airport	U	6	Yes	0	0	No	Yes	Yes
Perth	U	3	Yes	0	0	No	Yes	No
Esperance	М	1	Yes	0	0	No	Yes	No
Albany (port)	М	1	No	0	0	No	Yes	No
Dampier	L	0	No	0	0	No	Yes	No

			T			Ι		
Geraldton	М	2	Yes	0	0	No	Yes	No
Bunbury	н	1	Yes	0	0	No	Yes	No
Total Western Australia	2 High; 3 Medium	22	7	0	0	1	9	3
South Australian Port	S							
				10, + 30 at				
Port Adelaide	Н	6	Yes	depots	0	Yes	Yes	Yes
Whyalla	L	0	Yes	2	0	Yes	No	Self sometimes
Port Pirie	L	6	Yes	4	0	Yes	Yes	Self sometimes
Wallaroo	L	6	Yes	4	0	Yes	No	Self sometimes
Adelaide airport	U	0	Yes	6	0	Yes	Yes	Self sometimes
Total South Australia	1 High	18	5	56	0	5	3	1
Northern Territory Po	orts						I	
Darwin	М	3	Yes	0	4	Yes	Yes	Yes
Berrimah Farm	U	3	No	0	0	Yes	No	Yes
Airport	U	0	No	0	0	No	No	No
Groote Eylandt	М	0	No	0	0	No	No	No
Total Northern Territory	2 Medium	6	1	0	4	2	1	2
Tasmanian Ports								
Hobart	Н	8	Yes	0	0	Yes	Yes	Yes
Devonport	М	4	Yes	0	0	No	Yes	No
Burnie	L	4	Yes	0	0	No	Yes	No

Bell Bay	Н	7	Yes	0	0	Yes	Yes	Yes
Total Tasmania	2 High; 1 Medium	23	4	0	0	2	4	2

Target	Specimens examined	Comments
		2013
Apis cerana	34	<i>Apis cerana</i> specimens were examined from known samples (nests and swarms) in the Cairns region during the Asian Honey Bee Transition to Management Program until 30 June 2013.
Tracheal mite	100	Tracheal mite specimens examined included 30-50 bees from sentinel hives being randomly selected and morphologically dissected to determine Tracheal mite presence
Small hive beetle	39	Small hive beetle samples included oil traps and hive inspection of sentinel hives in Northern Territory, Tasmania and Western Australia
<i>Varroa</i> and tropilaelaps mites	368	129 additional sugar shaking and alcohol washing samples were collected from hives across Australia throughout 2013. Each sample included approximately 300 bees
Total	541	
		2014
Apis cerana	13	The development of floral maps and coordinated floral sweep netting began to be implemented in late 2014 around Australia for the detection of pest bees. This figure is the number of floral sweep netting surveillance runs conducted.
Tracheal mite	156	Tracheal mite specimens examined included 30-60 bees from sentinel hives being randomly selected and morphologically dissected to determine Tracheal mite presence
Small hive beetle	142	Small hive beetle samples included Apithor traps, oil traps and hive inspection of sentinel hives in WA, NT and Tas
<i>Varroa</i> and tropilaelaps mites	557	800 additional sugar shaking, alcohol washing and drone uncapping samples were collected from hives across Australia throughout 2014
Total	868	
		2015
Apis cerana	61	A total of 23 swarms of Asian honey bee (<i>Apis cerana</i> Java genotype) were collected in the Cairns port area in 2015 by Operational Science Services (OSS). Diagnostics were performed on the bees and no <i>Varroa</i> sp., <i>Tropilaelaps</i> sp or <i>Acarapis woodi</i> were detected. The development of floral maps and coordinated floral sweep netting began to be implemented in late 2014 around Australia for the detection of pest bees. This figure is the number of floral sweep netting runs conducted in 2015
Tracheal mite	160	Tracheal mite specimens examined included 30-60 bees from sentinel hives being randomly selected and morphologically dissected to determine Tracheal mite presence
Small hive beetle	138	Small hive beetle samples included Apithor traps, oil traps and hive inspection of sentinel hives in WA, NT and Tas
<i>Varroa</i> and tropilaelaps mite	580	814 additional sugar shaking, alcohol washing and drone uncapping samples were collected from hives across Australia

Table A1.3 Summary of results from surveillance undertaker	ו in	the NBP	SP (2013-2015).
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		throughout 2015. Of this, 669 were collected in Victoria as part of their routine sugar shaking program.
Total	939	

A1.4 Interception data 1995–2015

While only formally recognised as the nationally coordinated NBPSP since 2013, surveillance for bee pests and pest bees has been undertaken by the commonwealth government at Australia's border for several decades. Data from these activities are presented in Table A1.5, noting that any intercepts listed in this table have been eradicated once detected. These intercepts show that surveillance for bee pests and pest bees is essential given 73 Asian honey bee, 49 Giant honey bee and 12 Red dwarf honey bee detections have occurred in the period between 1995 and 2015. *Varroa* mites have been detected 13 times in this period.

DATE	DESCRIPTION	Australian Port	GENUS	SPECIES	Comments on interception
April 1995	Imported goods - Machinery	Brisbane, QLD	Apis	cerana	Alive
June 1996	?	South Australia	Apis	cerana	No further details
February 1997	?	Fremantle, WA	A.m	scutellata	Abandoned nest only
December 1997	?	Buderim, QLD	Bombus	vosnesenskii	?
June 1998	Nest discovered by local beekeeper	Darwin, NT	Apis	cerana	Eradicated
July 1999	Air freight of computer motherboards	Sydney, NSW	Apis	dorsata	Only bees
October 1999	Ship	Brisbane, QLD	Apis	cerana	Alive. Asian honey bee swarm (50-100) detected on ship. Absconded but follow up surveillance showed nothing.
December 1999	Imported goods - Machinery	Brisbane, QLD	Apis	cerana	Introduced with heavy earth moving equipment from Lae, PNG. Nest of 5000 bees which were <i>Java</i> <i>flores</i> type with <i>Varroa</i> <i>jacobsoni</i> .
March 2000	Shipping Container	Brisbane, QLD	Apis	dorsata	Alive. A swarm was found under a container at the Brisbane wharves and destroyed.
November 2000	Packing - Pallet	Brisbane, QLD	Apis	dorsata	Alive
January 2001	Ship	NT - Port not specified	Apis	dorsata	Alive
January 2002	Ship	Melbourne	Apis	cerana	Alive. Swarm on a container ship from PNG. Destroyed and inspection revealed <i>Varroa jacobsoni</i>
January 2002	Airport?	Richmond, NSW	Aethina	tumida	Means of arrival unknown. Established in Australia.
December 2002	ship	Brisbane, QLD	Apis	cerana	One bee found on ship from PNG. Follow up surveillance revealed nothing.
January 2003	Ship	Brisbane	Apis	cerana	Alive.
February	ship	Vessel off north	Apis	dorsata	Oil tanker from Singapore.

Table A1.5 Interception data surveillance for bee pests and pest bees (1995 – 2015).

DATE	DESCRIPTION	Australian Port	GENUS	SPECIES	Comments on interception
2003		of Australia			A 'quite large swarm' found by crew and inexpertly destroyed before arrival. Only dead bees found and no mites observed.
February 2003	ship	Vessel off north of Australia	Apis	dorsata	Vessel off north of Australia. Seven dead and one dying bee found. No evidence of swarm despite repeated checks. No mites found on inspection.
May 2003	?	Fisherman Island, QLD	Bombus	terrestris	A single bee found by Department of Agriculture
November 2003	Imported goods - Live Fish	Perth	Apis	dorsata	Alive
May 2004	?	Cairns, QLD	Apis	cerana	Vessel from PNG. Swarm of <i>Apis cerana</i> found in hold on arrival in port. Bees destroyed. Spread considered unlikely. No mites found on bees.
November 2004	Shipping Container - External	Brisbane	Apis	cerana	Vessel from PNG. Nest of Apis cerana found under a container in port. Bees destroyed. Spread considered unlikely. <i>Varroa</i> <i>jacobsoni</i> found on inspection. Surveillance for <i>Apis cerana</i> put in place with 6km radius for 12 months.
April 2005	Ship/ Vessel deck/structure	Brisbane	Apis	cerana	Alive and dead. <i>Varroa jacobsoni</i> found alive
April 2007	Ship/Vessel deck/structure	Perth	Apis	florea	Alive
May 2007	Yacht, dry dock	Cairns. QLD	Apis	cerana	A single nest of <i>Apis cerana</i> was detected on the mast of a yacht in dry dock. Eradication attempted, and failed. This bee is now spread throughout the Cairns region. No mites on the population.
February 2008	Air Container Internal	Adelaide	Apis	dorsata	Alive
March 2010	Aircraft pallets	Adelaide	Apis	dorsata	Alive
March 2010	Aircraft pallets	Adelaide	Apis	dorsata	Alive

DATE	DESCRIPTION	Australian Port	GENUS	SPECIES	Comments on interception
June 2010	Personal Effects	Brisbane	Apis	cerana	Unknown
March 2011	Ship/Vessel deck/structure	Darwin	Apis	florea	Alive
March 2011	Shipping Container	Brisbane	Apis	cerana	Alive + <i>Varroa jacobsoni</i> alive
July 2011	Packing - Plastic	Adelaide	Apis	dorsata	Alive
August 2011	Ship/Vessel deck/structure	Darwin	Apis	dorsata	Alive
January 2012	Shipping Container	Townsville	Apis	cerana	Alive + <i>Varroa jacobsoni</i> alive
June 2012	New Vehicles	Adelaide	Apis	<i>Florea</i> and <i>cerana</i>	Alive and Dead
July 2012	New Vehicles	Adelaide	Apis	cerana	Alive
September 2012	Vessel Galley	Port Hedland	Apis	cerana	Alive and Dead
November 2012	Ship/Vessel deck/structure	Sydney	Apis	cerana	Alive + <i>Varroa jacobsoni</i> alive
January 2013	Shipping Container - External	Townsville	Apis	cerana	Alive (4000 bees)
March 2014	Break Bulk machinery	Townsville	Apis	cerana	Alive + <i>Varroa jacobsoni</i> alive
June 2014	Break Bulk machinery - trans- shipped to NZ	Brisbane	Apis	cerana	Alive
July 2014	Ship/Vessel deck/structure	Darwin	Apis	cerana	Alive
October 2014	Ship/Vessel deck/structure	Cairns	Apis	cerana	Alive
March 2015	Packing - Wooden reel	Brisbane	Apis	cerana	Alive + <i>Varroa jacobsoni</i> alive

A1.5 Current NBPSP costs and program funding

A1.5.1 NBPSP costs

Contracts with each jurisdiction to conduct surveillance for the 2015/2016 financial year are currently valued at \$139,000 and the current total cost of the program is \$220,000 per annum. This funding is divided between states and territories and other agencies (i.e. Northern Australia Quarantine Strategy [NAQS]) in the following risk based approach: QLD (\$28,000), NSW (\$23,000), VIC (\$21,000), WA (\$18,000), NT (\$14,000), SA (\$14,000), TAS (\$14,000), NAQS (\$5,000) and ACT (\$2,000).

Other components of the NBPSP budget include:

- PHA time (0.2FTE) and incidentals (postage, teleconferences etc.) \$40,000
- PHA Contract with Bugs for Bugs for Tracheal mite diagnostics \$22,500
- PHA Contract with AUSVET Animal Health Services for data management \$12,500
- Purchase of sticky mats for sentinel hives \$3,000
- Purchase of chemical strips for sentinel hives \$3,000

It should be noted that the current costs for deployment of sentinel hives is estimated to be \$2,500 per hive. This is based on figures of \$1,000 for diagnosis, \$700 for hive maintenance and \$800 for inspection intervals of 6 weeks. Given the number of sentinel hives deployed within each jurisdiction (see Table 1) as well as the additional surveillance activities being undertaken in each region, it is apparent that considerable in-kind contributions are being provided by all agencies in the current program. Considerable levels of in-kind have also been provided by other agencies such as DAWR (NAQS) and PHA in contributions to surveillance and coordination of activities (see Table A1.6).

In a newly restructured NBPSP, it may be possible to make some efficiencies based on targeting the optimal arrangement of 6 hives per port and only placing hives at the highest risk ports for *A. mellifera* see Tables A1.2, A1.6 and A7.1), however some high risk ports currently have no sentinel hives and will need additional resourcing. Another issue is not all ports were considered in the analyses conducted by Caley *et al.* (2013) and additional ports could be required.

Table A1.6 Estimated costs of the sentinel hive components alone for state agencies in the NBPSP (based on a figure of \$2,500 per hive).

Component	Contracted 2015/16	Estimated cost of current numbers of sentinel hives	Estimated costs if only high risk ports included (6 hives/port)
Queensland	\$28,000	\$60,000	\$45,000
New South Wales	\$23,000	\$65,000	\$45,000
Victoria	\$21,000	\$80,000	\$45,000
Western Australia	\$18,000	\$55,000	\$30,000
South Australia	\$14,000	\$45,000	\$15,000
Tasmania	\$14,000	\$57,500	\$30,000
Northern Territory	\$14,000	\$15,000	No high rated risk ports
NAQS	\$5,000	Considerable additional in-kind has been contributed	\$5,000
Australian Capital Territory	\$2,000	\$5,000	No high rated risk ports
Plant Health Australia	\$40,000	\$40,000 (Considerable additional in-kind has been contributed for coordination)	\$40,000
Diagnostics – Bugs4Bugs (Tracheal Mite)	\$22,500	\$22,500	\$30,000
Chemical consumables	\$6,000	\$6,000	\$10,000
Data collection and management	\$12,900	\$12,900	\$12,900
Total	\$217,700	\$463,200	\$295,700

A1.5.2 NBPSP funding

The Department of Agriculture and Water Resources have funded the Program (formerly known as the National Sentinel Hive Program) in its entirety from 2000-2013. In 2008, when the More Than Honey report was released by the House of Representatives Standing Committee, an additional \$300,000 was provided to the program. This funding ran out on the 30th June 2013.

In June 2013, the Department of Agriculture and Water Resources committed an additional \$60,000 for the next two financial years (2013/2014 and 2014/2015) as well as providing in-kind support through their NAQS and operational sciences program for high risk locations.

Ports where assistance is provided by Departmental staff include Melbourne (VIC), Port Botany (NSW), Brisbane, Gladstone, Cairns and Weipa (QLD), Darwin (NT), Fremantle (WA) and Port Adelaide (SA).

PHA convened the National Bee Pest Surveillance Workshop in July 2012 to discuss the future of the program, as well as a future funding model. At this meeting both the Australian Honey Bee Industry Council (AHBIC) and Horticulture Innovation Australia Limited (HIAL) committed to each providing \$75,000 pa (\$150,000 in total) over the next two financial years for 2013/2014 and 2014/2015.

The Australian Honey Bee Industry Council (AHBIC) committed \$75,000 for the 2015/16 financial year, through the honey bee industry's Emergency Plant Pest Response (EPPR) levy fund. The Australian honey bee industry also provides a large amount of support to the program through in-kind services such as managing and conducting the surveillance on sentinel hives at specific ports.

Grains Producers Australia (GPA) committed \$10,000 for the 2015/16 financial year, through the grain industry's EPPR levy fund.

All states and territories (except ACT) are contracted by PHA to complete surveillance activities at specified ports as part of the NBPSP and PHA is working with ACT Beekeepers and the ACT Government to implement surveillance activities across ACT for exotic bee pests from 2015/16. This is in an effort to have coordinated surveillance activities conducted across all Australian jurisdictions. However, the funding provided does not pay for the entire Program in each state or territory, and is instead seen as a contribution towards conducting specific levels of surveillance. Each state and territory provides extensive in-kind commitment through apiary and biosecurity staff, as well as diagnostic support as part of the NBPSP. The NBPSP is just one component of bee surveillance (see Appendix 3 for Bee surveillance across the continuum).

Industries with high reliance on honey bees for pollination, such as almonds or avocados acknowledge that the key pest of concern is, Varroa. Industries with less clear reliance (e.g. pear and stonefruit) may receive significant benefits from incidental pollination provided by feral bees, and these industries are more likely to be negatively affected by the introduction of an exotic pest such as *Varroa* mite that destroys feral bees in the native and urban environments. Therefore, the impact and related cost incurred by *Varroa* mite is outlined below.

A1.6 Cost of a Varroa mite establishment

Industries with high reliance on honey bees for pollination, such as almonds or avocados, are more likely to recognise the need for, and maintain use of, paid pollination services. Industries with less clear reliance (e.g. pear and stonefruit) may receive significant benefits from incidental pollination provided by feral bees, and these industries are more likely to be negatively affected by the introduction of an exotic pest such as *Varroa* mite that destroys feral bees in the native and urban environments.

An incursion of *Varroa* mite will have significant impacts on the honey bee industry, however flow-on effects on pollination-reliant industries in agriculture and horticulture will have more far-reaching impacts. The costs of having *Varroa* mite in Australia can be divided into the direct costs attributable to beekeepers controlling *Varroa* mite and the loss of the free pollination services. While the costs of *Varroa* mite treatments can be estimated, the labour involved and the potential colony losses cannot be established without first studying beekeeping operations in Australia to gain an understanding of how *Varroa* mite treatments might be incorporated into business models. For this reason, the assumptions used are drawn from the New Zealand experience. The value of the lost pollination services will not be immediately replaced by paid pollination.

Based on figures from New Zealand, treatment costs for *Varroa* management in Australia are estimated to be approximately \$15.40 per hive or approximately \$8.5 million per annum. These figures are derived from the following assumptions:

- 2 brood box hives²⁷
- \$2.09 / Bayvarol (treatment) strip & 4 strips per box²⁸

²⁷ This figure needs to be converted for the percent of hives managed with just one brood box

²⁸ Current costs for Bayvarol[®]

- 550,00 hives in Australia²⁹
- 0.92 exchange rate (NZ\$ to Aus\$)
- Two treatments per year to manage Varroa mite³⁰

In addition to the projected costs estimated above, additional costs will be incurred as a result of travelling to hives to insert and remove strips 2 times per year. In theory these can be carried out while beekeepers are carrying out other beekeeping activities however in New Zealand about 25% of these visits occur as special trips. There is currently no data for New Zealand or Australia on how much these extra beekeeping trips would cost. There are also no data on hive losses due to colonies not being treated, treated at an incorrect time or *Varroa* mite being resistant to treatments, making it likely that the full costs for establishment of *Varroa* in Australia are likely to be an under-estimation.

In New Zealand, it was assumed that the cost of treatment, extra labour and hive losses would equate to \$50 per hive or \$AUS27 million. It has been estimated that the cost to pollination would be between \$21-50.5 million p.a. (Cook *et al.* 2007). Therefore, the economic costs avoided by preventing a *Varroa* mite incursion and subsequent establishment is estimated to be between \$50-75 million per year (Barry *et al.* 2010).

The potential present value of losses to producers and consumers of pollination-dependent crops from an unhindered *Varroa* mite spread could be expected to range from \$21.3–50.5 million per year or \$630 million–1.3 billion over 30 years depending on the port of entry (ABARES 2012; Hafi *et al.* 2012; Cook *et al.* 2007). However, if the spread of *Varroa* mite could be slowed through containment, it is estimated that the losses would range from \$630 million–0.93 billion, depending on the port of entry.

²⁹ A benefit–cost framework for responding to an incursion of *Varroa* destructor Ahmed Hafi, Nicola Millist, Kristopher Morey, Peter Caley and Benjamin Buetre

³⁰ This is what is currently required in New Zealand.

Appendix 2: Major bee pests and pest bees

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A2.1 High priority biosecurity threats for the honey bee and pollination-reliant industries

The NBPSP involves a range of surveillance methods conducted at locations considered to be of most likely entry of exotic bee pests and pest bees (Appendix 1). Major bee pests of concern that are targeted using these surveillance methods include:

- Varroa mites (Varroa destructor and V. jacobsoni)
- Tropilaelaps mites (*Tropilaelaps clareae* and *T. mercedesae*)
- Tracheal mite (Acarapis woodi)

Exotic pest bees targeted in the Program include:

- exotic Asian honey bee (Apis cerana)
- Giant honey bee (Apis dorsata)
- Red dwarf honey bee (Apis florea)
- Exotic strains of the European honey bee, including Africanized honey bees (*A. m. scutellata*) and Cape honey bees (*A. m. capensis*)

Regionalised pests that are contained to different regions within Australia are monitored in specific states and territories. These pests include:

- Braula fly (Braula coeca)
- Small hive beetle (Aethina tumida)
- Asian honey bee (Apis cerana)

Other pests of concern recommended for surveillance include:

- Asian Hornet (Vespa velutina)
- Viruses (Acute bee paralysis virus, Deformed wing virus, Slow bee paralysis virus)

The following sections provide information on high priority pest threats for the honey bee industry in Australia.

Invertebrate bee pests

A2.1.1 Varroa mite

Varroa mites are parasitic mites, which require a honey bee host to survive and reproduce. Although *Varroa* mites can feed and live on adult honey bees, they mainly feed and reproduce on larvae and pupae in the developing brood, causing malformation and weakening of honey bees as well as transmitting numerous viruses.

Colonies with low infestation generally show very few symptoms, however as the mite population increases symptoms become more apparent. Heavy *Varroa* mite infestations can build up in 3–4 years and cause scattered brood, crippled and crawling honey bees, impaired flight performance, a lower rate of return to the colony after foraging, a reduced lifespan and a significantly reduced weight of worker bees. Colony symptoms, commonly called Parasitic Mite Syndrome (PMS), include an abnormal brood pattern, sunken and chewed cappings and larvae slumped in the bottom or side of the cell. This ultimately causes a reduction in the honey bee population, supersedure of queen bees and eventual colony breakdown and death.

Varroa mite is present in all major beekeeping areas of the world except Australia and experts agree that it is only a matter of time before the mite arrives in Australia (Hafi *et al.* 2012).

Due to the nature of feral bee movement, the drifting and robbing behaviour of honey bees, *Varroa* is expected to move rapidly within regions. Unless *Varroa* is detected and movement controls can be implemented, human-assisted movement has the potential to spread an incursion quickly over larger areas as beekeepers move their hives on a regular basis to access flowering plants to feed their bees, and also more particularly through the movement of hives through commercial pollination services (Gordon *et al.* 2014). A study by Gordon *et al.* (2014) examined the movements of Australian beekeepers to determine their potential to assist the spread of pests and diseases. They found 147 beekeepers moving beehives in eastern Australia visited 488 locations, 288 of which were joined in an extended network spreading from central Queensland to western Victoria.

For eradication to be considered technically feasible, an incursion would need to be detected early, preferably surrounding a port area i.e. before it had a chance to spread widely and become established in a large number of bee colonies (Department of Agriculture 2011).

Based on the geographic distribution of the mite when detected, a decision would need to be made whether to eradicate an incursion or whether to adopt a management program to manage the spread. Given the mobility of honey bees and previous experiences both here and overseas, containing a major incursion is considered highly unlikely, the exception being significant geographical barriers such as Bass Strait or the Nullarbor Plain.

The notion of eradicating a *Varroa* mite incursion should not be taken lightly. If Australia succeeded in eradicating a *Varroa* mite incursion, it would be the first country to do so (Boland 2005). Many countries have tried and failed, some incurring difficult and costly eradication attempts (De Jong 1997). Eradicating an incursion would depend on the geographic distribution of the mite. Eradication of a *Varroa* mite incursion might be feasible if detected early enough (Boland 2005), e.g. the detection of *Apis cerana* in Darwin in 1998 which was detected early and successfully eradicated (Boland 2005). Eradication would involve not only the destruction of domestic hives within a declared zone but also the (much more difficult) attempted destruction of all feral colonies.

The *Varroa* mite was discovered in South Auckland, New Zealand in 2000 and has since spread throughout much of the North Island. Before this, New Zealand was considered to be free from *Varroa* mite. Although it is not possible to determine exactly how the mite entered the country, a potential route has been identified as the illegal importation of queen bees by a New Zealand beekeeper, either by post or as personal luggage. However, it has also been suggested that it might have arrived by the sea container pathway. The mite is thought to have been present in New Zealand for up to five years before being detected, by which time it was considered too late to eradicate³¹.

Based on the experience in other countries, if left unmanaged a *Varroa* mite infestation will destroy a honey bee colony completely within 2-3 years (Keogh *et al.* 2010). In New Zealand colonies are being observed to die within 12 months (Goodwin, pers. comm.). Given this, it is likely that feral honey bee populations in Australia will be severely impacted should *Varroa* mite become established, increasing the reliance and need for paid pollination services by many plant industries.

Surveillance methods for Varroa mites

Surveillance methods for *Varroa* mites (*Varroa destructor, V. jacobsoni*) include the use of Bayvarol[®] (Flumethrin) or Apistan[®] (Tau-fluvalinate) acaricide strips and sticky mats in sentinel hives, sugar shaking and alcohol washing of bees, drone uncapping and examination of swarms captured in and around high risk port areas (including from sweep netting, catchboxes/remote catchboxes) (see Appendix 3). These methods can involve commercial and hobby beekeepers, and state and territory Department of Agriculture members.

³¹ Case Study 4 Response to the Incursion, of the *Varroa* Bee Mite", page 80 (http://www.oag.govt.nz/2002/biosecurity---case---studies/docs/part4.pdf)

A2.1.2 Tropilaelaps mite

Tropilaelaps mites are native to Asia and naturally parasitise the brood of the Giant honey bees of Asia, such as *Apis dorsata*. Two species of Tropilaelaps mites (*Tropilaelaps clareae* and *T. mercedesae*) are able to parasitise European honey bees.

The life cycle of Tropilaelaps mites is very similar to that of *Varroa* mites in many ways, as both species of mites are external feeders which parasitise the brood stages of the honey bee. However, Tropilaelaps mites have a much shorter life cycle, and because of this, have a much greater reproductive rate than *Varroa* mites. Because of this greater reproductive rate, research has shown that in some hives there can be around 25 Tropilaelaps mites to every *Varroa* mite in a honey bee colony. However, unlike *Varroa* mites which can survive on adult bees for quite a few months, Tropilaelaps mites can only live for around 3 days on an adult worker bee as the adult Tropilaelaps mite mouthpiece cannot pierce the adult wall membrane, and therefore, cannot feed on adult worker bees. For this reason, Tropilaelaps mites spend the majority of their life in the brood and will continue to breed and survive in a honey bee colony as long as there is brood present.

Tropilaelaps mite infestation causes severe damage to honey bee colonies such as deformed pupae and adults (stunting, damaged wings/legs/abdomens), PMS and colony decline. The colony may also swarm or abscond, further spreading the mite to new locations. Tropilaelaps mites can also spread viruses which further affect the colony's health and disease susceptibility. If Tropilaelaps mites were to become established in Australia, they would cause significant losses to managed and wild honey bee colonies, crop pollination and yields of honey products.

Surveillance methods for Tropilaelaps mites

Surveillance methods for Tropilaelaps mites (*Tropilaelaps clareae* and *T. mercedesae*) include the use of Bayvarol[®] or Apistan[®] acaricide strips and sticky mats in sentinel hives (as per *Varroa* mite) and drone uncapping (see Appendix 3). Up to 97% of Tropilaelaps mites in a honey bee colony are found within capped brood cells. Tropilaelaps mites reproduce in both worker and drone cells, but as with Varroa, there is a preference for drone brood. Therefore, uncapping drone brood and examining pupae is one of the best methods for detection. This method is recommended as it is rapid and can be carried out easily as part of a routine hive inspection. The disadvantage of this method is that the drone brood are killed.

As only 3 to 4% of adult mites are reported to attach themselves to adult honey bees, sugar shaking and alcohol washing are unlikely to detect these mites.

A2.1.3 Tracheal mite (Acarapis woodi)

Tracheal mite is a microscopic, internal mite of the honey bee respiratory system, capable of infecting queen bees, drones and worker bees. Tracheal mite infects and reproduces inside the tracheae (breathing tubes) of the honey bee and feeds on the honey bee's haemolymph (blood).

The entire life cycle of the mite occurs within the honey bee's tracheae (breathing tubes), except for brief migratory periods. Within the 24 hours after worker bees emerge from their cells, female mites migrate between adult bees into their tracheae and remain there for their life span or until their host bee dies. The invading mites are attracted to the current of expired air coming from the first thoracic spiracle. Once inside the host bee, each female mite lays 5 to 7 eggs over a period of 3 to 4 days and continues to lay eggs throughout her life. Eggs hatch in 3 to 4 days and progress through a larval stage, then a nymphal stage before finally reaching adult form. The male takes 11 to 12 days to fully develop, whereas the female takes 14 to 15 days. Mating then occurs within the breathing tubes. The female is capable of laying almost one egg a day, each of which is about two thirds the weight of the female herself. There are usually 2 to 4 times more females present than males and as many as 21 offspring from each female are possible. Once mated, the female mites leave the tracheae, moving to the external surface of the bee to locate a new bee and begin the reproductive cycle again.

Infection affects the honey bee's capacity to breathe, opens the tracheal surface to pathogens and reduces capacity of air flow to the wing muscles. This results in weakened and sick honey bees which do not work as hard and have a significantly reduced lifespan.

When Tracheal mite infestation is combined with other stresses (e.g. disease, lack of pollen or nectar etc.) it can lead to the death of the colony. Once a honey bee colony is infested with Tracheal mite it remains infested, with impacts more significant over winter and early spring, contributing to high colony losses in severe cases.

Surveillance methods for Tracheal mite

Bees from sentinel hives or collected swarms can be examined for the presence of Tracheal mite. Due to their very small size ($\sim 120 - 180$ microns) and presence in honey bee tracheae, Tracheal mite can only be detected with bee dissection and examination under a microscope.

Two methods can be used for detecting Tracheal mite: individual dissection and examination of tracheae, or examination of stained thoracic discs. The method used will depend on the technical skills, equipment and preference of each laboratory, as well as the number of bees to be examined (see Appendix 3).

For testing of sentinel hives for the presence of Tracheal mite, bees are collected from hives at each eight week visit. Ideally, each sample will consist of approximately 50 bees from a randomly selected hive at each of the port areas. It has been demonstrated that a 1-2% rate of infection can be detected by sampling 50 bees (OIE 2008) and therefore, this should be viewed as the minimum for sampling for Tracheal mite.

Samples of bees are also taken from these sentinel hives every 6-8 weeks and submitted for dissection and examination for Tracheal mite (*Acarapis woodi*), which also could enter via exotic bees.

Exotic honeybee viruses

European honeybees are affected by numerous viruses. The Biosecurity plan for the honey bee industry (PHA 2013) identified three species (Acute bee paralysis Cripavirus; Deformed wing Iflavirus; Slow bee paralysis Iflavirus) as posing a significant threat to the honeybee industry (PHA 2013)

A2.1.4 Acute bee paralysis virus

Acute bee paralysis virus causes paralysis and death of adults and white eyed pupae (de Miranda *et al.* 2004). The virus can be vertically transmitted (i.e. from queen to offspring) (Chen *et al.* 2006a) and can be transmitted by *Varroa* mites (*Varroa* destructor) (Chen *et al.* 2006b, Bakonyi *et al.* 2002).

A2.1.5 Deformed wing virus

Deformed wing virus can be symptomatic or asymptomatic, typical symptoms include: deformed wings (often crumpled or greatly reduced), and shortened abdomens (Brown-Walker *et al.* 1999). The virus can be vertically transmitted (Chen *et al.* 2006a) or vectored by the *Varroa* mite (Brown-Walker *et al.* 1999)

A2.1.6 Slow bee paralysis virus

Slow bee paralysis virus causes paralysis of the front two pairs of legs of infected honeybees and eventually leads to the death of the infected honeybee (de Miranda *et al.* 2010). The virus is transmitted orally and by *Varroa* mites (de Miranda *et al.* 2010).

Surveillance methods for bee viruses

Honeybees from sentinel hives or collected swarms can be tested using PCR techniques for the presence of honeybee viruses.

Exotic bee pests

A2.1.7 Asian honey bee (Apis cerana)

There are exotic strains of Asian honey bee not currently present in Australia which pose a risk to honey bees as they are not only pests in their own right, but have the potential to carry exotic mites with them if they enter the country. In addition, the strain of Asian honey bee present in Queensland is not as aggressive as some exotic strains of *A. cerana* and is likely to have established from a small initial colony, resulting in in-breeding. Early detection of new incursions of Asian honey bee and testing for exotic mites may prevent establishment of these more aggressive strains and the exotic mites that may be carried on them.

The Asian honey bee produces less honey than the European honey bee and in other regions where this bee has established, it has shown the ability to rob the European honey bee of their honey stores. As a cavity nesting bee which is capable of nesting in smaller areas than the European honey bee, the Asian honey bee also has the potential to become a competitor for nectar, pollen and nesting sites in the natural environment, as well as nest in urban environments.

Surveillance methods for Asian honey bee

Asian honey bees can be detected through the capture of swarms or feral nests from in and around high risk port areas (method as per above), as well as floral sweep netting or rainbow bee-eater (*Merops ornatus*) surveillance (See Appendix 3).

A2.1.8 Giant honey bee (*Apis dorsata*)

Giant honey bees (*Apis dorsata*) are the largest of the honey bee species. The Giant honey bee is 17–20 mm long, however, their colour is quite similar to the European honey bee, with golden, black and pale bands on the abdomen and with a hairy thorax. Their forewing length can vary from between 12.5–14.5 mm. The Giant honey bee is widely distributed throughout south-east Asia, ranging from the Indian subcontinent, up to southern China and down throughout Indonesia and Malaysia.

The nests of giant honey bees are large single combs which can measure up to 1.5 m in width and 1 m in depth. This large single comb can contain upwards of 60,000 bees. Unlike dwarf honey bees or cavity nesting honey bee species, colonies of giant honey bees can be highly clustered in a specific location, with some trees in Asia (termed 'bee trees') containing multiple nests in a single tree, sometimes up to 50 nests.

Giant honey bee nests are usually built in exposed places far off the ground, sometimes 20–40 m high on thick branches of tree limbs, overhanging rocks or cliffs, or on buildings or other man-made structures. The key difference between dwarf honey bees and giant honey bees, apart from their nest size, is that giant honey bee nests hang underneath a structure such as a branch, whereas dwarf honey bee nests are wrapped around a structure such as a branch. Giant honey bee colonies can be quite aggressive, and because of this, around three quarters of the population of a giant honey bee colony are engaged in colony defence, forming a protective curtain around the nest that is three to four bees thick.

Giant honey bees are mainly tropical and in most places they migrate seasonally. Colonies are capable of migrating great distances, sometimes up to 200 km, as they follow the wet and dry seasons. Colonies will travel for many months, resting in trees along the way, building combs and honey reserves and then moving on to new locations as the forage decreases, before setting up new nests for the mass flowering of the monsoon season. Some evidence suggests that the bees are capable of returning to the same nest sites as previous years, even though all of the original bees in the process may be replaced. This mechanism of memory retention within the honey bee colony remains a mystery.

One of the major risks, is if giant honey bees were to enter Australia, the exotic parasitic mites that nests or swarms with this species (*Tropilaelaps clareae*, *T. mercedesae*, *T. thaii* and *T. koenigerum*), maybe capable of parasitising European honey bees as well.

Surveillance methods for Giant honey bee

The Giant honey bee can be detected through the capture of swarms or feral nests from in and around high risk port areas or through floral sweep netting (see Appendix 3).

A2.1.9 Red dwarf honey bee (Apis florea)

Dwarf honey bees are by far the most common honey bees throughout tropical Asia. The most common of the dwarf honey bees, is the Red dwarf honey bee (*Apis florea*) which is naturally distributed from the Indian subcontinent throughout south-east Asia through to the Malaysian peninsular.

Given that dwarf honey bee colonies are usually very small (usually only a few thousand bees), and that they only produce a single comb with very little honey, dwarf honey bees have not been domesticated for honey production or pollination services. Apart from their small size and simple single comb exposed nests, much of their life cycle, biology and behaviour is similar to that of other *Apis* species.

Dwarf honey bees typically establish their colonies in cryptic nest sites, and due to the fact that they are not very aggressive, they can easily stay undetected for a long time. It is believed that this behavioural trait has assisted in the spread and expansion of the Red dwarf honey bee throughout the Middle East and into eastern Africa.

The Red dwarf honey bee is now widely present in the Middle East, including Iran, Iraq, Israel, Jordan, Yemen, and Saudi Arabia as well as in Sudan in eastern Africa where populations of Red dwarf honey bee have been accidentally introduced. Reports from these areas suggest that the Red dwarf honey bee is continually expanding westward in an invasive manner and has even started to rob European honey bee hives, even in areas where there are dense populations of European honey bees.

One of the major risks for Australia, is if dwarf honey bees were to enter the country is the exotic parasitic mites that a nest may carry, maybe a potential problem for Australia's bee population. The Red dwarf honey bee is parasitised by *EuVarroa wongsirii* and *EuVarroa sinhai*, both of which are close relatives to *Varroa* mites.

Some research has been conducted on the ability of Eu *Varroa* to parasitise European honey bees and survive on European honey bee adults, however, research into this area is very limited, and the scenario of Eu *Varroa* parasitising European honey bees is considered highly unlikely. The ability of Eu *Varroa* to parasitise European honey bees in the natural environment of Asia, where the native dwarf honey bees exist with the introduced European honey bees, has not been observed or reported to date. Despite dwarf honey bees being parasitised by these mites, reports suggest that they cause minimal impact for dwarf honey bee colonies as they are restricted to reproducing on drone brood.

In addition to the Eu*Varroa* mites, Tropilaelaps mites (*Tropilaelaps clareae*) have been observed on Red dwarf honey bee colonies. Tropilaelaps mite is capable of jumping over to European honey bee colonies. Parasitic mites such as these pose a constant threat to Australia's honey bee population.

Surveillance methods for Red Dwarf honey bee

The Red dwarf honey bee can be detected through the capture of swarms or feral nests from in and around high risk port areas or through floral sweep netting (see Appendix 3).

A2.1.10 Africanized honey bees (Apis mellifera scutellata)

The Africanised honey bee is a hybrid of several European honey bee subspecies (*Apis mellifera mellifera, A. m. carnica, A. m. caucasia* or *A. m. ligustica*) and the African honey bee (*A. m. scutellata*). The Africanised honey bee occurs naturally throughout sub-Saharan Africa.

In the 1950s the African honey bee was introduced into Brazil in South America for breeding purposes. Unfortunately, the African honey bee escaped the breeding trial and starting breeding with the local populations of European honey bee. This was able to occur because all subspecies of *Apis mellifera* are capable of interbreeding or hybridising. Consequently, African honey bee hybridisation with European honey bees became frequent, as the African honey bee moved into areas which were previously occupied by European honey bees.

The Africanised honey bees have a much greater aggressive and defensive behaviour than European honey bees and because of this rapid hybridisation, they were quickly able to out-compete the European honey bee. As of 2012, the Africanised honey bees had saturated Central and South America and had established in many southern states of the USA.

The main differences between the Africanised honey bee and the European honey bee are displayed through their behavioural traits including:

- Africanised honey bees swarm and abscond much more frequently than other races of European honey bees. Typical European honey bee colonies will swarm once every 12 months, while Africanised honey bees are capable of swarming every month or two which saturates the area with Africanised honey bees.
- Africanised honey bees have a heightened defensive behaviour compared to other European honey bees. This can result in the Africanised honey bees defending a greater radius around their nest and attacking with many more individual bees than European honey bees would. Although they have been termed 'killer bees' in the USA, they do not have a more potent or a larger amount of venom than other honey bees, they just attack more aggressively with more individual bees.
- Africanised honey bees are less selective with nesting sites, and can nest in much smaller volumes than European honey bees.
- Africanised honey bees are more 'flighty' than European honey bees and commonly leave the hive when it is being inspected.
- Africanised honey bee colonies produce more drones per colony than European honey bees and their colonies grow faster and tend to be smaller than European honey bees. Africanised honey bees also store less honey than European honey bees.

Surveillance methods for Africanised honey bees

Africanized honey bees can be detected through the capture of swarms or feral nests from in and around high risk port areas, from catchboxes or remote surveillance catchboxes placed at high risk port locations. Any honey bees captured from swarms, nests or catchboxes should be tested for exotic external and internal mites (including Braula fly in mainland Australia) (see Appendix 3).

A2.1.11 Cape honey bees (*Apis mellifera capensis*)

The Cape honey bee (*Apis mellifera capensis*) is a subspecies of the European honey bee and is native to the Eastern and Western Cape provinces of South Africa. In its natural environment (the Fynbos region of South Africa), the Cape honey bee can be readily managed for the purposes of honey production and pollination, just like other races of the European honey bee.

The Cape honey bee has a distinct reproductive system which makes it unique amongst other races of *Apis* species. The worker bees have the ability to lay unfertilised diploid eggs which can still develop into worker bees or queen bees. This unique reproductive system causes major problems for beekeepers in its natural environment of South Africa where it acts as a 'social parasite'. Cape honey bees have been moved by beekeepers into areas where African honey bees naturally occur, the Cape honey bee has started to socially parasitise African honey bee colonies. The increased distribution of the Cape honey bee has therefore allowed for clonal lineages of Cape worker bees to establish, which have become widespread as reproductive (social) parasites within African honey bee populations.

Cape honey bees present a major problem for beekeepers in South Africa, and could pose a major problem to other beekeepers around the world if they were to spread to other regions. The ability of Cape honey bees to drift and parasitise other honey bee colonies, causing these colonies to dwindle or die is of great concern. It is perceived as such a threat in South Africa that local beekeepers believe that Cape honey bees pose a greater threat to beekeeping than even the deadly *Varroa* mites.

The main differences between the Cape honey bee and the European honey bee are displayed through their behavioural traits including:

- Cape honey bees swarm and abscond much more frequently than other races of European honey bees. Typical European honey bee colonies will swarm once every 12 months, while Cape honey bees are capable of swarming every month or two.
- Cape honey bees are more 'flighty' than European honey bees and commonly leave the hive when it is being inspected.
- Cape honey bee colonies grow faster and tend to be smaller than European honey bees. Cape honey bees also store less honey than European honey bees.
- Unlike the closely related African honey bee, the Cape honey bee is quite docile.

Surveillance methods for Cape honey bees

Cape honey bees can be detected through the capture of swarms or feral nests from in and around high risk port areas, from catchboxes or remote surveillance catchboxes placed at high risk port locations. Any honey bees captured from swarms, nests or catchboxes should be tested for exotic external and internal mites (including Braula fly in mainland Australia) (see Appendix 3).

A2.1.12 Asian hornet (Vespa velutina)

Asian hornet (*Vespa velutina*) is native to southern Asia from Afghanistan east to Indonesia but has recently been introduced into Europe where it is spreading rapidly in France and Spain (Villemant *et al.* 2011). This species is a significant pest of Asian honeybees in its native range and of European honeybees in its introduced range (Tan *et al.* 2007).

The life cycle of Asian hornet begins in spring when mated queens start new colonies. As workers are produced the colony rapidly grows until autumn. At this time the colony will often contain 6,000 individual wasps. New queens and males develop and mate in autumn. During winter the workers, brood and males die leaving only mated queens, which found new colonies the following spring (Monceau *et al.* 2014).

Asian hornet larvae require protein to develop, which the Asian hornet can collect by foraging or preying on other insects such as honeybees (Monceau *et al.* 2014). The Asian hornet preys on workers returning to the colony (Tan *et al.* 2007). Overtime this predation weakens the colony and increases the chance of collapse during winter (Monceau *et al.* 2014).

Given its rapid spread in Europe and impact on honeybees the Asian hornet is considered to be a major exotic threat to honeybees in Australia.

Surveillance methods for Asian hornet

Asian hornet has been shown to respond to traps and attractants have been developed (see: <u>www.veto-pharma.com/products/trap-and-hornet-attractant/</u>).

There is potential for this trap and attractant to be used for the surveillance of Asian hornets in Australian ports.

Regionalised pests

A2.1.13 Braula fly (Braula coeca)

The Braula fly is a small species of wingless fly that lives in honey bee colonies. The Braula fly is not considered to be a serious threat to commercial beekeeping as it does not damage or parasitise any stage of the honey bee life cycle. Instead, it is considered to be a minor pest as the Braula fly larvae damage the appearance of the wax cappings on honey comb and adult Braula flies steal small amounts of food from adult honey bees.

The Braula fly is currently widespread overseas and has been reported from all continents. In Australia it is only known to occur in Tasmania. In mainland Australia, Braula fly is a reportable pest.

The following is a summary of the life cycle of Braula flies:

- Eggs are laid on various surfaces of the hive, but only eggs that are laid on capped honey will hatch.
- Eggs hatch 2–7 days after they have been laid. The time required to hatch is determined by the temperature that the egg is exposed to.
- Once the egg has hatched, the larvae tunnel under the wax cappings, leaving a narrow tunnel that is visible across the surface of the honey comb.
- Larvae feed on honey and pollen, while tunnelling and undergo three larval stages before pupating when they are 7–11 days old.
- Braula fly remain as pupa for 1–3 days before emerging as an adult.
- Females mate shortly after emerging.
- The adult then has to quickly find an adult honey bee to carry it. Without a host to steal food from the adult Braula flies will survive for less than a day.
- Once it has found an adult honey bee, the adult Braula fly uses specialised claws to hold onto the honey bee's hair and rides on the bee's thorax or abdomen. The Braula fly will move to the bee's head to steal food while the bee is feeding itself or other bees.

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Braula flies take between 10 and 21 days to develop from eggs to adults. The time required is dependent on temperature. Braula flies are able to survive in the absence of brood but require the presence of adult honey bees to survive. Adult Braula flies are thought to overwinter on adult honey bees. After conditions have become favourable again the females lay eggs and the life cycle continues.

Surveillance methods for Braula fly

Surveillance methods for Braula fly includes examination of swarms captured in and around high risk port areas (including from catchboxes/remote catchboxes) (as per *Varroa* mite, see Appendix 3).

A2.1.14 Small hive beetle (Aethina tumida)

Small hive beetle (*Aethina tumida*, SHB) is a small brown-black beetle with clubbed antennae that originated from sub-Saharan Africa. In Africa, the SHB is not a significant honey bee pest species; however, since arriving in Australia in 2002, the SHB has caused a major impact to honey bee colonies throughout the warm and humid coastal strip between Victoria and North Queensland.

The larval stage of the SHB life cycle causes the majority of damage to active hives by burrowing into combs, eating brood, honey and pollen. Unlike some other honey bee pest species, SHB is preferentially attracted to active hives because of the availability of food. Whilst feeding the larvae also carry a yeast species (*Kodamaea ohmeri*) which contaminates the honey causing it to ferment. Heavy infestations cause the hive to become 'slimed out' and may cause the colony to die or abscond.

The development of SHB throughout its lifecycle depends primarily on humidity, temperature and food availability. As such SHB has the greatest impact in the warm and humid coastal strip between Victoria and north Queensland, but its presence has also been detected in all states and territories of Australia, except Tasmania, Northern Territory and southern parts of Western Australia. The SHB lifecycle can take between 3–12 weeks and has four stages: egg, larva, pupa, and adult beetle.

Female SHBs can lay approximately 1000 eggs in their lifetime. The number of eggs that will hatch depends primarily on the relative humidity, with some evidence suggesting at 30° C, no eggs will hatch at or below 34 per cent relative humidity. It takes approximately 1–6 days for larvae to emerge from the eggs, though most hatch within 3 days within a hive.

The larval stage of the SHB lifecycle is the most damaging because the larvae immediately start to burrow through combs and cappings, and consume honey bee eggs, pollen and honey. They also defecate throughout the comb, releasing the yeast *K. ohmeri*, which contaminates the honey in both active hives and stored combs. This yeast causes the honey to ferment, which may cause the hive to become 'slimed out' and die or abscond.

The developmental period for the larvae depends on the temperature and the availability of food but generally takes between 8–29 days. After a feeding period of between 6–14 day larvae enter a 'wandering' phase where they could travel up to 200m outside the honey bee colony to find an appropriate site for pupation, typically moist soil. When larvae cannot find an appropriate site for pupation they are able to pause development for a period of time until suitable conditions arise.

Once the larvae find a suitable site, they will burrow approximately 5–20cm into the soil and construct a smooth-walled pupation chamber. Moist soil and warm temperatures are critical for successful pupation and the emergence of the adult beetle. Pupation can take between 2–12 weeks depending on these environmental factors. During cold periods of less than 10°C pupation can take up to 100 days.

Adult SHBs are able to fly up to 15km to locate a honey bee colony to infest. Adult beetles prefer weak hives in spring and summer, but strong hives in autumn where the higher honey bee numbers keep them warm. It is believed that the SHB adults find the hives by detecting the odour of adult bees and hive products (honey and pollen).

Adult beetles reach sexual maturity at seven days and mate within the honey bee colony. Adult beetles can survive up to six months feeding on honey and up to 50 days feeding on an old empty brood comb.

In June 2013, surveillance for SHB was incorporated into the Program for Tasmania and the Northern Territory, where it is currently not present, and also Western Australia, where it is currently restricted in distribution to the northern part of the state (Kununurra). Hives are tested every two months using oil traps or Apithor harbourages (containing the insecticide fipronil) (see Appendix 3).

A2.1.15 Asian honey bee (Apis cerana)

Asian honey bee (*A. cerana* Java Genotype) is currently established in the Cairns region in the state of Queensland. The *A. cerana* Java genotype is a tropical strain of Asian honey bee and most likely arrived in Cairns in 2008 via a ship from Papua New Guinea or Indonesian Papua. This genotype cannot be managed

for honey production and pollination services due to its frequent swarming and tendency to abscond. The Asian honey bee produces less honey than the European honey bee European honey bee and in other regions where this bee has established, it has shown the ability to rob the European honey bee of their honey stores. As a cavity nesting bee which is capable of nesting in smaller areas than the European honey bee, the Asian honey bee also has the potential to become a competitor for nectar, pollen and nesting sites in the natural environment, as well as nest in urban environments.

Appendix 3: Surveillance methods for bee pests and pest bees

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As described in Appendices 1 and 2, surveillance for bee pests and pest bees and hornets is comprised of a number of different activities and occurs across the continuum of pre-border, border and post-border in Australia. An overview of the targets at different points in the continuum is described in Table A3.1 and in the following sections.

	Offshore surveillance (note not all pests are surveyed in all countries)	Border surveillance	Onshore surveillance (NBPSP and industry programs, BeeAware)	Export market notification and/or freedom required?
	Surveillance at this part of the continuum supports early warning of biosecurity threats to Australia. Surveillance targets are selected through negotiations with the relevant country and so may include pests of concern for them.	Border surveillance aims to intercept biosecurity risks that present at airports, seaports, mail centres and along Australia's coastline. Surveillance complements compliance and inspection activities.	Surveillance at this part of the continuum supports early detection of biosecurity threats once they enter Australia. Activities are conducted/supported by the Commonwealth, state and territory governments, industry and the community.	Trading partners often require declarations of pest absence in a commodity and/or notification of pest detections to enable trade and meet our international obligations. Target pests are at the discretion of the trading partner.
		Pest bees		
Africanised bees <i>Apis mellifera</i> exotic subspecies				Freedom required
Asian honey bees <i>Apis cerana</i>		Surveillance targets all	Curveillance terreste all	
Cape honey bees Apis mellifera capensis		bees and bee swarms. Import conditions for live queen honey bees cover Cape bees and Africanised bees	Surveillance targets all bees and bee swarms (n.b. Asian honey bee is established in parts of Australia)	Freedom required
Dwarf honey bees Apis florea				
Giant honey bees				
Apis dorsata				
		Bee pests and disease	es	
Acariosis		Covered by import conditions for live queen		Notification and freedom required

Table A3.1 Surveillance targets at each point of the continuum (Coloured boxes indicate the most effective points for surveillance for each target).

Acarapis woodi	honey bees		
Acute bee paralysis virus (APBV)			
American foulbrood <i>Paenibacillus</i> <i>larvae</i>	Covered by import conditions for live queen honey bees	Established in parts of Australia	Notification required
Asian hornet <i>Vespa</i> <i>mandarinia</i>			
Black queen cell virus (BQCV)		Established in parts of Australia	
Braula fly <i>Braula coeca</i>	Covered by import conditions for live queen honey bees	Established in parts of Australia	
Brood mite <i>EuVarroa sinhai</i>			Freedom required
Chalkbrood <i>Ascosphaera</i> <i>apis</i>		Established in parts of Australia	Notification required
Chronic bee paralysis virus (CBPV)			
Deformed wing virus (DWV)			
European foulbrood <i>Melissococcus</i> <i>plutonius</i>	Covered by import conditions for live queen honey bees	Established in parts of Australia	Notification required
Israeli acute paralysis virus (IAPV)			
Kashmir bee virus (KBV)			
Lake Sinai Virus 1 (LSV1) and Lake Sinai Virus 2 (LSV2)			

			1
Large hive beetle Oplostomus fuligineus			
Nosemosis <i>Nosema apis</i> <i>Nosema ceranae</i>		Established in parts of Australia (<i>N. Ceranae</i> absent from WA)	
Sacbrood virus (SBV)		Established in parts of Australia	Freedom required for Thai sacbrood
Slow paralysis virus (SPV)			
Small hive beetle Aethina tumida	Covered by import conditions for live queen honey bees	Established in parts of Australia	Notification required
<i>Tropilaelaps</i> spp.	Covered by import conditions for live queen honey bees		Notification and freedom required
Varroa spp.	Covered by import conditions for live queen honey bees		Notification and freedom required
Wax moths <i>Galleria</i> <i>mellonella and</i> Achroia grisella		Established in parts of Australia	

A3.1 Bee surveillance off-shore

The Department of Agriculture and Water Resources conducts annual surveillance activities across Timor-Leste, Papua New Guinea (PNG) and the Solomon Islands for the early detection and monitoring of priority pest bees and bee pests.

Through these activities, bee and larvae samples are collected for molecular analysis of *Nosema apis* and *Nosema ceranae* (microsporidian gut-parasites that cause Nosemosis) and 10 honey bee viruses: Acute bee paralysis virus; Kashmir bee virus; Israeli acute paralysis virus; Sacbrood virus; Black queen cell virus; Deformed wing virus; Slow paralysis virus; Chronic bee paralysis virus; Lake Sinai Virus 1 and Lake Sinai Virus 2 by CSIRO molecular diagnostics specialists. Brood inspections are also conducted for hives in both PNG and Solomon Islands to assess the presence of parasitic mites (*Varroa destructor, V. jacobsoni* and *Tropilaelaps* spp.) in PNG and Solomon Islands, and for hive pests (small hive beetle and wax moth) and brood diseases (chalkbrood, American foulbrood and European foulbrood) in Solomon Islands.

An emerging risk identified through the offshore surveillance activities is the spread of *Tropilaelaps mercedesae*. This pest (which is established in Papua New Guinea) is spreading with the population expanding along the Highlands highway. *T. mercedesae* is moving towards Papua New Guinea's trade centre of Lae; and primary honey bee production region in Goroka. This presents the risk of the mite entering several pathways if left unmonitored. For example, *Tropilaelaps* spp. can move between *A. mellifera* and

A. cerana and *A. cerana* has a natural pathway through the Torres Strait to Australia (based on evidence from recent interceptions).

A3.2 Bee surveillance onshore

Onshore, the department works cooperatively with other Australian Government agencies, state and territory governments and industry partners to prepare for, detect and respond to pests and diseases. Key activities include the development of:

- A honey bee industry and pollination continuity strategy should *Varroa* mite become established in Australia, released in May 2011.
- The National Bee Biosecurity Program and associated Biosecurity Code of Practice.
- A Biosecurity Manual for Beekeepers and Industry Biosecurity Plan for the Honey Bee Industry.
- BeeForce, a community engagement project that examined the potential of urban hobby beekeepers in a post-border surveillance program for the early detection of *Varroa* mites around high-risk entry points in two locations in Victoria. BeeForce identified how community detective networks, or BeeForce task groups, could be recruited, structured, trained and motivated to assist in monitoring for a high priority honey bee pest over a two-year initiative in both Melbourne and Geelong.
- The BeeAware website was developed to boost preparedness for an incursion of *Varroa* mite or another exotic pest of bees. Subsequently the scope of the site was extended to include information on established pests already affecting honey bees in Australia, as well as pollination information for a range of plant industries. Launched in July 2014, BeeAware was developed and is maintained by PHA. Funding for the site was provided by the <u>Australian Honey Bee Industry Council</u>, the Australian Government <u>Department of Agriculture and Water Resources</u>, <u>Rural Industries Research and</u> <u>Development Corporation</u> and <u>Horticulture Innovation Australia</u>.
- The employment of Bee Biosecurity Officers within the Department of Primary Industries of each state government, funded through a combination of beekeeper levies and in-kind state primary industry agency contributions. The Bee Biosecurity Officer's key responsibilities include:
 - Implementation of the Biosecurity Code of Practice
 - Extension Officer for bee biosecurity
 - Assist in the management of the National Bee Pest Surveillance Program
 - \circ $\;$ Emergency Response assistance for bee biosecurity threats and incidents

A3.3 Bee surveillance at the border

Border activities seek to intercept biosecurity risks that present at airports, seaports, mail centres and along Australia's coastline. Routine inspection is undertaken of passengers, mail, goods and vessels for exotic pests and diseases.

- Pre-arrival reporting is mandatory for vessels over 25m arriving in Australia requiring reporting on whether any insects, including bees, were discovered onboard during the voyage to Australia.
- Ongoing strong engagement with port operators and workers to raise awareness of the risk associated with bees and to encourage reporting of bees or bee swarms.
- Exotic bees intercepted are exterminated and submitted to entomologists for identification and dissection for the presence of *Varroa* mite and other parasitic mites, such as tracheal mites.

Under the *Quarantine Act 1908* and *Quarantine Proclamation 1998* 'bee products' are prohibited entry to Australia unless granted a permit to do so. The Proclamation exempts a number of apiary products from this requirement as long as the products are 'pure and free from extraneous material'. This includes honey.

Strict biosecurity regulations and standards are imposed on importation of live honey bees to Australia. Currently there are no import conditions for bee semen (note the recent release of a draft review which includes proposed conditions). <u>http://www.agriculture.gov.au/biosecurity/risk-analysis/ira/current-animal/honey-bee-semen</u>.

Regular surveillance of Australia's northern coastline, stretching from Cairns to Broome and including the Torres Strait to monitor for targeted pests and disease, including pests of bees to provide early warning and enable rapid response.

A3.4 Surveillance methods

A3.4.1 Sentinel Hives

The NBPSP is currently primarily based on sentinel hives, which are hives of European honey bees (*Apis mellifera*) of a known health status placed in key locations and monitored for the detection of new pests such as *Varroa* mite. As of February 2016, there were over 160 sentinel hives maintained at air and sea ports around Australia that receive a significant volume of cargo and are believed to be of high risk (see section 3.3.1 and Appendix B1.2). The sentinel hives are provided, managed and tested by cooperating beekeepers under the support of AHBIC, or in some cases, the hives are provided, managed and tested by the respective State/Territory Departments of Agriculture. The operation of sentinel hives has included the use of the acaricides Bayvarol® and Apistan® with hives tested every 6-8 weeks to provide a means of early detection of *Varroa* mites (*Varroa destructor* and *V. jacobsoni*) which could potentially enter via exotic bees on a vessel or transported cargo. Within Australia, operation of sentinel hives has relied on the assumption of no acaricide resistance being present in any incursion of Varroa. Populations of acaricide resistant *Varroa* are increasing in frequency around the world however and alternate treatments for *Varroa* are recommended for sentinel hives.

A3.4.2 Swarm/feral nest capture and catchboxes

A swarm is a group of bees searching for a new nesting site. Swarms can be found hanging from any object (e.g. tree branch, house gutter, fence) as a dense cluster around a queen that can vary in size from hundreds to thousands of bees. Swarming usually occurs in spring but sometimes occurs at other times of the year when local conditions permit. They may remain in place for a few hours to up to 1–2 days, while scout bees are sent out to seek a new nesting site. Swarming bees are much less defensive than they would be if still protecting combs with brood and stored pollen and honey.

Collection of swarms and/or feral nests in and around high risk ports as well as from catchboxes (including remote catchboxes) placed at high risk locations, supplements sentinel hive surveillance activities. Honey bees collected through these methods can be examined for the presence of exotic mites using the alcohol washing procedure and Tracheal mite examination.

A3.4.3 Catchboxes

Catchboxes or bait hives (empty hives) positioned in high risk port areas provide a means of early detection of exotic species of *A. mellifera* including Africanized honey bees (*A. m. scutellata*) and Cape honey bees (*A. m. capensis*). Newly arriving swarms of European honey bee (i.e. inadvertently imported on cargo/vessels) as well as the local *A. mellifera* population may also be picked up using catchboxes and can subsequently be sampled for exotic mites on a regular basis. Catchboxes are not used to detect Asian honey bee (*A. cerana*)

due to their different nesting requirements, or the Giant honey bee (*A. dorsata*) or Red dwarf honey bee (*A. florea*) as these species are not cavity nesting.

Advantages of catchboxes include:

- Catchboxes can be set up with remote surveillance (see section A3.4.4)
- Fewer resources are required when compared with sentinel hives as bee colonies are not being maintained and monitored in a catchbox.

Disadvantages for use of catchboxes as a surveillance method include:

- The effectiveness of catchboxes is dependent on nesting site availability (less likely to capture swarms if many refuges are available).
- The appropriate density, and sensitivity, of catchboxes is yet to be determined
- Unless remote surveillance capability is linked with the catchbox, they require inspection almost daily, and skill to observe bee flight to and from a catchbox and to detection occupation
- Endemic *A. mellifera* swarms can be captured, thus becoming time consuming to test, clean the catchbox, and replace
- Boxes require monitoring and costs in labour and travel

A3.4.4 Remote surveillance catchboxes

A remote surveillance catchbox is an empty hive with a mobile phone camera and sensors that can detect when honey bees are present in the hive. The phone captures an image at frequent intervals and performs image analysis to determine the presence of a swarm. The phone uploads an image on a daily basis or if activity is detected by image analysis. Power to the phone is provided by a solar panel and batteries in the catchbox lid. An electronic door on the catchbox entry can be triggered remotely to close and open the hive door.

The effectiveness of remote surveillance catchboxes is dependent on nesting site availability (less likely to capture swarms if many refuges are available).

Disadvantages of remote surveillance catchboxes include:

- The appropriate density, and sensitivity, of remote surveillance catchboxes is yet to be determined
- Expensive set up costs and ongoing data transmission costs
- Endemic *A. mellifera* swarms can be captured, thus becoming time consuming to test, clean the catchbox, and replace
- The national pilot project to assess remote surveillance catchboxes has not captured any bees.
- The sensitivity of the sensors needs refining

A3.4.5 Hobby beekeeper involvement

A BeeForce community engagement pilot has been operating 2010 - 2012 to test the involvement of urban hobbyist and professional beekeepers in a passive surveillance program for *Varroa* mites in both Melbourne and Geelong in Victoria. BeeForce is now being implemented nationally. The BeeForce pilot demonstrated not only that an active task force could be enlisted and trained, but that after two years of testing in two separate locations, participants were still willing to be involved in such an initiative. It has enabled a higher number of sentinel hives to be deployed.

The pilot program also demonstrated that if this strong motivation is encouraged and nurtured, then the BeeForce model of community surveillance could provide a reliable task force on which government agencies could draw on, not only for early surveillance initiatives as part of the NBPSP, but also for eradication or surveillance in an emergency response for a honey bee emergency plant pest.

As part of the NBPSP, coordinators in each state/territory actively seek the involvement of local beekeepers in high risk port areas. They then provide awareness training about exotic bee pests and show the beekeepers how to conduct routine surveillance on their hives.

This method of surveillance requires ongoing oversight, which would likely fall on state and territory apiary officers or active beekeeping associations. It also requires access to skilled diagnostic support and only allows for less effective methods of surveillance (i.e. sugar shaking).

A3.4.6 Floral sweep netting

Floral sweep netting consists of operators using insect sweep nets over floral sources where bees are present. This technique can be used for capturing *A. mellifera* individuals for further assessment for bee pests using techniques such as alcohol wash, sugar shake or dissection methods for internal pests, however is seen as most useful for capturing pest bees such as Asian honey bee.

During the Asian honey bee response in Cairns, Biosecurity Queensland and the Northern Australian Quarantine Strategy (NAQS) demonstrated that floral sweep netting was the most efficient and effective sampling method to confirm the presence of Asian honey bee in the Cairns port area.

Considering the work by NAQS and the reality that another incursion of pest bees poses a significant risk to Australia's honey bee and pollination-reliant industries, floral sweep netting has been proposed as the main surveillance method to provide early detection of exotic pest bees at high risk ports.

For this reason, PHA have built on the work conducted by NAQS and developed a floral sweep netting and mapping method that targets all pest *Apis* spp., (Asian honey bee (*Apis cerana*), Giant honey bee (*A. dorsata*), Red dwarf honey bee (*A. florea*)) as well as Bumblebees (*Bombus* spp). This method is not effective for *A. mellifera* as mainland *A. mellifera* would provide numerous false samples.

Potential disadvantages for this method include:

- Knowledge of floral resources required to be mapped in risk areas
- Influenced by seasonal/temporal factors
- Only detects foraging bees
- Labour intensive
- Bee foraging can occur at heights
- Need to know where, what and when bees forage on in an area
- Diagnostic support required
- A beeline (direct observation of bee flight) has to be conducted to identify the nest

A3.4.7 Sugar shaking

Sugar shaking of honey bees is a quick and easy method used to detect *Varroa* mites. About 300 bees (1/2 a cup) are removed from a hive or swarm and shaken in a closed container with pure icing sugar. The method works by the fine sugar particles dislodging *Varroa* mites by stopping their sticky pads (feet)

gripping onto honey bees and also potentially by stimulating grooming behaviour of honey bees which then assists remove mites. The sugar is then separated from the bees and inspected for mites.

This method does not kill the bees and where *Varroa* are present, it is estimated that it removes 70-90% of external *Varroa* mites present on adult honey bees. As a surveillance tool in a management program, it is recommended that it should be conducted on at least 10% of hives in an apiary. Note that this method will not detect very low infestations of *Varroa* mites in hives.

A3.4.8 Alcohol washing

Alcohol washing is a quick method for detecting the presence of *Varroa* mites, as well as monitoring colony mite levels. A minimum of 300 bees (1/2 a cup) is removed from a hive or swarm and placed in a container with 70% alcohol (or methylated spirits). The alcohol wash method can remove 70-80% of external *Varroa* mites present on adult honey bees. This technique is more effective when little brood is present in the sample assessed, however, it will provide improved results when there are also significant quantities of brood and the sample bees are taken from the centre of the brood nest as this is where the majority of *Varroa* mites will occur in a hive (see Section 3.4.9). This method kills the bees that are sampled.

A3.4.9 Drone uncapping

Drone uncapping is a surveillance technique for detection of *Varroa* mite and also for Tropilaelaps mite. For *Varroa* mite, up to 85% of mites in a honey bee colony are found within capped brood cells, with a preference for drone brood. Therefore, uncapping drone brood and visual examination of pupae is another method for detection of *Varroa* mites. With appropriate training, this technique could be conducted by beekeepers as it is rapid assessment and can be carried out easily as part of a routine hive inspection. The disadvantage of this method is that the drone brood are killed. The preference of *Varroa* for drone brood is strongest in the spring and decreases towards the end of the drone rearing season. Therefore, this technique is most sensitive when conducted in early spring.

For Tropilaelaps mites, symptoms such as deformed pupae and adults (stunting, damaged wings/legs/abdomens), Parasitic Mite Syndrome (PMS) and colony decline could be undertaken with visual observation of hive health and drone uncapping. Due to the small size of Tropilaelaps mites, confirmation of an infestation would require magnification to determine mites were present.

A3.4.10 Rainbow bee-eater pellets

Rainbow bee-eater pellets provide a tool for determining the presence of pest bees such as Asian honey bee (*Apis cerana*). The method was developed following the 1998 incursion of *A. cerana* in Darwin and further developed to be used during the eradication efforts of the 2007 Cairns incursion.

The rainbow bee-eater is widespread across much of mainland Australia and occurs on several near-shore islands. It is absent from Tasmania, and is thinly distributed in the most arid regions of central and Western Australia. It breeds throughout most of its range, being present in many northern locations throughout the year. The birds in southern Australia, however, migrate north during the winter months, resulting in larger populations in northern Australia between March and November. Although rainbow bee-eaters eat a variety of insects, their diet consists mainly of bees and wasps. When roosting, they regurgitate non-digestible parts of their prey (such as bee wings) in the form of a pellet. As pellets fall to the ground, they can be collected and the contents examined for the presence of *A. cerana* wings (Bellis and Profke 2003). The rainbow bee-eaters will forage over a range of several kilometres and may collect bees from areas inaccessible to humans. These features make rainbow bee-eaters useful for establishing the presence of *A. cerana* in an area.

A report detailing the efficacy of detection methods for Asian honey bee during the Transition to Management program in Queensland noted that rainbow bee-eater (*Merops ornatus*) surveillance was the most efficacious method of detection where roosts were present.

Disadvantages of this surveillance technique include that it requires the presence of a local population of rainbow bee-eaters. For early detection purposes, this local population should ideally be situated as close as possible (within a few kilometres) to a high risk port area. Furthermore, the identification stage is labour intensive and requires a great deal of technical skill. One of the significant unknowns about bee-eaters is the length of time between ingestion of bees by the bird and the disgorging of the pellet. As populations may move seasonally, it is possible that a migrating bird could contribute into the testing system a pellet representing bee populations hundreds of kilometres away. A bird flying south from, say, Saibai Island in the Torres Strait could be disgorging pellets rich in *A. cerana* in Cairns within a day or two.

A3.4.11 Apithor harbourages for detection of small hive beetle

Specific surveillance methods are required for Small hive beetle (SHB (*Aethina tumida*)) as it can be very difficult to detect low numbers of SHB in hives and therefore trapping is the most useful tool for early detection. Traps currently available include the Apithor harbourage that contains an insecticide (Fipronil) to kill trapped beetles. Non-chemical traps usually contain oil to drown trapped SHB, although lime or diatomaceous earth can also be used. From 2013, surveillance for SHB was formally integrated into the NBPSP and began in Tasmania and Northern Territory where it is currently not present, as well as through Western Australia where SHB has a restricted distribution (north of Kununurra).

The Apithor harbourage is comprised of two black, rigid moulded plastic shells that hold a Fipronil treated 4 mm corrugated cardboard insert. This insert is located 10 mm back from the 3 mm wide entrance slots. Size differences between the beetles and the honey bees and the precise dimensions of the harbourage prevent the honey bees from contacting the cardboard insert but allow easy access for SHB.

The plastic shells are ultra-sonically welded to produce a tamperproof device so that the harbourage can be safely handled without fear of contacting the insecticide. For information on where to purchase and the cost of Apithor harbourages. Apithor is now registered for use in all states and territories of Australia (as of 9/12/2013).

Appendix 4: Varroa Incursion Model

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Development of a *Varroa* Incursion Model was initiated to undertake an analysis of the sentinel hive component of the NBPSP with a particular emphasis on detection of *Varroa* mites to determine if improvements could be recommended to make surveillance more cost-effective, efficient or robust. The project was coordinate by PHA and involved; CSIRO who led the statistical element of the project, generating models and formulas for determining effectiveness of a range of surveillance methods and how it can be applied to the NBPSP, Queensland University of Technology for statistical expertise in determining effectiveness of a range of surveillance to ensure the surveillance undertaken will detect an exotic bee pest within a set period of time.

A4.1 Scope of the Varroa Incursion Model

To design a cost effective surveillance programme to enable the detection *Varroa* mite early enough that eradication can be considered.

1. The overall approach to the design of the surveillance program was as follows:

- a. Specify high risk entry sites.
- b. List potential pathways (modes of entry, points of release) for Varroa mite.
- c. Determine what is currently known about these pathways.
- d. Prioritise modes of entry with respect to risk.
- e. Determine the set of high priority points to include in the surveillance program.
- f. Characterise the geographic risk zones around each priority point with respect to variables that influence the spread and establishment of *Varroa* mite.
- g. Characterise the range of Varroa mite infestation on the pathway at those points

2. Specify potential surveillance components:

- a. List potential surveillance components.
- b. Select the set of most effective components for use in the surveillance program.
- c. Determine the characteristics of these components with respect to sensitivity (probability of detecting the pest), footprint (range of detection), cost (dollar and non-dollar), etc.
- **3.** Specify measures of power of detection:
 - a. Define a set of important endpoints.
 - b. Define a set of desired outputs from the analysis of the surveillance design.

4. Model the potential power of detection for a proposed surveillance scheme:

a. For the selected entry sites, based on the characteristics identified in Step 1, propose a surveillance plan using the surveillance components identified in Step 2.

b. Create an agent-based model that computes the power of detection at that site, using the endpoints and outputs identified in Step 3.

c. Apply the model to each of the high priority entry points.

5. Optimise the surveillance design:

a. For a given surveillance strategy, determine the overall power of detection across all high priority sites.

- b. Repeat Step 3 using different surveillance strategies.
- c. Choose the preferred plan based on the compiled results.

A4.2 Model description and assumptions

The modelling process consists of three main activities, namely (1) Developing a stochastic spatial spread model for *Varroa destructor* within and between beehives, (2) Calibrating this spread model to known incursion events of *Varroa* mite, and (3) Running this model forward to evaluate the surveillance effectiveness of various sentinel hive surveillance options.

The modelling builds and improves on the earlier work of Clifford *et al.* (2011) in two keys ways as recommended by Barry *et al.* (2010). First, it incorporates more realism into the spatial spread processes

and dynamics of *Varroa* mite, enabling more realistic and quantitative assessments of sentinel hive surveillance designs. Second, it uses data from New Zealand to calibrate model parameters empirically. Despite this, the resulting model does have its limitations that are described below.

Parameter values assessed for surveillance performance using simulation are shown in Table A4.1.

Factor	Description	Levels			
		1 hive			
		2 hives			
Number of		4 hives			
sentinel hives*		6 hives			
		9 hives			
		12 hives			
		1 km			
Sentinel hive	Distance between sentinel	2 km			
spacing	hives	3 km			
		5 km			
		1 month			
Inspection interval	Time between hive inspection	2 months			
		4 months			
Apistan®	The reduction in susceptibility	0 equating to P(Kill of phoretic mite)=0.70			
resistance	to Apistan [®] arising from genetic resistance	50% equating to P(Kill of phoretic mite)=0.35			
Distance to	Distance to the nearest	1 km			
hobbyists	suburbs where hobbyist bee keeping occurred	5 km			
		Single bee hosting 6 mites			
Incursion type	Characteristics of incursion event	Swarm hosting 100 mites			
	event	Swarm hosting 1,000 mites			
Incursion location	The spatial location of incursion in relation to the port of arrival	Random bearing from port with distance drawn from a Uniform (0,5) distribution.			
Course in a	Density of hives (domestic	5 km ⁻² (low density scenario)			
Carrying capacity	and feral combined)	10-20 km ⁻²			

Table A4.1 Factors	, their description	and levels for sentin	el hive scenarios	examined by simulations.
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* 'Bespoke' arrangements such as Brisbane with two sites containing three hives each were als assessed.

A4.3 Model results

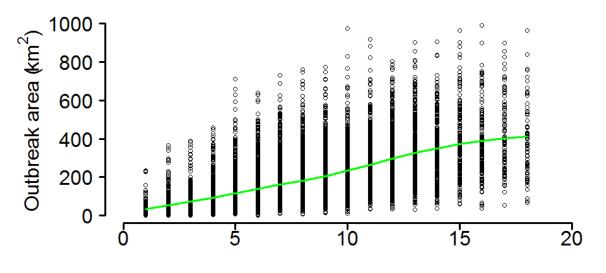
Unless otherwise stated, results presented assume the strain of *Varroa* mite introduced have no Apistan[®] resistance. Furthermore, a standard reference sentinel hive arrangement will consist of 6 hives spaced at equal 2 km, inspected at 1-2 monthly intervals (effectively 6 weeks as is the current protocol). Note this by no means is an endorsement of such an arrangement. The effect of other factors (e.g. type of incursion) will be marginalised (averaged) out. Conversion table for outbreak area and diameter are presented to help the reader (Table A4.2 and Table A4.3).

Diameter (km)	10	12	14	16	18	20	22	30	40	50
Area (km²)	78.5	113	154	200	254	314	452	707	1257	1963

Table A4.2 Relation between area and diameter for hypothetical outbreaks.

Area (km²)	100	200	300	400	500	750	1000	1250	1500	2000
Diameter (km)	11.3	16.0	19.5	22.6	25.2	30.9	35.7	39.9	43.7	50.5

There is a roughly linear relationship between the size of the incursion at first detection and the time to first detection in sentinel hives (Figure A4.1). Although it is often stated that it is critical to detect *Varroa* mite early, this really speaks to detecting *Varroa* mite at an eradicable incursion size.



Time to first detection (mths)

Figure A4.1 Relationship between the size of *Varroa* mite infestation at first detection in sentinel hives and the time to first detection in sentinel hives. Solid line is smoothed fit.

In general, we use the median and the 95% percentile to summarise the time to first detection and outbreak size at first detection. The median is easier to calculate and more robust for small sample sizes. It is also important to note the response variables are distributions. For example, 2 km arrays of 2, 6 or 12 sentinel hives inspected at two monthly intervals can all potentially detect a *Varroa* mite incursion within a month of it arriving, though with differing probabilities (Figure A4.2a). The median time to first detection, however, are quite different, being 8 months for the two hives, 5.5 months for six hives and four months for the 12 hive array (Figure A4.2b). Both the 6 hive and 12 hive array will detect and incursion within 12 months at least 95% of the time (Figure A4.2c).

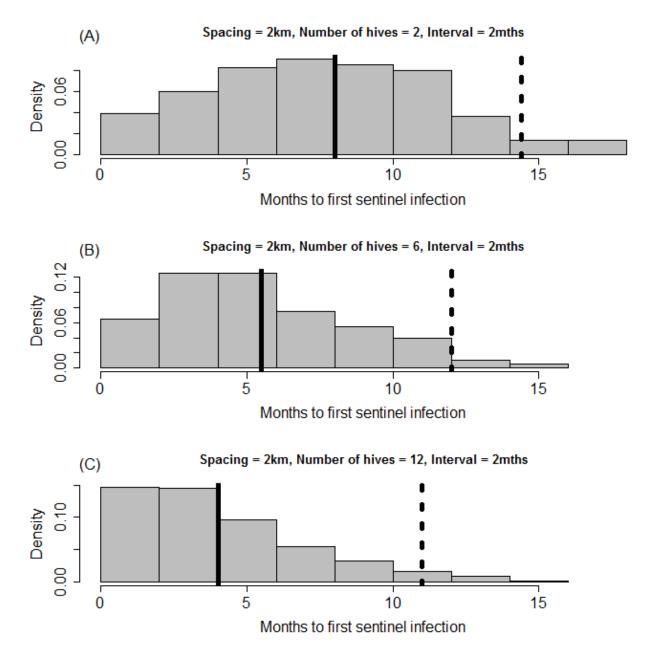


Figure A4.2 Distribution of times to first detection within a sentinel hive for (A) 2, (B) 6 and (c) 12 sentinel hives located on a 2 km array and inspected at 2 monthly intervals. Vertical solid line denotes the median and vertical dashed line the 95th percentile.

A4.3.1 General effects

General effects of parameters were examined by fitting a linear model relating the incursion size at first detection to a linear combination of parameters. Although the effects probably aren't strictly linear, as a first-order approximation it is useful to fit such a model and examine the direction and relative size of the regression coefficients.

Unsurprisingly given the high number of simulations, all estimated coefficients for scenario variables were statistically significant (P<0.001), however it is the size and direction of the coefficients that are of interest (Table A4.2). Note what follows are marginalized over all parameter combinations. For example, each month increase in the interval between inspections increases the time to first detection by 0.6 months. Likewise, each kilometre increase in the spacing between hives increases the delay by 0.5 months. The greater the number of mites arriving, the shorter the delay compared to a single bee. Halving the mortality of phoretic mites arising from Apistan® increases the delay by 0.3 months. Finally, each unit increase in the density of hives (either feral or hobbyist) increases the time to first detection in sentinel hives by 0.1 month (Table A4.2).

Parameter	Estimated coefficient	Std Error	Ρ
Inspection interval (mths)	0.6	0.046	<0.001
Hive spacing (km)	0.5	0.040	<0.001
Number of sentinels hives	-0.3	0.007	<0.001
Single bee (6 mites)	0.0	_	_
Swarm with 100 mites	-2.6	0.10	<0.001
Swarm with 1000 mites	-5.6	0.10	<0.001
No Apistan® resistance	0.0	-	_
50% Apistan® resistance	0.3	0.09	<0.001
Hive density (ferals and hobbyist)	0.1	0.01	<0.001

Table A4.2. Scenario parameters and their estimated coefficients for their contribution to the time (in months) to first sentinel hive detection, fitted over all simulation results.

A4.3.2 Hobbyist beekeeper proximity

In nearly all instances, a substantial number of hobbyist hives were also infested by the time *Varroa* mite infestation was detected in sentinel hives, particularly when hobbyists were located close to port facilities. Typically, if hobbyist hives were distributed to within 1 km of the port, about 100 hives would be infested by the time *Varroa* mite was first detected in a sentinel hive. In the case of the nearest hobbyist hives being 5 km from the port, the number drops to around 25.

A4.3.3 Inspection interval

Increasing the inspection interval from one to two months had little effect on the outbreak area at first detection, though doubling the inspection interval again to four months resulted in a jump in size (Figure A4.3). The current protocol is for 6-weekly inspections. With the model running on a monthly time step, this was not possible to simulate in the available time, so to examine the effectiveness of the current inspection protocol the results for monthly and 2-monthly inspections were pooled.

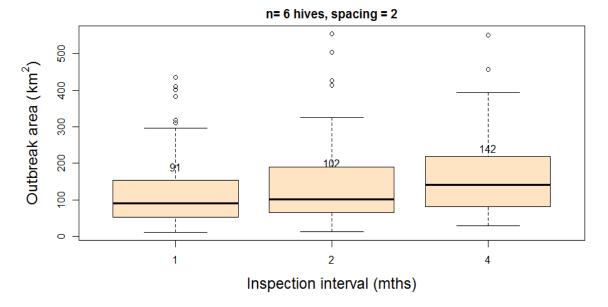


Figure A4.3 The effect of the interval between inspection on the median outbreak area (solid horizontal line) at first sentinel hive detection.

For a 6 sentinel hive array inspected at 6-weekly intervals, the delay between first sentinel hive infestation and first detection was 1.7 months. This increased to 3.1 months if the inspection interval was increased to 4 months.

A4.3.4 Apistan[®] resistance

Halving the expected mortality rate of phoretic mites due to Apistan[®] resistance increases the delay to the first detection of *Varroa* mite in sentinel hives and hence the incursion size, though not by a large amount. For a 4 hive by 2 km layout inspected at approximately 6 weekly intervals, the mean incursion size increases from 109 km² to 128 km² (Figure A4.4). The underlying reason for this would be the exponential growth of mites within the hive, such that the number mites in sentinel hives soon reach levels that even a less sensitive test can detect.

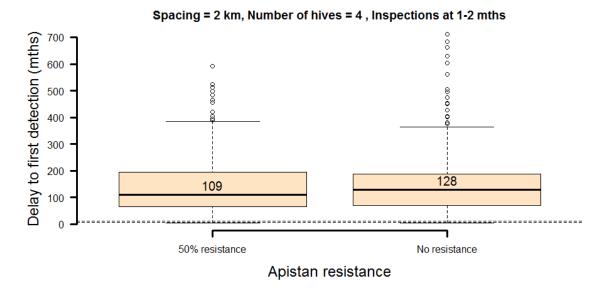


Figure A4.4 Effect of 50% resistance to Apistan[®] on the median incursion area (solid horizontal line) at first detection of *Varroa* mite in a sentinel hive.

A4.3.5 Effect of sentinel hive numbers and spacing

A summary of the median and 95th percentile for incursion area and diameter at first sentinel infection is shown in A4.3. For a given number of hives, the best surveillance performance occurred at a spaced of two km when four, six and 12 sentinel hives were deployed. If the number of sentinel hives was restricted to two, then a three kilometre spacing was best.

Single sentinel hives still provided a measure of surveillance, though the incursion size at first detection was about double that of a 6 hive array (Table A4.3). More detailed analysis of the interplay between spacing and number of hives can be found in the Appendix C.

Table A4.3 Median (50th percentile) and 95th percentile areas (km²) and diameters (km) of *Varroa* mite outbreaks at first detection in sentinel hives as a function of the number of sentinel hives and array spacing. Data are summarised over inspections at either 4 weeks or 8 weeks. There is assumed to be no Apistan[®] resistance. The best performing array spacing for a fixed number of hives is highlighted in bold.

	Number of sentinel hives										
		1 hive	*	2 hive	S	4 hive	S	6 hive	S	12 hives	
Array spacing		50 th	95 th								
1 km	Area	194*	(567)	187	(552)	129	(411)	112	(414)	84	(298)
	Diameter	15.7	(26.9)	15.4	(26.5)	12.8	(22.9)	12.0	(23.0)	10.3	(19.5)
2 km	Area	174	(534)	173	(537)	109	(370)	96	(325)	78	(306)
	Diameter	14.9	(26.1)	14.8	(26.1)	11.8	(21.7)	11.0	(20.3)	10.0	(19.7)
3 km	Area		_	165	(476)	138	(404)	107	(370)	98	(356)
	Diameter			14.5	(24.6)	13.3	(22.7)	11.6	(21.7)	11.2	(21.3)
5 km	Area		_	185	(545)	170	(416)	133	(426)	139	(441)
	Diameter			15.4	(26.4)	14.7	(23.0)	13.0	(23.3)	13.2	(23.8)
* For inc	lividual hive	s, the a	array spa	acing re	efers to d	istance	from por	t centr	e (c.f. spa	acing)	

A4.3.6 Hobbyist and feral hive involvement

Feral hives are invariably involved by the time of first sentinel hive detection, by virtue of the fact that the same methods of transmission apply. Under the reference sentinel hive setup, typically about 200 feral hives would be infested at the time of first sentinel detection (Figure A4.5).

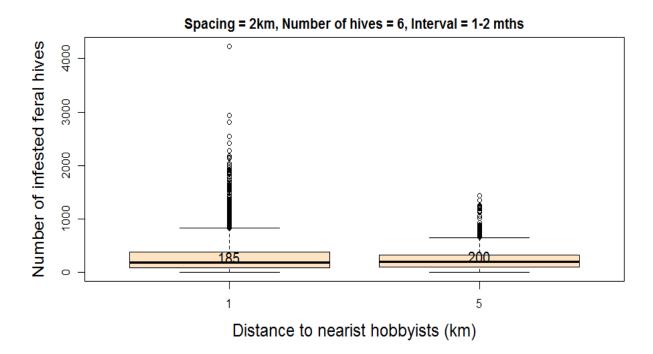


Figure A4.5 Median number of feral hives with *Varroa* mite infestation at the time of the first detection in sentinel hives in relation to the distance to the start of the suburbs (with associated urban beekeeping).

Similarly, a substantial number of hobbyist hives were also infested by the time *Varroa* mite infestation was first detected in sentinel hives, particularly when hobbyists were located close to port facilities (Figure A4.6). Typically, if hobbyist hives were distributed to within 1 km of the port, about 100 domestic hives would be infested by the time *Varroa* mite was first detected in a sentinel hive (Figure A4.6). In the case of the nearest hobbyist hives being 5 km from the port, the number drops to around 20 (Figure A4.6).

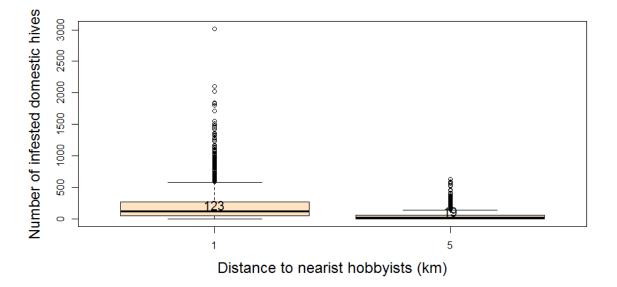


Figure A4.6 Median number of domestic hives with *Varroa* mite infestation at the time of the first detection in sentinel hives in relation to the distance to the start of the suburbs (with associated urban beekeeping).

A4.4 Implication of current bespoke arrangements

Some ports currently have differing arrangements of hives. For example, at Brisbane, the sentinel hive arrangement consists of three hives in each of two locations. This has a median incursion size at detection of 121 km² (95% percentile 376 km²) compared to 95.7 km² (95% percentile 325 km²) for the reference 6 hives at 2 km layout.

The expected size of a *Varroa destructor* incursion at first sentinel hive detection for a typical surveillance setup up (6 hives spaced on a 2 km array and checked 6 weekly) is in the order of 100 km² following a delay of 6 months since introduction. This is equivalent to a circular infestation area of diameter *c*. 11 km. By this stage, a considerable number of feral (100s) and hobbyist (10s to 100s) hives will also be infested, depending on how close suburbs are from the port. Intuitively, the reason for the high involvement of feral and/or hobbyist hives by the time of the first detection in sentinel hives is that all hives are infested by the same method. Furthermore, it is unlikely to have infestation in sentinel hives prior to infestation in feral hives, particularly when the latter are assumed much more numerous. This assumption (regarding density of feral hives around ports) needs checking.

Increasing the number of sentinel hives to 12, and using the best performing array spacing of 2 km still resulted in a median incursion size at sentinel detection of c. 80 km².

The frequency of movement of hives maintained by commercial beekeepers was estimated using hive movement data collected during surveys of all beekeepers in Australian managing greater than 100 hives (see Gordon *et al.* 2014 for full details). As part of a much broader set of survey questions, beekeepers were asked "On average, how many times would you relocate your hives in a year?" and "Do you use a net when transporting bees?" A random sub-sample (n=263) of the beekeeper surveys were used to characterize the frequency of movement and propensity to net bees during moving.

A small percentage (16.5%) of beekeepers used nets when moving bees. The mean number of moves per year was 3.6, although this was skewed somewhat by an individual who reported moving hives 50 times per year. There is probably some ambiguity in the question as to the number of times an individual lot of hives is moved (the question of interest here) as opposed to the number of moves a beekeeper conducts (not necessarily on the same hives). After the removal of this extreme data point, the highest number of moves per year was 10, with about 30 percent of beekeepers reporting no moves (Figure A4.7). The data are clearly bi-modal. For those beekeepers that did move hives, the mean frequency was 4.6 moves per year. This is equivalent to a hive of bees being moved every 2.6 months.

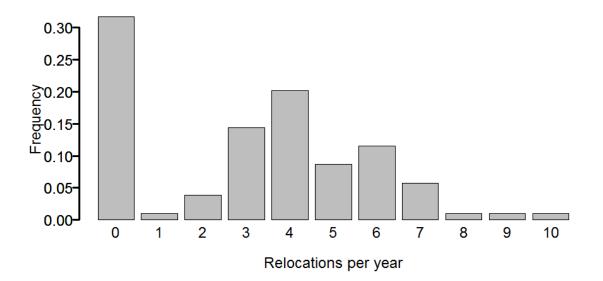


Figure A4.7 Yearly frequency of relocation of bee hives obtained from a sample of beekeepers managing > 100 hives.

The exact implications of commercial beekeeper movement is difficult to quantify without explicitly incorporating such operations into the simulation model, with all the complexity of integrating over yard sizes, location in relation to the port of interest and timing of movements. However, some robust generalisations can be made. First, given the preponderance for a considerable number of feral and hobbyist hives to be infested at the time of first sentinel hive detection, it stands to reason that a commercial apiary would also be infested at this time. The key issue then relates to the timing of the move, if it occurs.

The worst case scenario is that the commercial hives have been present from the initial incursion, and are infested immediately. This being the case, there is still about a 30% chance the beekeeper involved is one of those that does not move his bees, in which case no export of *Varroa* has occurred. If not, the chance of the hives being shifted and exporting *Varroa* in the expected 6 months delay before sentinel detection (and hive movement controls) is about 90% based on the movement rate. The distances involved are likely in the order of 100s of kilometres (see Gordon *et al.* 2014), and the possibility of contamination en route high due to the infrequent use of netting to prevent losing bees.

A4.5 Conclusions from new incursion simulation model

The results presented here incorporate considerably more realism than previous treatments of the problem, both in terms of model detail and the underlying data. That said, uncertainty remains in the parameterisation of the spread of *Varroa*, arising in part from irreducible uncertainty in the details of the New Zealand incursions used for model calibration. Our analysis has propagated this uncertainty through to the outputs.

- All else being equal, a spacing of 2 km between sentinel hives seems optimal.
- Surveillance performance starts to deteriorate noticeably once the interval between hive inspections exceeds two months.
- If the number of sentinel hives are limited to 4 or less, then a wider spacing is more effective than smaller.
- Fewer hives at more locations is better than multiple hives at fewer locations.
- Moderate levels of Apistan[®] resistance (e.g. a 50% reduction in toxin-induced mortality) will have a small to moderate impact on surveillance sensitivity and hence the size of outbreak at first detection.
- At the time of first detection of *Varroa* mite in sentinel hives, the number of infested feral hives will number in the 100s.
- Depending on how close domestic beekeeping occurs to the port environment, the number of infested domestic hives ranges from 10's to 100s. This clearly represents of risk of generating satellite foci of infestation through hive movement.
- To successfully eradicate such a number of infested feral hives will undoubtedly require the use of toxins (e.g Fiprinol) in combination with effective management of hobbyist hives. Effective move movement control would be critical.

The number of feral and/or hobbyist hives has a major impact on detection statistics. The greater the number of hives, the faster the rate of spread, the later the first detection in sentinel hives, and the larger the infestation area at first sentinel hive detection. Consideration of suppression of feral colonies around ports may be warranted, along with inspection regimes for hobbyist hives close to.

A4.6 How do these results indicate an incursion is detected early enough

Few data exist on the technical feasibility of eradicating *Varroa* mite. Note in the opinion of one of the authors, the incursion into the South Island of New Zealand would have been eradicable using the technical plan prepared (see Goodwin *et al.* 2006). The scale of the Nelson incursion at the time of detection involved 653 managed colonies (not all infected) spread over an area of approximately 10.3 km. However, the incursion was only in one direction, due to terrain influences.

An expert elicitation exercise to estimate the probability of eradicating *Varroa destructor* incursions into Australia under varying scenarios was undertaken by Penrose & Caley (2011). As there were no real data to estimate these probabilities (of successful eradications), expert opinion was sought instead. Experts were surveyed for their opinions on the probability of eradicating incursions under 32 hypothetical scenarios that differ for variables thought to affect eradication (Table A4.4). The data from the surveys were used to estimate the parameters of an additive linear regression equation with which to predict the odds (probabilities of eradication over failure to eradicate) of eradicating *Varroa*, given specified scenarios.

	• • • •	
Possible values	Variable	
In eight geometric increments from 40 to 4000 km2	Area of infestation	
Low (5– 20%), High (20–75%)	Proportion of bushland around incursion area	
Single	Number of foci in incursion area	
Multiple		
Excellent	Delimitation of incursion area	
Average		
Low	Feral hive density around incursion area	
High		
Yes	Commercial Beekeeper involvement in setting regulations	
No		
High (>95%)	Compliance of hive owners with regulations	
Moderate (>75%)		
Optimal	Eradication treatments	
Suboptimal		
20 field person equivalents	Number of persons managing incursions	
100 field person equivalents		

Table A4.4. Variables defining the 32 *Varroa* mite incursion scenarios in the expert opinion survey. Reproduced from Penrose & Caley (2011).

The analysis revealed that all the variables in Table A4.4 bar "Delimitation of incursion area" had a significant influence on the experts' beliefs in the probability of *Varroa* mite eradication. Of particular note were that the results were very pessimistic, with eradication of *Varroa* mite only considered likely in a scenario with an outbreak of no more than about 40 km², with all other variables being favourable. It is worth noting, however, that the background material provided to experts detailed the destruction of feral hives as occurring via bee lining for detection followed by manual destruction. Although the experts were asked to use their judgement in factoring in efficiencies likely to be gained as the eradication progressed, they weren't alerted to the possibility or remote poisoning. The work of Taylor (2003) in New Zealand clearly demonstrates the potential of remote poisoning for destroying feral bee colonies. The key finding then, from

the work of Penrose & Caley (2011) is that eradication will be very difficult and unlikely to be successful without the use of remote poisoning.

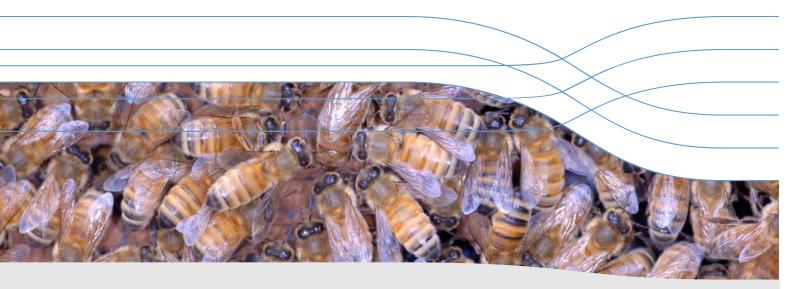
Appendix 5: Technical Report – Varroa Spread Model

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Peter Caley, Mark Stanaway & Simon C Barry April 2016 Prepared for: Horticulture Innovation Australia Ltd

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A5.1 Introduction

This technical report describes the technical details of modelling and simulation underpinning evaluation of the survey sensitivity of sentinel hives operated within the National Bee Pest Surveillance Program.

The research builds and improves on the earlier work of Clifford *et al.* (2011) in two keys ways as recommended by Barry *et al.* (2010). First, it incorporates more realism into the spatial spread processes and dynamics of *Varroa* within and between bee hives, enabling more realistic and quantitative assessments of sentinel hive surveillance designs. Second, it uses data on *Varroa* incursions from New Zealand to calibrate model parameters empirically.

A5.2 Methods

A5.2.1 Overview

The modelling process consists of three main activities, namely (1) Developing a stochastic spatial spread model for *Varroa destructor* within and between beehives, (2) Calibrating this spread model to known incursion events of *Varroa*, and (3) Running this model forward to evaluate the surveillance effectiveness of various sentinel hive surveillance options.

A5.2.2 Model components

Simulation background

Calculations were undertaken on a 30 km x 30 km area. A slightly larger area would have been preferable to better define how large outbreaks could be, however this would have slowed computation times considerably. In addition, early discussions suggested an outbreak of 30 km diameter would be the maximum considered eradicable.

Bee hives were randomly allocated across the introduction landscape according to the chosen carrying capacity. Suburban areas containing hobbyist beekeepers were started at either a 1 km radius from the port (similar to Port Botany and Melbourne) or a 5 km radius (similar to Brisbane). Where hobbyists occurred, they were assumed to compromise 50% of beehives – the remainder of hives were considered as being feral.

Incursion seeding

Incursions were seeded either by a single bee hosting 6 mites (probably the maximum possible), a swarm hosting 100 mites, or a swarm hosting 1,000 mites. The location of the incursion was identified by choosing a random bearing from the port in combination with a random jump uniformly distributed from zero to five kilometres. If the incursion was a swarm, it was assumed to settle permanently at that location. If the incursion was a single bee, it was assumed to search for and settle in the nearest available hive.

Within hive Varroa dynamics

The rate of increase of *Varroa* mites within a hive was fixed at 0.15 wk⁻¹, the mid-point of the range $(0.105-0.214 \text{ wk}^{-1})$ given by Harris *et al.* (2003). The probability of a hive dying was set to 0.2 yr⁻¹ following Clifford *et al.* (2011). The average maximum number of mites per hive was set to 30,000, with within-hive mite populations growing in a logistic manner.

Swarming

When a hive swarms, it is assumed that the swarm takes with it *Varroa* at the same prevalence as the parent hive. There are few data on the distances travelled by honey bee swarms. For *A. mellifera*, the most detailed empirical study observed that swarms prefer nearby nest sites if they are available, with the majority of swarms moving within about 2 km (Seeley and Morse 1977). A Weibull distribution with shape parameter 1.24 and scale parameter 1.21 provided a reasonable fit to the data provided in Fig. 1 of Seeley and Morse (1977), including the additional data in this figure from Lindauer (1955) (Figure A5.1). This parameterisation was used in the port risk assessment for bee pests and pest bees of Heersink *et al.* (2016).

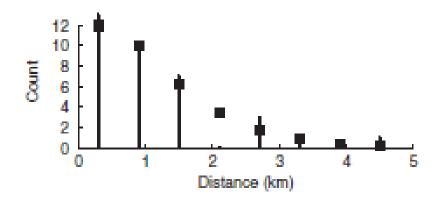


Figure A5.1 Distribution of observed (vertical bars) and modelled (squares) swarming distances for *A. mellifera swarms derived from the literature.*

Drifting

Drifting, particularly by drones, is thought to be a key mechanism of hive-to-hive spread of *Varroa*. The model used an exponential form for the drift, in combination with a y-intercept (the probability of drifting at zero distance between hives – e.g. hives side-by-side). Formally, the drifting distribution is: $f_D(d) = P(D = d) = p_0 e^{-\mu d}$

where *d* is the distance, μ denotes the rate of reduction with distance and p_0 the drift probability at zero distance. This process can be conceptualised easily but does not account for the number of hive in the vicinity that are available to drift to. For example, if there are no hives to drift to, bees would be modelled to drift regardless and eventually die. The proportion drifting will be the drifting distribution integrated over all possible distances.

The current approach is to calculate an intensity of drifting of bees and rescale. For the t^h hive, the unscaled intensity is:

$$I_i = \sum_{j=1}^{N} d_{ij} f_D(d_{ij}) V_j$$

Where:

N = total number of hives (includes ferals, domestic & sentinels)

 d_{ij} = distance between l^{th} and j^{th} hives [note can have i=j for reasons outlined below].

 V_{j} = the number of mites in the j^{th} hive.

This measure is unscaled, in that it is highly sensitive to the number of hives – the more hives there are, the more bees drift – potentially way more than the number available to drift. This largely makes

no sense. One solution that seems reasonable is to specify the number of bees that drift from a particular hive as Vp_d where $p_d = \int_0^\infty f_d(d)$. Crucially, bees are allowed to "drift" back to their own hive. Normalising over all hives, we get:

$$I_{i,scaled} = \frac{I_i}{\sum_{j=1}^N I_j} \sum_{j=1}^N V_j p_d$$

Appealing to the Poisson approximation to the Binomial, in each time step the number of *Varroa* infested bees is random Poisson ($I_{iscaled}$) draw.

Running simulations

The model was run with a monthly time step, with all parameters scaled accordingly. Each simulation run was based on a parameter values drawn from prior distributions (see Table A5.1), which were held constant for the duration of that simulation.

Parameter	Description	Prior value	Rationale
<i>p</i> ₀	Monthly drifting probability at zero distance	Uniform(0.0025,0.05)	Much lower than drifting rates observed in identical neighbouring hives.
a	Rate of exponential decay for drifting probability with distance	Uniform(0.5,5.0)	Chosen to encompass observed possible extremes in rate of spread.
Κ	Carrying capacity for feral and hobbyist hives within port environment and surroundings	Uniform(10,20)	From feedback from state bee biosecurity officers.
Incursion type	single bee hosting 6 mites swarm hosting 100 mites swarm hosting 1,000 mites	0.333 0.333 0.333	Type of incursion into Auckland unknown

Table A5.1 Statistical distributions used as priors for model parameters.

* For the Nelson incursion the apiarist at the apparent center of the outbreak was thought to have captured a swarm from the wharf area.

A5.2.3 Model calibration

New Zealand incursion data

Available data collected around the initial incursion into Auckland in 2000 can be used to estimate the rate of spread of *Varroa*, though little else. Stevenson *et al.* (2005) analysed the results of a delimiting survey of honey bee apiaries in the greater Auckland area to estimate the maximum rate of local spread of *Varroa* to be in the order of 19 km over estimated 19 months, equivalent to 12 km/year (interquartile range 10–15 km/year). Note, however, that the characteristics (swarm, imported queen, stray bee etc.) of the index case for the Auckland incursion remains unknown. For model calibration, for each simulation run we seeded the outbreak with either a single bee hosting 6 mites, a swarm

hosting 100 mites, or a swarm hosting 1,000 mites. These were selected with equal probability. The density of hives (feral and domestic combined) was assumed to be 15 hives km^{-2} .

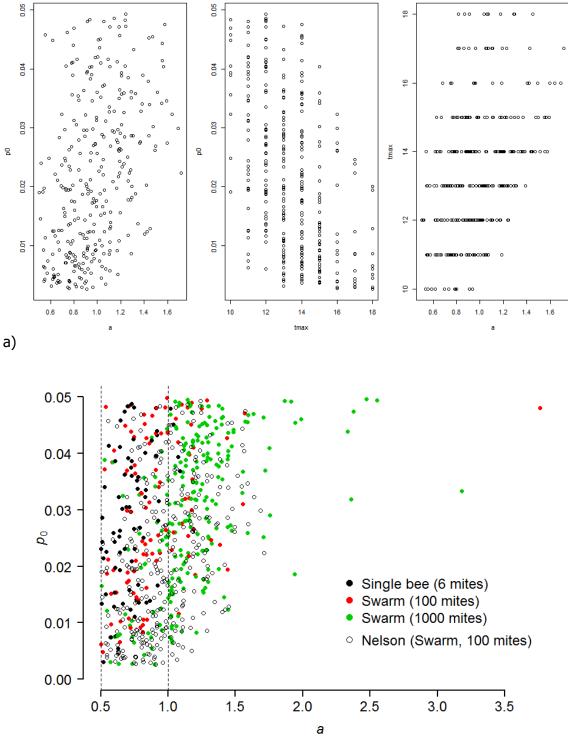
The data collected during the subsequent incursion in Nelson (New Zealand South Island) provides more detailed information on the number of mites in hives at an apiary level, including zero counts, though again the timing of the introduction was somewhat uncertain. The density of hives was 2.5 hives km⁻² (domestic and known feral hives combined). This much lower density to extent reflects the impact of farmland not having suitable nesting hollows for feral bees, and New Zealand forests (particularly introduced *Pinus spp*.) also having few nesting locations.

Calibration process

Approximate Bayesian Computational methods were used to calibrate the spread model to the two New Zealand incursions. In simple terms, this involved running the spread model using prior distributions on parameters (Table A5.2), and only accepting parameter combinations that resulted in an incursion with a similar rate of spread, or for the case of the Nelson incursion, the mean number of mites within infected apiaries.

From the accepted matches we have what is termed a posterior distribution, which contains combinations of parameters that resulted in incursions whose dimensions fell within the accepted tolerance.

The effect of incursion characteristic on the drifting kernel is considerable – for a single bee incursion the rate of drift needs to be higher to achieve the same incursion size after 19 months as observed during the Auckland incursion (Stevenson *et al.* 2005). This is reflected in the joint posterior for the parameters governing the drift kernel – accepted simulations for single bee introductions tended to have a lower exponential rate of decay (bees more likely to drift further in a given time) (see b)).



b)

Figure A5.2 a) Joint posterior distribution for parameters governing the monthly probability of bees drifting based on the Nelson data. b) Joint posterior distribution for parameters governing the monthly probability of bees drifting. Parameter p0 on the y-axis is t

A5.2.4 Surveillance scenarios

A summary of the sentinel hive parameters is contained in Table A5.1.

Factor	Description	Levels
Number of		1 hive
sentinel hives*		2 hives
		4 hives
		6 hives
		9 hives
		12 hives
Sentinel hive	Distance between sentinel	1 km
spacing	hives	2 km
		3 km
		5 km
Inspection	Time between hive inspection	1 month
interval		2 months
		4 months
Apistan®	The reduction in susceptibility	0 equating to P(Kill of phoretic mite)=0.70
resistance	to Apistan® arising from genetic resistance	50% equating to P(Kill of phoretic mite)=0.35
Distance to	Distance to the nearest	1 km
hobbyists	suburbs where hobbyist bee keeping occurred	5 km
Incursion type	Characteristics of incursion	Single bee hosting 6 mites
	event	Swarm hosting 100 mites
		Swarm hosting 1,000 mites
Incursion location	The spatial location of incursion in relation to the port of arrival	Random bearing from port with distance drawn from a Uniform (0,5) distribution.
Carrying capacity	Density of hives (domestic and	5 km ⁻² (low density scenario)
	feral combined)	10-20 km ⁻²

Table A5.1. Factors, their description and levels for sentinel hive scenarios examined by simulations.

Sentinel hives for simulation runs were arranged on a rectangular grid. Examples are shown in Figure A5.1.

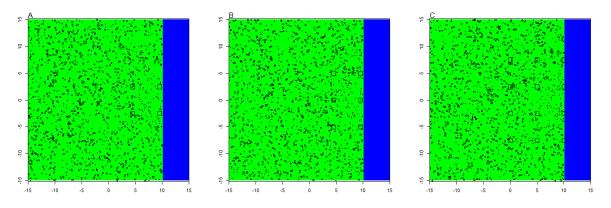


Figure A5.1 Diagrammatic representation of arrangements of (A) 4 hives, (B) 6 hives and (C) 12 hives.

In current practice, the actual number of sentinel hives ranges from a single hive to 11, and multiple hives are located at a reduced number of sites. For example, at Brisbane Port there are 3 hives located at each of 2 sites (Figure A5.2). Townville has 6 sentinel hives arranged roughly in a grid (Figure A5.3), whereas Geelong has about a dozen sentinel hives with a cluster adjacent to the port environs and others spread more widely (Figure A5.4).

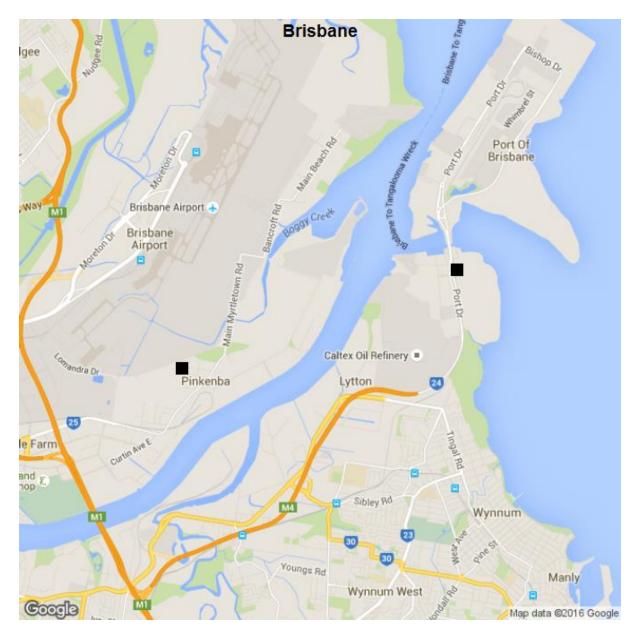


Figure A5.2 Sites (square dots) of sentinel hives at Port of Brisbane. Each site contains three hives.



Figure A5.3 Locations of sentinel hives (square dots) associated with Townsville port.

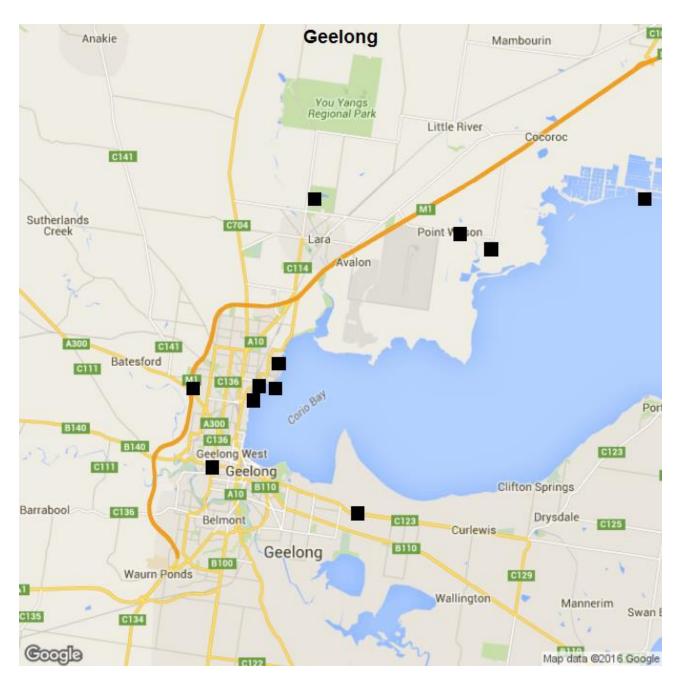


Figure A5.4 Locations of sentinel hives (square dots) surrounding the port of Geelong.

A5.2.5 Running simulations forward

A minimum of 100 simulations were run for all combinations of parameters in Table A5.1. For each simulation, a set of parameter values to be used were first drawn from the posterior distributions obtained from model calibration to both the Auckland and Nelson incursions, which were weighted equally. For each simulation run, the infestation status of all hives was retained, and in the case of sentinel hives, subject to a simulated hive inspection using Apistan® and sticky mats. The timing of the first sentinel hive inspection (regardless of interval) was selected randomly.

For each simulation at the time of first detection, the width, breadth and area of the incursion was recorded, along with the number of mites in each hive (sentinels, ferals & domestics). Simulations were run for 18 months. Introductions for which the mite failed to establish (sub-critical chains) were removed prior to analysis.

General effects of parameters were examined by fitting a linear model relating the incursion size at first detection to a linear combination of parameters. Although the effects probably aren't strictly linear, as a first-order approximation it is useful to fit such a model and examine the direction and relative size of the regression coefficients.

All analyses were undertaken in the computing environment R (R Development Core Team 2014).

A5.3 Results

Unless otherwise stated, results presented assume the strain of *Varroa* mite introduced have no Apistan® resistance. Furthermore, a standard reference sentinel hive arrangement will consist of 6 hives spaced at equal 2 km, inspected at 1-2 monthly intervals (effectively 6 weeks as is the current protocol). Note this by no means is an endorsement of such an arrangement. The effect of other factors (e.g. type of incursion) will be marginalised (averaged) out. Conversion table for outbreak area and diameter are presented to help the reader (Table A5.3 & Table A5.4).

Diameter (km)	10	12	14	16	18	20	22	30	40	50
Area (km²)	78.5	113	154	200	254	314	452	707	1257	1963

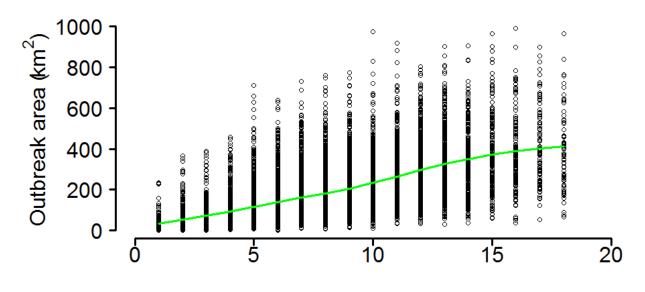
Table A5.2 Relation between area and diameter for hypothetical outbreaks.

Table A5.3 R	Relation	n betweer	n area and	d diamete	r for hyp	othetical	outbreaks	; .
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Area (km ²)	100	200	300	400	500	750	1000	1250	1500	2000
Diameter (km)	11.3	16.0	19.5	22.6	25.2	30.9	35.7	39.9	43.7	50.5

A5.3.1 Surveillance measures – incursion size versus time to first detection

There is a roughly linear relationship between the size of the incursion at first detection and the time to first detection in sentinel hives (Figure A5.5). Although it is often stated that it is critical to detect *Varroa* early, this really speaks to detecting *Varroa* at an eradicable incursion size.



Time to first detection (mths)

Figure A5.5 Relationship between the size of *Varroa* infestation at first detection in sentinel hives and the time to first detection in sentinel hives. Solid line is smoothed fit.

In general we use the median and the 95% percentile to summarise the time to first detection and outbreak size at first detection. The median is easier to calculate and more robust for small sample sizes. It is also important to note the response variables are distributions. For example, 2 km arrays of 2, 6 or 12 sentinel hives inspected at 2 monthly intervals can all potentially detect a *Varroa* incursion within a month of it arriving, though with differing probabilities (Figure A5.8). The median time to first detection, however, are quite different, being 8 months for the two hives, 5.5 months for six hives and four months for the 12 hive array (Figure A5.8). Both the 6 hive and 12 hive array will detect an incursion within 12 months at least 95% of the time (Figure A5.8).

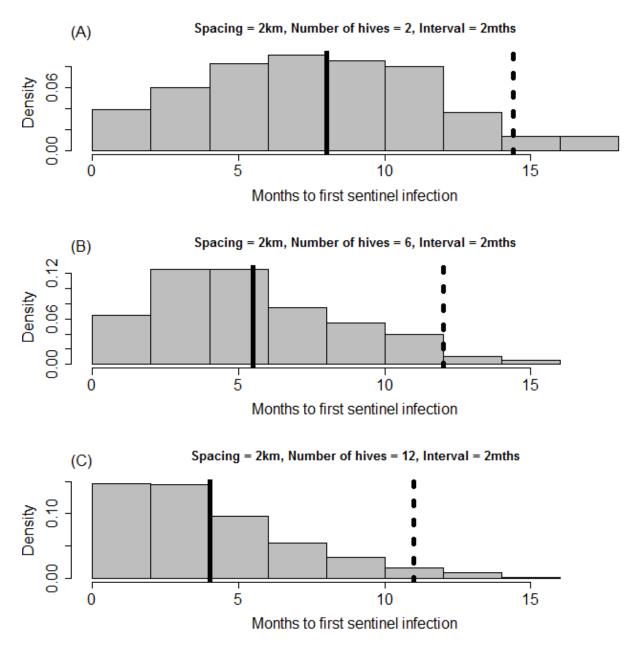


Figure A5.6. Distribution of times to first detection within a sentinel hive for (A) 2, (B) 6 and (c) 12 sentinel hives located on a 2 km array and inspected at 2 monthly intervals. Vertical solid line denotes the median and vertical dashed line the 95th percentile.

A5.3.2 General effects

Unsurprisingly given the high number of simulations, all estimated coefficients for scenario variables were statistically significant (P<0.001), however it is the size and direction of the coefficients that are of interest (Table A5.5). Note what follows are marginalized over all parameter combinations. Also, although the effects are probably not strictly linear, it is instructive to think of them as such. For example, each month increase in the interval between inspections increases the time to first detection by 0.6 months. Likewise, each kilometre increase in the spacing between hives increases the delay by 0.5 months (but note nuances below at A5.3.5 Hive spacing). The greater the number of mites arriving, the shorter the delay. Halving the mortality of phoretic mites arising from Apistan® increases the delay by 0.3 months. Finally, each unit increase in the density of hives (either feral or hobbyist) increases the time to first detection in sentinel hives by 0.1 month (Table A5.5).

Table A5.4 Scenario parameter and their estimated coefficients for time to first sentinel hive detection (in months), fitted over all simulation results.

Pa	rameter	Estimated coefficient	Std Error	Р
Inspection interval		0.6	0.046	<0.001
Hive spacing		0.5	0.040	<0.001
Number of sentinels hi	ves	-0.3	0.007	<0.001
Incursion type	Single bee with 6 mites	0.0	-	-
	Swarm with 100 mites	-2.6	0.10	<0.001
	Swarm with 1000 mites	-5.6	0.10	<0.001
Apistan® resistance	0% (none)	0.0	-	-
	50%	0.3	0.09	<0.001
Hive density (ferals an	d hobbyist)	0.1	0.01	<0.001

A5.3.3 Hobbyist and feral hive involvement

Feral hives are invariably involved by the time of first sentinel hive detection, by virtue of the fact that the same methods of transmission apply. Under the reference sentinel hive setup, typically about 200 feral hives would be infested at the time of first sentinel detection (Figure A5.7).

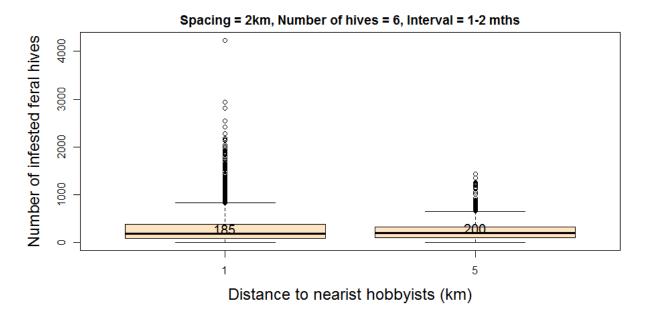


Figure A5.7. Median number of feral hives with *Varroa* infestation at the time of the first detection in sentinel hives in relation to the distance to the start of the suburbs (with associated urban beekeeping).

Similarly, a substantial number of hobbyist hives were also infested by the time *Varroa* infestation was first detected in sentinel hives, particularly when hobbyists were located close to port facilities (Figure A5.8). Typically, if hobbyist hives were distributed to within 1 km of the port, about 100 hives would be infested by the time *Varroa* was first detected in a sentinel hive (Figure A5.8). In the case of the nearest hobbyist hives being 5 km from the port, the number drops to around 20 (Figure A5.8).

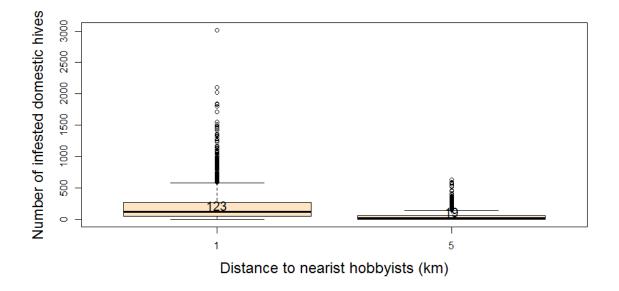


Figure A5.8 Median number of domestic hives with *Varroa* infestation at the time of the first detection in sentinel hives in relation to the distance to the start of the suburbs (with associated urban beekeeping).

A5.3.4 Inspection interval

Increasing the inspection interval from one to two months had little effect on the outbreak area at first detection, though doubling the inspection interval again to four months resulted in a jump in size (Figure A5.9). The current protocol is for 6-weekly inspections. With the model running on a monthly time step, this was not possible to simulate in the available time, so to examine the effectiveness of the current inspection protocol the results for monthly and 2-monthly inspections were pooled.

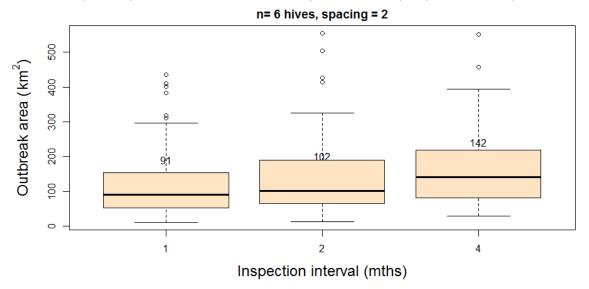


Figure A5.9 The effect of the interval between inspection on the median outbreak area (solid horizontal line) at first sentinel hive detection.

A5.3.5 Hive spacing

Ultimately, increasing the spacing between sentinel hives increases the incursion area at first detection (Figure A5.10), although across all numbers of sentinel hives examined, a 2 km spacing either nearly equalled or outperformed a 1 km spacing. This is due to the initial incursion being able to leapfrog over the array of sentinel hives when the inter-hive spacing was small. The fewer the sentinel hives, the greater the effect (Figure A5.10). Using a large number of sentinel hives (e.g. 12, Figure A5.10 A) removed the effect. Indeed, if the number of sentinel hives is limited, it appears a slightly wider spacing is preferable, with a hive spacing of 3 km being optimal if only 2 hives were deployed (Figure A5.10 D).

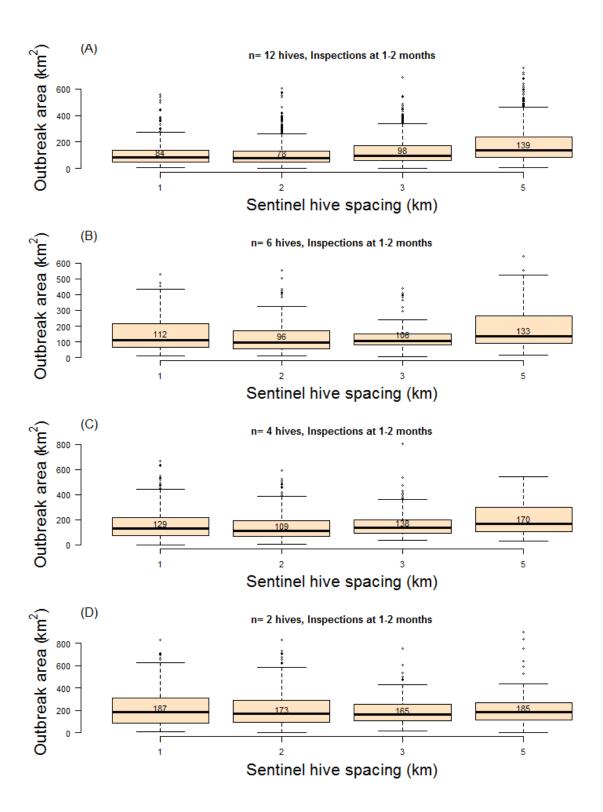


Figure A5.10. Effect of sentinel hive spacing on the median (solid horizontal line) outbreak size at first detection for (A) 12 hives, (B) 6 hives, (C) 4 hives, and (D) 2 hives. Numbers show median.

A5.3.6 Hive numbers

Increasing the number of sentinel hives decreases the outbreak size at first detection, particularly at reduced hive spacing (Figure A5.11). The best performing array considered consisted of 12 hives at a 2 km spacing, with a median incursion size at detection of 78 km² (Figure A5.11B). For a six hive array, again the best performance occurred at a spacing of 2 km (Figure A5.11B).

As spacing reaches 5 km, there appears to be no difference in the performance of 6 hives versus 12 hives (Figure A5.11D).

Although not a spacing *per se,* single sentinel hives were placed a 0.5 km, 1.5 km and 2.5 km from the port. Averaged over these distances the performance of a single sentinel hive (median = 184 km^2 , 95^{th} percentile 535 km²) was slightly worse than for 2 sentinel hives (median = 165 km^2 , 95^{th} percentile 498 km²).

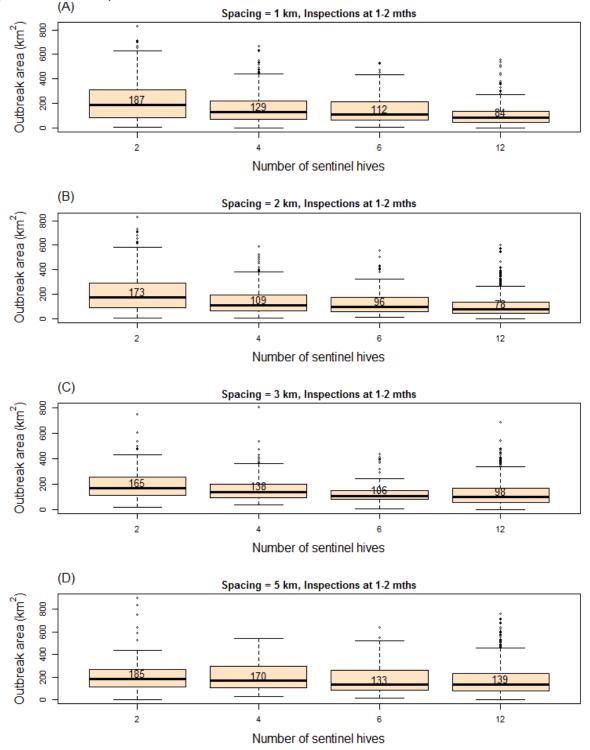


Figure A5.11 Effect of the number of sentinel hives on the median (solid horizontal line) outbreak size at first detection for (A). 1 km hive spacing, (B) 2km and (C) 3 km hive spacing.

A5.3.7 Incursion characteristic

Uncertainty in the nature of the *Varroa* introduction into Auckland manifests in the uncertainty in the expected outbreak size at first detection in sentinel hives. Incursions arising from a swarm with numerous mites are predicted to be detected much sooner, and somewhat counter-intuitively at a smaller outbreak size (Figure A5.12). This arises at least partly from the model parameterisation having a faster spread rate for a single bee incursion. Note this results from uncertainty as to the origins of the Auckland incursion to which the model was partially calibrated.

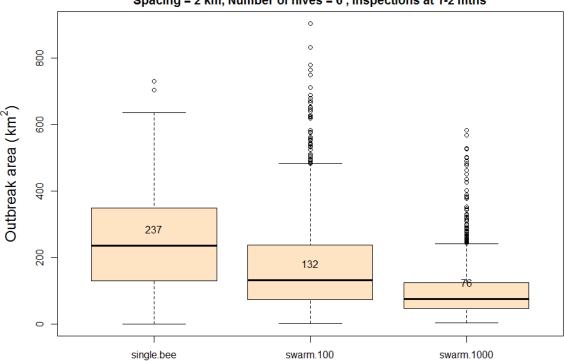




Figure A5.12 Effect of incursion characteristic on the median outbreak area at first detection.

A5.3.8 Apistan® resistance

Halving the expected mortality rate of phoretic mites due to Apistan® resistance increases the delay between infestation and first detection of *Varroa* in sentinel hives from 1.8 months (\pm 0.02 sem) to 2.2 months (\pm 0.05 sem), or about 6 days. Note this is an average across all sentinel hive numbers and hive spacings with 6-weekly inspections. This leads to an increase in the incursion size at first detection, though not by a large amount. For a 4 hive by 2 km layout inspected at approximately 6 weekly intervals, the mean incursion size increases from 109 km² to 128 km² (Figure A5.15). The underlying reason for this smaller than expected increase in the delay would be the exponential growth of mites within the hive, such that the number mites in sentinel hives soon reach levels that even a less sensitive test can detect.



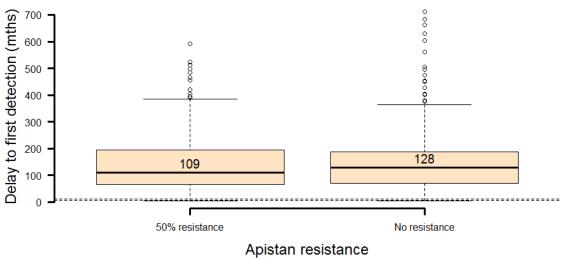


Figure A5.15 Effect of 50% resistance to Apistan® on the median incursion area (solid horizontal line) are first detection of *Varroa* in a sentinel hive.

A5.3.9 General

The median and 95th percentile areas and diameters at first sentinel detection for all combinations of sentinel hive numbers and spacing is provided in the Appendix (Table A5.6).

A5.3.10 Current bespoke arrangements

The Brisbane sentinel hive arrangement of three hives in each of two locations, has a median incursion size at detection of 121 km² (95% percentile 376 km²) compared to 95.7 km² (95% percentile 325 km²) for the reference 6 hives at 2 km layout. A single sentinel hive adjacent to the port, as is currently in place at Esperance, has a median time to detection of 9 months (95th percentile 16 months) at which time the median infestation size is estimated to be 185 km² (95th percentile 558 km²).

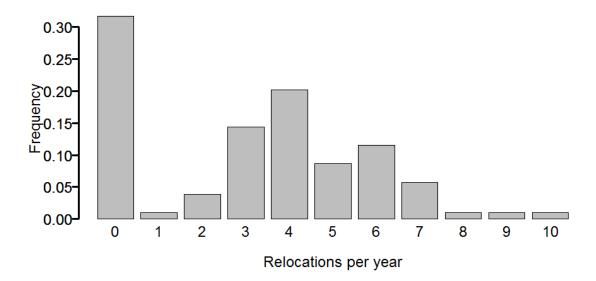
The performance of other sentinel hive arrangements can be estimated by cross-referencing the layout to the most representative sentinel hive arrangement results in Appendix (Table A5.6).

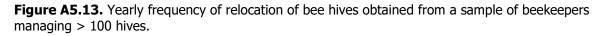
A5.3.11 Involvement of commercial beekeepers

The frequency of movement of hives maintained by commercial beekeepers was estimated using hive movement data collected during surveys of all beekeepers in Australian managing greater than 100 hives (see Gordon *et al.* 2014 for more details). As part of a much broader set of survey questions, beekeepers were asked "On average, how many times would you relocate your hives in a year?" and "Do you use a net when transporting bees?" A random sub-sample (n=263) of the beekeeper surveys were used to characterize the frequency of movement and propensity to net bees during moving.

A small percentage (16.5%) of beekeepers used nets when moving bees. The mean number of moves per year was 3.6, although this was skewed somewhat by an individual who reported moving hives 50 times per year. There is probably some ambiguity in the question as to the number of times an individual lot of hives is moved (the question of interest here) as opposed to the number of moves a beekeeper conducts (not necessarily on the same hives). After the removal of this extreme data

point, the highest number of moves per year was 10, with about 30 percent of beekeepers reporting no moves (Figure A5.13). The data are clearly bi-modal. For those beekeepers that did move hives, the mean frequency was 4.6 moves per year (0.38 moves mth⁻¹). This is equivalent to a hive of bees being moved every 2.6 months.





The exact implications of commercial beekeeper movement are difficult to quantify without explicitly incorporating such operations into the simulation model, with all the complexity of integrating over different numbers of hives, location in relation to the port of interest and timing of movements etc. However, some robust generalisations can be made. First, given the preponderance for a considerable number of feral and hobbyist hives to be infested at the time of first sentinel hive detection, it stands to reason that a commercial apiary would also be infested at this time. The key issue then relates to the timing of the move, if it occurs.

The worst case scenario is that the commercial hives have been present from the initial incursion, and are infested immediately upon *Varroa* entering. This being the case, there is still about a 30% chance the beekeeper involved is one of those that does not move his bees, in which case no export of *Varroa* has occurred. If not, the chance of the hives being shifted and exporting *Varroa* in the expected 6 months delay before sentinel detection (and hive movement controls) is about 90% based on the reported movement rate. The distances involved are likely in the order of 100s of kilometres (see Gordon *et al.* 2014), with the possibility of generating infestation foci en route due to the infrequent use of netting to prevent losing bees.

A5.4 Discussion & Conclusions

The results presented here incorporate considerably more realism than previous treatments of the problem, both in terms of model detail and the underlying data. That said, uncertainty remains in the parameterisation of the spread of *Varroa*, arising in part from irreducible uncertainty in the details of the New Zealand incursions used for model calibration. Our analysis has propagated this uncertainty through to the outputs.

The expected size of a *Varroa destructor* incursion at first sentinel hive detection for a typical surveillance setup up (6 hives spaced on a 2 km array and checked 6 weekly) is in the order of 100 km² following a delay of 6 months since introduction. This is equivalent to a circular infestation area of

diameter *c*. 11 km. By this stage, a considerable number of feral (100s) and hobbyist (10s to 100s) hives will also be infested, depending on how close suburbs are from the port of entry. Intuitively, the reason for the high involvement feral and/or hobbyist hives by the time of the first detection in sentinel hives is that all hives are infested by the same method. Furthermore, it is unlikely to have infestation in sentinel hives prior to infestation in feral hives, particularly when the latter are assumed much more numerous. This assumption (regarding density of feral hives around ports) needs checking.

Increasing the number of sentinel hives to 12, and using the best performing array spacing of 2 km still resulted in a median incursion size at sentinel detection of c. 80 km².

It is not the intention of this technical report to canvas effective eradication options, however it is suffice to say that remote area poisoning methods will be required (see Taylor 2003).

Some conclusions:

- All else being equal, a spacing of 2 km between sentinel hives seems optimal.
- Surveillance performance starts to deteriorate noticeably once the interval between hive inspections exceeds two months.
- If the number of sentinel hives are limited to 4 or less, then a wider spacing is more effective than smaller.
- Fewer hives at more locations is better than multiple hives at fewer locations.
- Moderate levels of Apistan® resistance (e.g. a 50% reduction in toxin-induced mortality) will have a small to moderate impact on surveillance sensitivity and hence the size of outbreak at first detection.
- At the time of first detection of *Varroa* in sentinel hives, the number of infested feral hives will number in the 100s. Depending on how close domestic beekeeping occurs to the port environment, the number of infested domestic hives ranges from 10's to 100s.
- To successfully eradicate such a number of infested feral hives will undoubtedly require the use of toxins (e.g. Fipronil) in combination with effective management of hobbyist hives. Effective move movement control would be critical.
- The number of feral and/or hobbyist hives has a major impact on detection statistics. The greater the number of hives, the faster the rate of spread, the later the first detection in sentinel hives, and the larger the infestation area at first sentinel hive detection. Consideration of suppression of feral colonies around ports may be warranted, along with inspection regimes for hobbyist hives close to ports.
- If present within *c*. 20 km of the port environs, commercial beekeeping operations could potential result in the establishment of additional infestation foci through hive movements.

A5.5 References

- Barry, S., Cook, D., Duthie, R., Clifford, D., and Anderson, D. (2010). Future surveillance needs for honeybee biosecurity. RIRDC Publication No 10/107. (Rural Industries Research and Development Corporation: Canberra).
- Clifford, D., Barry, S., Cook, D., Duthie, R., and Anderson, D. (2011). Using simulation to evaluate time to detect incursions in honeybee biosecurity in Australia. *Risk Analysis* 31, 1961-1968. doi: 10.1111/j.1539-6924.2011.01607.x
- Gordon, R., Bresolin-Schott, N., and East, I. J. (2014). Nomadic beekeeper movments create the potential for widespread disease in the honeybee industry. *Australian Veterinary Journal* 92, 283-290.

- Harris, J. W., Harbo, J. R., Villa, J. D., and Danka, R. G. (2003). Variable population growth of *Varroa destructor* (Mesostigmata: Varroidae) in colonies of honey bees (Hymenoptera: Apidae) during a 10-year period. *Environmental entomology* 32, 1305-1312.
- Heersink, D. K., Caley, P., Paini, D. R., and Barry, S. C. (2016). Quantifying the establishment likelihood of invasive alien species introductions through ports with application to honeybees in Australia. *Risk Analysis* 36, 892-903. doi: 10.1111/risa.12476

Lindauer, M. (1955). Schwarmbienen auf Wohnungssuche. Z. vergl. Physiol 37, 263-324.

R Development Core Team (2014). R: A language and environment for statistical computing. <u>http://www.R-project.org</u>

Seeley, T. D., and Morse, R. A. (1977). Dispersal behavior of honey bee swarms. *Psyche* 84, 199-209.

- Stevenson, M. A., Benard, H., Bolger, P., and Morris, R. S. (2005). Spatial epidemiology of the Asian honey bee mite (Varroadestructor) in the North Island of New Zealand. *Preventive Veterinary Medicine* 71, 241-252.
- Taylor, M. A. (2003). Field testing a proposed method for destroying feral bee colonies. Client Report 10: 2003/9723. (HortResearch: Auckland).

A5.6 Appendix

Table A5.5. Median and 95th percentile areas (km²) and diameters (km) of *Varroa* outbreaks at first detection in sentinel hives as a function of the number of sentinel hives and array spacing. Data are summarised over inspections at either 4 weeks or 8 weeks. There is assumed to be no Apistan® resistance.

				Nur	nber of s	entine	l hives				
		1	hive*	2	2 hives 4 hives		6	hives		12 hives	
Array spacing		50 th	95 th								
1 km	Area	194*	(567)	187	(552)	129	(411)	112	(414)	84	(298)
	Diameter	15.7	(26.9)	15.4	(26.5)	12.8	(22.9)	12.0	(23.0)	10.3	(19.5)
2 km	Area	174	(534)	173	(537)	109	(370)	96	(325)	78	(306)
	Diameter	14.9	(26.1)	14.8	(26.1)	11.8	(21.7)	11.0	(20.3)	10.0	(19.7)
3 km	Area			165	(476)	138	(404)	107	(370)	98	(356)
	Diameter			14.5	(24.6)	13.3	(22.7)	11.6	(21.7)	11.2	(21.3)
5 km	Area			185	(545)	170	(416)	133	(426)	139	(441)
	Diameter			15.4	(26.4)	14.7	(23.0)	13.0	(23.3)	13.2	(23.8)

* For individual hives, the array spacing refers to distance from port centre (c.f. spacing)

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Appendix 6: Strategies for Surveillance Design for NBPSP based on the *Varroa* **Spread Model**

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A6.1 Introduction

This document provides some further analysis of the output of the agent based simulation model that was designed to estimate the impact of a *Varroa* incursion into Australia.

The document comprises three main parts. This section (Introduction) and the next section (Characteristics of the Model) are introductory.

Section 3 provides some further summary statistics of the model output, with particular focus on the predicted hazard associated with different designs. Details of the hazards and designs considered in the model are described in Section 2. Section 3 also provides a comparative assessment of the relative merits of the different designs, based on the obtained summary statistics.

Section 4 provides a decision-tree analysis of the model outputs in order to identify nonlinear relationships between the input factors and predict the resultant hazards.

Section 5 provides some commentary on ways in which the analyses could be used to develop a national perspective, noting that the existing model is based on sentinel hives at a single port. Options for reducing the predicted hazard through the use of other surveillance components and other entry points are also discussed. These are not pursued in this report due to a lack of information.

The report ends with a short conclusion.

A6.2 Characteristics of the Model

The agent-based *Varroa* Spread Model developed in this project was developed to estimate the potential hazard of an arrival of *Varroa* at a port, based on a set of external assumptions, geographic features of the port, and design components.

The hazard is described in terms of a set of measures, each of which is represented as a probability distribution. The median and 95% percentile of the distribution is used to predict the respective hazard.

Predicted hazard:

- Time to first detection (months)
- Outbreak area (km²)
- Number of infested hives

The input variables to the VIM can be grouped as follows:

External assumptions:

- 1. Apistan[®] resistance: 0, 50% (i.e., Pr(kill mite) = 0.70, 0.35, respectively)
- 2. Incursion type: single bee with 6 mites, swarm with 100 mites, swarm with 1000 mites

Geographic features:

- 3. Distance to hobbyists, to the nearest suburb: 1km, 5km
- 4. Carrying capacity, i.e. density of feral and domestic hives: 10-20km²

Design components:

- 5. Number of sentinel hives: 1, 2, 4 6, 9, 12
- 6. Spacing, i.e., distance between hives: 1km, 2km, 3km, 5km
- 7. Inspection interval, i.e., time between hive inspections: 1, 2, 4 months.

A6.3 Further summary analysis of simulations

The following analyses are based on summary statistics obtained from the agent based simulation model outputs. The aim is to provide further information in the form of plots and tables could be used to identify possible sentinel array designs that would meet specified thresholds, e.g. no more than a specified median number of months to first detection in a sentinel hive or no more than a specified median area of infection at the time of first detection in a sentinel hive.

Note that the same analyses could be undertaken using the 95th percentile if this were of interest.

A6.3.1 Analyses of sentinel array designs

The following two plots show the median number of months to detection and the median area infected, for the 23 sentinel array designs (no. hives and spacing) considered in the simulation model (e.g. 12at1 means 12 hives at 1km spacing, etc.). Note that in this first part, the simulation outputs are combined for the three inspection intervals considered (1, 2, 4 months). (Hence 12at1 designs with 1 month, 2 months and 4 month inspections are combined, etc.). Figures and plots are given later for sentinel array separated by inspection interval.

Examples

What array designs result in a median 3 months or less to first detection in a sentinel hive?

Based on the plots and tables below, the corresponding designs are 12at1, 12at2 and 4at2, that is:

- 12 hives at 1km and 2km spacing
- 4 hives at 2km spacing

What array designs result in a median 150km² or less area infected at first detection in a sentinel hive?

It can be seen that the designs 12at1, 12at2, 12at3, 4at1, 4at2, 4at3, 6at1, 6at2, 6at3, 6at5, 9at1, 9at2, 9at3 satisfy this criterion, that is:

- 12 hives at 1, 2 or 3km spacing
- 4 hives at 1, 2 or 3km spacing
- 6 hives at 1, 2 3 or 5km spacing
- 9 hives at 1, 2 or 3 km spacing

The first figure shows the median number of months to detection for each of the sentinel array designs. For space reasons, the x label is given as numbers 1-23, which correspond to the sentinel array designs listed in the following table. The second figure shows the area infected at first detection for the same designs. The tables that appear after the figures give the actual numbers corresponding to the plots.

Hence, for instance, for the design 12at1 (12 sentinel hives at 1km spacing), the median number of months to first detection in a sentinel hive is 3 months, and the median area infected at this time is 93km².

The sentinel array design IDs are as follows:

ID	1-4	5-9	10-14	15-19	20-23
Design array	12at1 12at2	1at0 1at1	2at3 2at5	4at5 6at1	9at1 9at2
(no. hives at	12at3 12at5	1at2 2at1	4at1 4at2	6at2 6at3	9at3 9at5
spacing)		2at2	4at3	6at5	

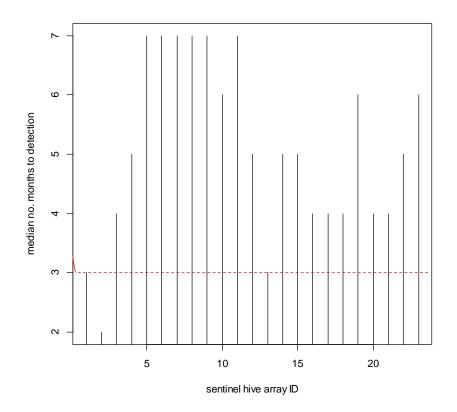


Figure A6.1 Median number of months to first detection for each sentinel hive array design (see ID labels above). Red dotted line is an example of a criterion of max. 3 months: vertical lines not exceeding this threshold indicate possible designs that meet this criterion (i.e. no's 1, 2, 13 which equate to designs 12at1, 12at2, 4at2; see text).

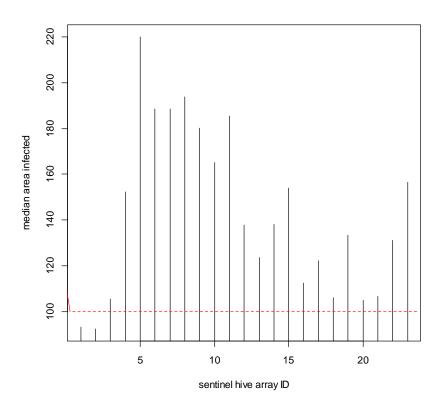


Figure A6.2 Median area infected at first detection, for each sentinel hive array design (see ID labels above). Red dotted line is an example of a criterion of max. 100 km²: vertical lines not exceeding this threshold indicate possible designs that meet this criterion; see text.

The following tables give the actual values corresponding to the vertical lines in the above figures.

Note: medians can be rounded so small differences (e.g. ±1 month in median time to detection) may not be substantive.

No. months to first detection by sentinel array

12at1	12at2	12at3	12at5	1at0	1at1	1at2	2at1	2at2	2at3	2at5	4at1	4at2	4at3	4at5	6at1	6at2	6at3	6at5	9at1	9at2	9at3	9at5
3	2	4	5	7	7	7	7	7	6	7	5	3	5	5	4	4	4	6	4	4	5	6

Size of area at first detection by sentinel array

12at1	12at2	12at3	12at5	1at0	1at1	1at2	2at1	2at2	2at3	2at5	4at1	4at2	4at3	4at5	6at1	6at2	6at3	6at5	9at1	9at2	9at3	9at5
93	92	106	152	220	189	189	194	180	165	185	138	124	138	154	112	122	106	133	105	107	131	156

No. months to first detection, by inspection interval

Interval	1month	2months	4months
Median months	4	5	4

Size of area of infection (km²) by inspection interval

Interval	1month	2months	4months
Median area	110	132	155

A6.3.2 Analyses of Sentinel array and Inspection interval

The following analyses expand on the preceding analyses by taking into account the inspection interval. The x axis represents the 23 sentinel array combinations, for inspection interval = 1 month, then for 2 months, for 3 months.

If a certain threshold is required (e.g., median area < 100km²), the table below the plot can be inspected, or a horizontal line can be drawn on the plot, and the combinations that meet this threshold could be identified.

Examples

What designs would provide a median 3 months or less to first detection in a sentinel hive?

Based on the plot and table below, the designs that meet this criterion are:

12at1.1	12 hives at 1 km spacing, inspected at 1 month.
12at2.1, 12at3.1	12 hives at spacing of 2 or 3 km, inspected at 1 month
12at1.2, 12at2.2	12 hives at spacing of 1 or 2 km, inspected at 2 months
12at1.4, 12at2.4, 12at3.4	12 hives at spacing of 1, 2 or 3 km, inspected at 4
	months
4at2.1, 4at2.4	4 hives at spacing of 2 km, inspected at 1 or 4 months.

What designs would provide a median 100km² or less area infected at first detection in a sentinel hive?

Based on the plot and table below, the designs that meet this criterion are:

12at1.1, 12at2.1., 12at3.1	12 hives at 1, 2 or 3 km spacing, inspected at 1 month
4at2.1, 6at2.1	4 or 6 hives at 2 km spacing, inspected at 1 month
9at1.1, 9at2.1	9 hives at 1 or 2 km spacing, inspected at 1 month
12at1.2, 12at2.2	12 hives at 1 or 2 km spacing, inspected at 2 months

The first figure below shows the median number of months to detection for each of the combinations of sentinel arrays and inspection intervals. For space reasons, the x label is given as numbers 1-23 for inspection interval of 1 month, then 24-46 for inspection interval of 2 months, then 47-69 for inspection interval of 4 months, where the 23 numbers in each group correspond to the sentinel array designs, as listed in the following table. The second figure shows the area infected at first detection for the same designs. The tables that appear after the figures give the actual numbers corresponding to the plots.

Hence, for instance, for the design 12at1.1 (12 sentinel hives at 1km spacing, inspected at 1 monthly intervals), the median number of months to first detection in a sentinel hive is 3 months, and the median area infected at this time is 82km².

ID	Insp. Int=1	1-4	5-9	10-14	15-19	20-23
ID	Insp. Int=2	24-27	28-32	33-37	38=42	43-46
ID	Insp. Int=4	47-50	51-55	56-60	61-65	66-69
Design array		12at1 12at2	1at0 1at1	2at3 2at5	4at5 6at1	9at1 9at2
(no. hives at		12at3 12at5	1at2 2at1	4at1 4at2	6at2 6at3	9at3 9at5
spacing)			2at2	4at3	6at5	

The sentinel array design IDs are as follows:

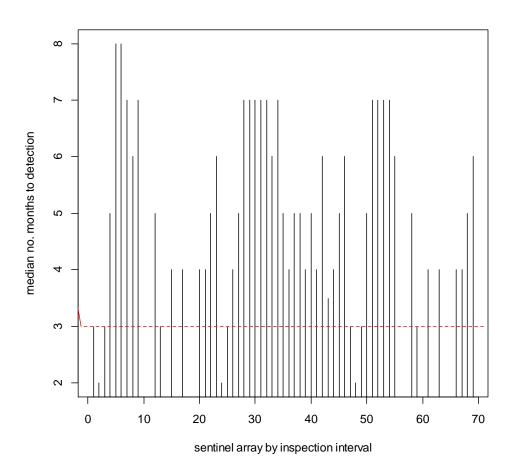


Figure A6.3 Median number of months to first detection for each sentinel hive array and inspection interval design (see ID labels above). Red dotted line is an example of a criterion of max. 3 months: vertical lines not exceeding this threshold indicate possible designs that meet this criterion (see text).

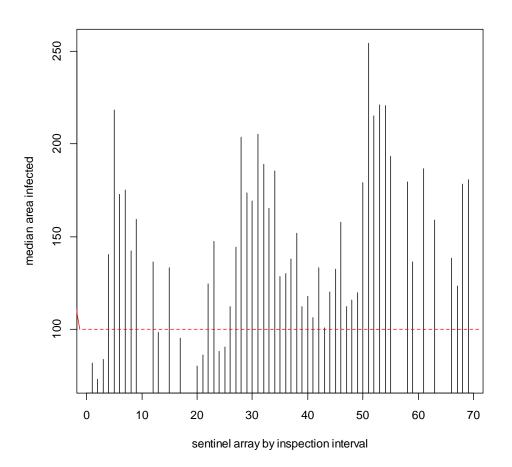


Figure A6.4 Median area infected at first detection, for each sentinel hive array and inspection interval design (see ID labels above). Red dotted line is an example of a criterion of max. 100 km²: vertical lines not exceeding this threshold indicate possible designs that meet this criterion; see text.

The following tables give the actual values corresponding to the vertical lines in the above figures.

Note: medians can be rounded so small differences (e.g. ±1 month in median time to detection) may not be substantive.

Median no. months to first detection:

Note: medians can be rounded so a difference of 1 month not be substantive.

1-month inspection:

12	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
at1	at2	at3	at5	t0	t1	t2	t1	t2	t3	t5												
3	2	3	5	8	8	7	6	7	Ν	na	5	3	na	4	na	4	na	na	4	4	5	6
									а													

2-month inspection:

12	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
at1	at2	at3	at5	t0	t1	t2	t1	t2	t3	t5	t1	t2	t3	t5	t1	t2	t3	t5	t1	t2	t3	t5
2	3	4	5	7	7	7	7	7	6	7	5	4	5	5	4	5	4	6	3. 5	4	5	6

4-month inspection:

12	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
	12	12	12	10	-	10		24				-	-	-	ou		ou	ou	20			
at1	at2	at3	at5	t0	t1	t2	t1	t2	t3	t5	t1	t2	t3	t5	t1	t2	t3	t5	t1	t2	t3	t5
3	2	З	5	7	7	7	7	6	na	na	5	З	na	4	na	4	na	na	4	4	5	6
5	2	,	5	'	,	,	'	0	пü	ų	,	,	iu		liu	•	nu	Ĩ	•		,	0

Median area of infection:

1-month inspection:

12	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
at1	at2	at3	at5	t0	t1	t2	t1	t2	t3	t5												
82	73	84	14	21	17	17	14	15	Ν	Ν	13	99	Ν	13	Ν	95	Ν	Ν	80	86	12	14
			0	8	3	5	2	9	а	а	6		а	3	а		а	а			5	8

2-month inspection:

12	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
at1	at2	at3	at5	t0	t1	t2	t1	t2	t3	t5												
88	90	11	14	20	17	17	20	18	16	18	12	13	13	15	11	11	10	13	10	12	13	15
		2	4	4	3	0	5	9	5	5	9	0	8	2	2	8	6	3	1	0	2	8

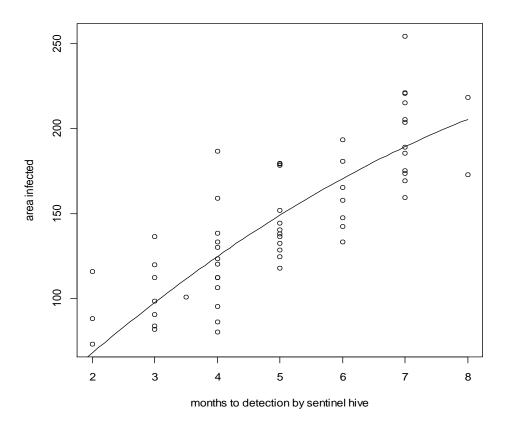
4-month inspection:

12	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
at1	at2	at3	at5	t0	t1	t2	t1	t2	t3	t5												
15	11	12	17	25	21	22	22	19	Ν	Ν	18	13	Ν	18	Ν	15	Ν	Ν	13	12	17	18
2	6	0	9	4	5	1	1	3	а	а	0	6	а	7	а	9	а	а	9	4	8	1

A6.3.3 Relationship between median no. months to detection and median area of infection

This is fairly linear as indicated in the report, but there is some curvature.

Let x = median months to detection: Estimated median area of infection = $36.6559 * x - 1.3729*x^2$



A6.3.4 Cost versus Hazard

The following plots indicate how the trade-off between cost (in terms of number of sentinel hives) can be weighed against hazard (here, median number of months to first detection in a sentinel hive and median size of infection at first detection in a sentinel hive). The plot, like those above, can be evaluated to determine sentinel arrays that satisfy an acceptable cost/hazard threshold.

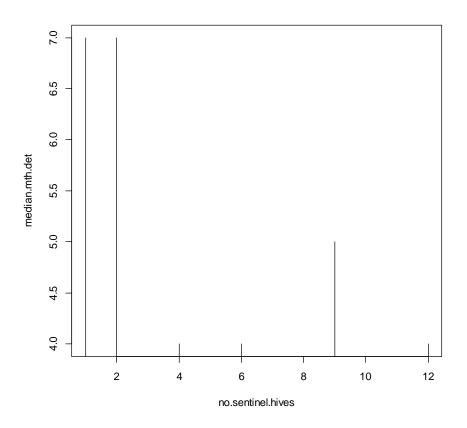


Figure A6.5 Median number of months to first detection by a sentinel hive (*hazard*) associated with the number of sentinel hives (*cost*).

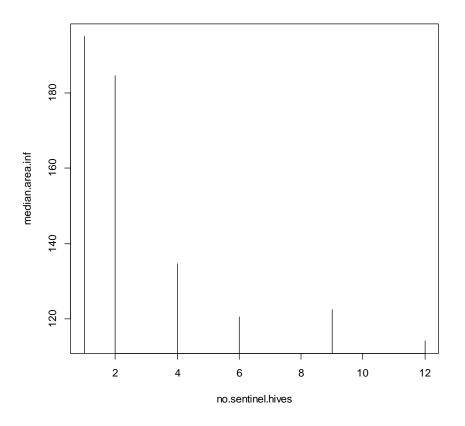


Figure A6.7 Median area infected at time of first detection by a sentinel hive (*hazard*) associated with the number of sentinel hives (*cost*).

Overall, it looks like at least 3, and preferably 4 or 6, sentinel hives are recommended, with respect to median months to detection and median area of infection at first detection in a sentinel hive. The number depends on the acceptable level of hazard (median months to detection, median area of infection).

A6.3.5 Probability of meeting thresholds

The following probabilities are based on the simulated outputs, by determining the number of simulations that meet a specified threshold.

Probabilities exceeding an arbitrary value of 70% chance of meeting the stated criterion are bolded.

Example: If sentinel hives alone are used, what designs will give us at least 70% chance of no more than 150km² (median) of infected area before detection by a sentinel hive?

From the tables below, averaging over the inspection interval, the preferred plans that meet this criterion are:

• 12 hives at 1 or 2km spacing; 6 hives at 3km spacing; 9 hives at 1km spacing.

Taking inspection interval into account, the sentinel array and inspection interval setups that meet the criterion are as follows:

• 1-month inspection: 12at1, 12at2, 12at3, 6at1, 9at1, 9at2, 9at3

12 hives at 1km, 2km or 3km spacing; 6 hives at 1km spacing; 9 hives at 1km, 2km or 3km spacing

• 2-month inspection: 12at1, 12at2, 6at3, 9at1

12 hives at 1 or 2km spacing; 6 hives at 3km spacing; 9 hives at 1km spacing

• 4-month inspection: none

The following tables show the chance of meeting the stated criterion. Note that the 'chance' is displayed as a percentage, which is the probability multiplied by 100 (e.g. 70 is 70% chance, i.e. probability 0.70).

Chance a sentinel array design meets the following criterion: area of infection < 100km², ignoring inspection interval

12a	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
t1	at2	at3	at5	t0	t1	t2	t1	t2	t3	t5												
.54	.54	.47	.26	22	22	23	25	27	22	18	37	40	31	27	40	40	45	32	47	48	34	25

Chance a sentinel array design meets the following criterion: area of infection < 150km², ignoring inspection interval

12 at	12 at	12 at	12 at	1a t0	1a t1	1a t2	2a t1	2a t2	2a t3	2a t5	4a t1	4a t2	4a t3	4a t5	6a t1	6a t2	6a t3	6a t5	9a t1	9a t2	9a t3	9a t5
1	2	3	5																			
74	73	66	49	υ n	о С	4	3 0	4 0	4 r	3	5	5	5 -	4 0	6	5 0	74	5 (70	6 L	5 0	4
				3	ð	0	9	2	2	5	4	9	5	9	1	9		6		5	9	/

Chance of meeting the following criterion: area of infection < 150km², taking the inspection interval into account

1 month:

12	12	12	12	1	1	1	2	2	2	2	4	4	4	4	6	6a	6	6	9a	9a	9a	9
at	t2	at	at	t1	t2	t3	at															
1	2	3	5	0	1	2	1	2	3	5	1	2	3	5	1		3	5				5
80	82	74	54	3	4	4	5	4	Ν	Ν	5	6	Ν	6	Ν	7	Ν	Ν	7	7	7	5
				6	0	6	2	7	а	а	4	8	а	1	а	1	а	а	9	6	1	3

2 months:

12	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
at 1	at 2	at 3	at 5	t0	t1	t2	t1	t2	t3	t5	t1	t2	t3	t5	t1	t2	t3	t5	t1	t2	t3	t5
77	74	64	52	3 2	4 2	4 5	3 7	3 8	4 2	3 5	5 7	5 9	5 5	5 0	6 1	6 1	74	5 6	77	6 1	6 1	4 9

4 months:

12	12	12	12	1a	1a	1a	2a	2a	2a	2a	4a	4a	4a	4a	6a	6a	6a	6a	9a	9a	9a	9a
at1	at2	at3	at5	t0	t1	t2	t1	t2	t3	t5												
66	62	61	40	29	33	28	30	40	Ν	Ν	43	53	na	35	na	45	na	Ν	55	57	42	40
									а	а								а				

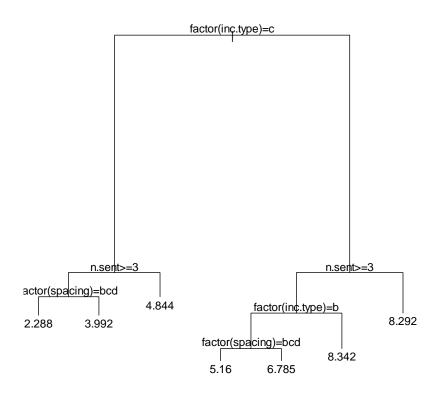
A6.4 Decision tree analyses to identify important factors

A6.4.1 Full set analysis

The results of the *Varroa* Spread Model were analysed to reveal the more influential factors in predicting the hazard. A regression tree model was used for this analysis, via the RPART package in R. The major factors were also confirmed using a boosted regression analysis, via the gbm package in R.

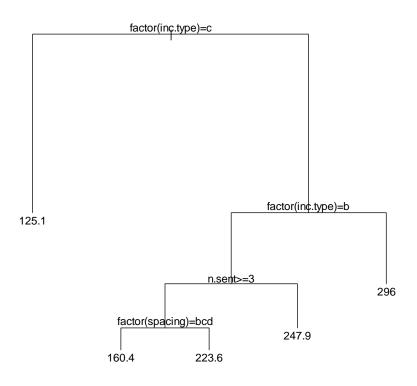
Time to First Detection in a Sentinel Hive:

- The major influential factor on time to detection is the incursion type: substantially less time to detection if it is (c) a swarm with 1000 mites, compared with (a) a swarm with 100 mites or (b) single bee with 6 mites.
- If the incursion type is (c), the next most important factor is the number of sentinel hives. The shortest time to detection is for at least 3 sentinel hives and spacing of at least 2 km.
- If the incursion type is (a) or (b), the next most important factor is again the number of sentinel hives. The shortest time to detection is for a swarm of 100 mites and spacing of at least 2 km.
- If the aim is to minimize the incursion time, then based on the *Varroa* Spread Model the best design is at least 3 sentinel hives and spacing of at least 2 km.
- With 1 or 2 sentinel hives, the expected time to detection is 4.84months for an incursion of a swarm of 1000 mites (c), and 8.3months for a swarm of 100 mites (a) or a single bee with 6 mites (b).
- With 4 or more sentinel hives and a spacing of at least 2 km, the expected time to detection is up to 4 months for incursion type (c), up to 6.8 months for (b) and 8.3 months for (a).



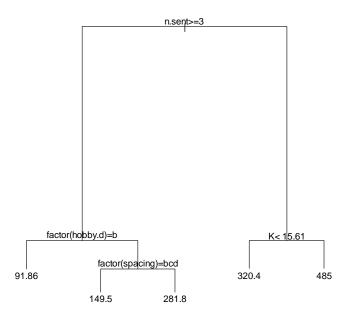
Area of infestation at time of first detection in a sentinel hive:

- The major influential factor on area of infestation is the incursion type: substantially less time to detection if it is (c) a swarm with 1000 mites, compared with (a) a swarm with 100 mites or (b) single bee with 6 mites.
- The smallest area is for incursion type (c), with an expected area of 125.1 km².
- If the incursion type is (b), the smallest area occurs with at least 3 sentinel hives and at least 2km spacing between them. The expected area under this configuration is 160.4, compared with 248 for less than 3 sentinel hives.
- If the incursion type is (a), the expected area is 298 km².
- If the aim is to minimize the area of infestation at first detection by a sentinel hive, then based on the *Varroa* Spread Model the best design is at least 3 sentinel hives and spacing of at least 2 km.
- With 1 or 2 sentinel hives, the expected time to detection is 4.84 months for an incursion of a swarm of 1000 mites (c), and 8.3months for a swarm of 100 mites (a) or a single bee with 6 mites (b).
- With 4 or more sentinel hives and a spacing of at least 2 km, the expected time to detection is up to 4 months for incursion type (c), up to 6.8 months for (b) and 8.3 months for (a).
- If the aim is to minimize the number of domestic hives infested, then based on the *Varroa* Spread Model the best design is at least 3 sentinel hives with hobby farmers at least 5km apart.
- With 1 or 2 sentinel hives, the number of domestic hives infested depends on the carrying capacity K.



Number of domestic hives infested at first detection in a sentinel hive:

- The major influential factor on number of domestic hives infested at the time of first detection of *Varroa* in a sentinel hive is the number of sentinel hives.
- If the number of sentinel hives is three or more, the next most important factor is the distance between hobby farmers. The least number of domestic hives infested is found with a distance of 5km (as opposed to 1km).
- If the number of sentinel hives is 1 or 2, the next most important variable factor is the carrying capacity (K). The largest number of hives infested is predicted for less than 3 sentinel hives and a carrying capacity of K>15.6.



A6.5 Important Factors: Design Components

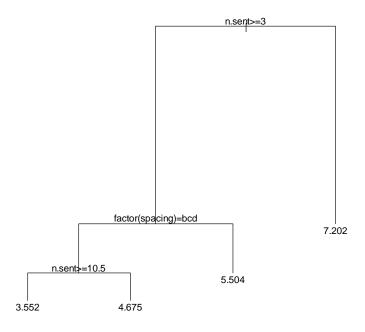
The above results are based on a model that includes all possible factors as described in Section 1 of this report. These include external and geographic factors that are not under the control of the biosecurity design.

The regression tree analyses were undertaken with just the design characteristics: number of sentinel hives, spacing and inspection interval.

As above, the analyses were performed using the RPART package in R.

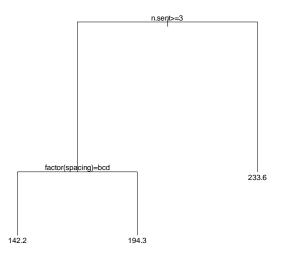
Time to First Detection in a Sentinel Hive:

- The major influential factor on time to detection is the number of sentinel hives.
- If the number is less than 4, the expected time to detection is 7.2 months.
- With 4 or more sentinel hives, the next most influential factor is spacing: if the spacing is 2 km or more, the expected time to detection is 5.5 months. If it is less than 2km, then if there are more than 10 sentinel hives, the expected time to detection is 3.6 months; otherwise if there are 2-10 sentinel hives then 4.7 months.
- If the aim is to minimize the spread time, then based on the *Varroa* Spread Model the best design is more than 10 sentinel hives with a hive spacing of 2 or more km.



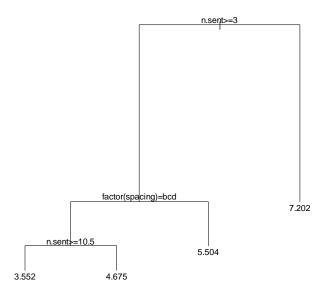
Area of infestation at time of first detection in a sentinel hive:

- The major influential factor on area of infestation is the number of sentinel hives.
- The smallest expected area is 142, which is obtained with at least 3 sentinel hives and a spacing of at least 2km.
- The largest expected area is 233, which is obtained with less than 3 sentinel hives.
- If the aim is to minimize the area of infestation at first detection by a sentinel hive, then based on the *Varroa* Spread Model the best design is at least 3 sentinel hives and spacing of at least 2km.



Number of domestic hives infested at first detection in a sentinel hive:

- The major influential factor on number of domestic hives infested at the time of first detection of *Varroa* in a sentinel hive is the number of sentinel hives.
- The smallest expected number of infested hives is 4 (rounded), which is found for at least 10 hives with a spacing of at least 2km.
- The largest expected number of infested hives is 7, which is obtained with 1 or 2 sentinel hives.
- If the aim is to minimize the number of domestic hives infested, then based on the *Varroa* Spread Model the best design is at least 10 sentinel hives spaced at least 2km apart.



A6.5.1 Important Factors – Targeted Outcome

We focus here on designing to achieve a specific expected hazard. Here, the hazard is measured by the area of infestation.

Three levels of hazard are considered:

- Predicted area of infestation is 100km²
- Predicted area of infestation is 150km²
- Predicted area of infestation is 200km²

For a given level of hazard (i.e. specification of area), the predicted area of infestation from each simulation of the *Varroa* Spread Model is coded as follows:

1 = area is less than specification

0 = area exceeds specification.

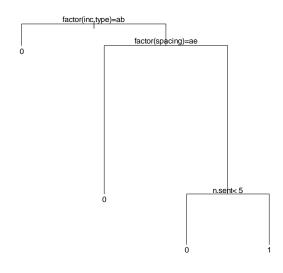
Following the above procedure, a decision tree (CART) model used for this analysis, using two sets of inputs:

- Full set: external factors, geographic characteristics, design options
- Reduced set: design options only

Area of infestation: 100km²

<u>Full set</u>

The decision tree for this level of hazard is given by

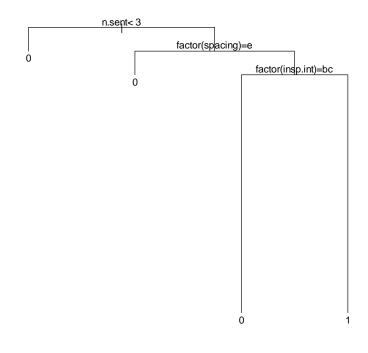


Conclusions:

- To meet this specification with reasonable probability, it has to be assumed that a large incursion occurs (swarm with 1000 mites) and that there are 5 or more sentinel hives with 'medium' spacing (2 or 3 km). Under this design, there is a 74% chance of meeting the specification.
- If the incursion is smaller (single bee or swarm with 100 mites), there is only a 26% chance of meeting the specification.
- If the incursion is large, but less than 5 sentinel hives are used, there is only a 43% chance of meeting the specification.

Reduced set

If only surveillance options are included in the analysis (i.e. averaging over all external factors and geographic characteristics, the following tree is obtained:



Conclusions:

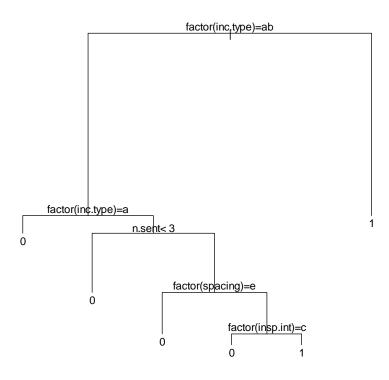
The following design is required to obtain more than 50% chance of meeting the specification. Note, however, that some of the probabilities of meeting the required specification are only marginally greater than 50%, so this design is not very satisfactory.

- at least 3 sentinel hives
- spacing (distance between hives) less than 5km
- inspection interval 1 or 2 months.

Area of infestation: 150km²

Full set

The decision tree for this level of hazard is given by

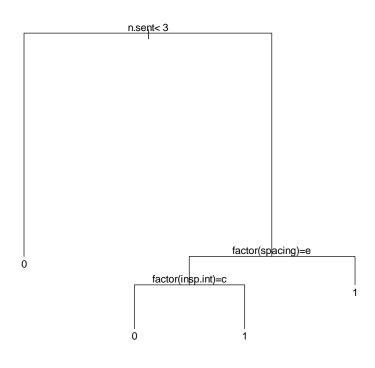


Conclusions:

- If the incursion type is large (swarm with 1000 mites), there is a 72% chance that it will be detected within 150km² even with one (or more) sentinel hives.
- If the incursion type is small (single bee), there is only a 20% chance of meeting the specification even with the maximum number of 12 hives.
- If the incursion type is a swarm with 100 mites, only the following design gives more than 50% chance of meeting specifications: 4 more sentinel hives with spacing of less than 5 km, and an inspection interval of 1 or 2 months. Under this design, there is a 63% chance of meeting specification.

Reduced set

If only surveillance options are included in the analysis (i.e. averaging over all external factors and geographic characteristics, the following tree is obtained:



Conclusions:

The following design gives approximately 60% chance of meeting specifications.

 at least 3 sentinel hives, spacing (distance between hives) of 5 km and inspection interval 1 or 2 months

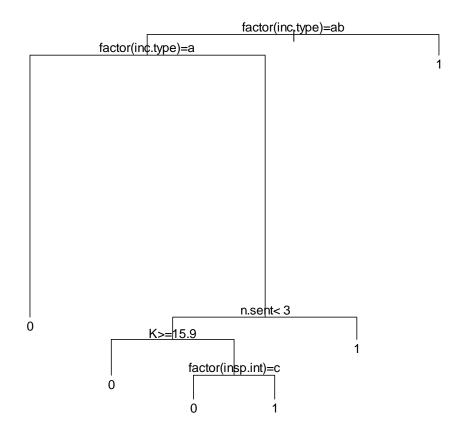
or

• at least 3 sentinel hives, spacing (distance between hives) of less than 5 km

Area of infestation: 200km²

Full set

The decision tree for this level of hazard is given by



Conclusions:

- If the incursion type is large (swarm with 1000 mites), there is a 69% chance that it will be detected within 200km² even with one (or more) sentinel hives.
- If the incursion type is small (single bee), there is only a 32% chance of meeting the specification even with the maximum number of 12 hives.
- If the incursion type is a swarm with 100 mites, only the following two designs give more than 50% chance of meeting specifications:
 - 4 more sentinel hives (68% chance of meeting specification)
 - 1-2 sentinel hives with high carrying capacity (K>15.9) and an inspection regime of 1 or 2 months (57% chance of meeting specification).

A6.5.2 Important Factors – Targeted Outcome, with sentinel array

The following analyses are repeats of the analyses undertaken in section 3B, with number of sentinel hives replaced by the actual sentinel array.

Area of infestation: 100km²

The following set of designs give a 60% chance of meeting this specification:

• Design array: 12at1,12at2,12at3, 4at2, 6at1,6at2,6at3, 9at1,9at2 *and* Inspection interval of 1 month.

Area of infestation: 150km²

The following designs gives an average 69% chance of meeting this specification:

• Sentinel array: 12at1, 12at2, 12at3, 4at2, 6at1, 6at3, 6at5, 9at1, 9at2, 9at3 *and* Inspection interval of 2 or 4 months

A smaller set of designs and a more stringent inspection interval gives an average 76% chance of meeting specification:

• Sentinel array: 12at1, 12at2, 6at3 or 9at1 and Inspection interval of 1 month

Area of infestation: 200km²

The following array designs give an average 79% chance of meeting this specification:

• Sentinel array: 12at1,12at2,12at3,4at2,4at3,6at1,6at2,6at3,9at1,9at2,9at3

Note: this supports the general conclusions made in the report about using an array of 6 hives with 2km spacing.

A6.6 Future Work: National Analyses

A6.6.1 Aggregation of Individual Analyses

The *Varroa* Spread Model can be used to develop a surveillance plan for incursion of *Varroa* at a port. The design of the plan depends on whether the pivot, or focal point of specification is cost of surveillance, cost of eradication, or both.

- The cost of surveillance is determined by the design components listed above: the number of sentinel hives, the distance between hives and the inspection interval. The cost can be focused on one or more of these components, and can be measured in monetary or other terms (e.g., there could be other strategic considerations in choosing the number of sentinel hives at certain locations).
- The cost of eradication is determined by the predicted hazard listed above: the time to first outbreak, outbreak area and number of infested hives. As above, the cost can be focused on one or all of these hazards.

In all of the following plans, the external assumptions and geographic features are considered to be fixed. This can be achieved in a number of ways. For example, a scenario can be selected by specifying one or more of these parameters (e.g., no resistance, incursion of a single mite, 5km

distance between hobbyists, low density of hives) and the plan can be determined for this scenario. The plan will be averaged over all combinations of any unspecified parameters (e.g., if the focus is on a specific location and only the geographic features are specified, the plan will be averaged over all combinations of resistance and incursion).

Design focused on cost of surveillance

Under this approach, the focus – and hence the point of choice in the plan – is the estimated overall cost of surveillance.

The cost of the surveillance plan is specified, which determines the choice of the design components – the number of sentinel hives, spacing and inspection interval. This choice in turn results in a predicted hazard from the model and hence a predicted total cost of eradication.

The process can be depicted as follows.



Design focused on cost of eradication

Under this approach, the focus – and hence the point of choice in the plan – is the predicted overall cost of eradication.

The cost of eradication is specified, based on an acceptable level of hazard – the time to first detection, outbreak area and/or number of infested hives. The design components that result in this hazard are then determined from the model. Given these components – the number of sentinel hives, spacing and inspection interval – the overall cost of the surveillance plan can be determined.

The process can be depicted as follows.



Design focused on combination of cost of surveillance and eradication

Under this approach, the focus – and hence the point of choice in the plan – is a combination of cost of surveillance and eradication.

For each set of design components – the number of sentinel hives, spacing and inspection interval – an overall cost can be determined. This cost can focus on one design component (e.g. number of sentinel hives) or on the collection of components.

For each set of hazard measures – time to first detection, outbreak area, or number of infested hives – an overall cost of eradication can be determined. As above, this cost can focus on one hazard measure (e.g., outbreak area) or on the collection of components.

A series of plots can then be constructed based on the outputs of the Varroa Spread Model:

- Overall cost of surveillance versus overall cost of eradication
- Specific surveillance components (e.g. number of hives) versus specific predicted hazards (e.g., outbreak area).

Tolerable combinations of cost and hazard can then be identified from these plots. This will determine the design components and the corresponding potential hazard. The process can be depicted as follows.



Examples of the type of plots that can be helpful here are given in the first section of this report.

A6.6.2 Combined approach

- A national surveillance design for *Varroa* can be evaluated by extending the above approach. A number of suggestions are proposed here:
- a) Focus on port-specific surveillance cost: For each port, use the cost to determine a surveillance plan and corresponding hazard. Then calculate a weighted sum of these hazards, where the weight is based on a measure of risk of arrival and establishment of *Varroa* at that port.
- b) Focus on port-specific hazard
 For each port, use the specified hazard to determine a surveillance plan and corresponding cost (and/or number of surveillance components). Then calculated a weighted sum of these costs (and/or components), where the weight is as above.
- c) Focus on overall surveillance cost: Apportion this cost to each port and proceed as in (a) above.
- d) *Focus on overall hazard:* Apportion this hazard to each port and proceed as in (b) above.

5.3. Incorporating other surveillance components and entry points

The *Varroa* Spread Model used to derive the above surveillance plans is focused on: sentinel hives as surveillance components, and ports as points of entry.

Ports are a major point of entry, but other points such as airports and postal services have also been proposed as non-ignorable.

Moreover, sentinel hives are a major surveillance component, but current bee surveillance programs include a range of components:

- sentinel hives
- swarm/feral nest capture
- catchboxes
- remote surveillance catchboxes

- floral sweep netting
- hobbykeeper involvement, and
- monitoring of other bee pests such as sugar shakes, alcohol wash, drone uncapping and analysis of tracheal mites.

Different points of entry and/or States and Territories have developed specific surveillance plans, commensurate with perceived needs and available funds. These include different combinations of the above surveillance components. Importantly, many plans do not include sentinel hives.

Details of the current plans for specified ports are given in Table 3 of the report. The following table provides an overall summary, highlighting the range of components and number of plans that do not include sentinel hives

	Qld	NSW	Vic	WA	SA	NT	Tas
No. ports	8 ports, 4 major	10 ports, 3 major	5 ports, 3 major	9 ports, 3 major	8 ports, 3 major	3 ports, 2 major	4 ports, 2 major
No. ports with sentinel hives							
No. sentinel hives	24	27	32	27	24	6	23
No. with Swarm capture	8/8	8/10	5/8	9/9	8/8	1/3	4/4
No. catchboxes	11	50	54	2	26 + 30 at depots	0	0
Remote surveillance catchboxes	15	0	0	0	0	4	0
No. with floral sweep netting	5/8	3/10	1/5	1/9	8/8	2/3	2/4
No. with hobby beekeeper involvement	8/8	10/10	5/5	9/9	3/5	1/3	4/4
No. with tracheal mite analysis	4/8	3/10	4/5	4/9	1/8	2/3	2/4

Two approaches are proposed below that can take this additional surveillance activity into account in determining the overall hazard and surveillance design.

Neither of these methods has been implemented due to lack of information or agreement about the sensitivity, footprint and cost of each activity, where:

(i) footprint: area (m²) covered by the surveillance activity

(ii) sensitivity: probability that the activity detects *Varroa* if it is in the footprint

(iii) relative cost: note that this can include not only dollar cost, but also other resources, comparative difficulty, environmental or other impact, etc.

There is a lack of definitive information about these figures in the literature or among experts. Indeed, there is some disagreement among experts and states about the efficacy (sensitivity) and cost of particular activities. This may be due to geographic and environmental factors, as well as the organization of the industry across the country.

The following table shows the type of information that would be required to implement the proposed approaches.

Surveillance activity	Footprint (local, regional)	Sensitivity (Prob. Detection)	Cost (relative units)	Hazard Reduction (%)
Sentinel hive				
Swarm/feral nest capture				
Catchbox				
Remote surveillance catchbox				
Floral sweep netting				
Hobby beekeeper involvement				
Tracheal mite analysis				
Sugar shaking, alcohol washing, drone uncapping				
Other				

A6.6.3 Direct adjustment approach

The above non-sentinel-hive activities can be considered to directly modify the predicted hazard (estimated time to detection) obtained from the *Varroa* Spread Model.

Given that different activities are undertaken at different locations for cost, logistic and geographic reasons, the modifications in predicted hazard are best undertaken for each location. This can be achieved by including an additional parameter, "Modified hazard", in the "Geographic Location" factors listed in Section 1 above.

The "Modified Hazard" can be calculated as follows.

• For each port:

• If the port has sentinel hives, calculate the hazard associated with the sentinel hives. If there are no sentinel hives, select a small baseline probability reflecting the chance that *Varroa* would be detected without any of the listed surveillance activities.

- $_{\odot}$ $\,$ Identify existing and/or possible surveillance activities from the above list.
- Attach a value to each activity that reflects the reduction in hazard achieved by undertaking that activity. This value can be expressed in two ways:
 - An absolute reduction, i.e., a value between 0 and 1, or
 - A relative reduction, i.e., between 0% and 100%
- $\circ~$ Use these values to adjust the hazard for that port. This may require assuming independence between the activities.
- Combine the modified hazards to compute a national hazard, using one of the methods describe above, to obtain an overall hazard and related design and cost.

Alternatively, a surveillance strategy can be developed based on the designs described in Sections 2 and 3 above, using the modified hazard value of M instead of T.

A6.6.4 Power-focused approach

As described in Section 3 of this document, one approach to surveillance is to focus on the overall features of a desired surveillance strategy and apportion the resultant surveillance design components to the different locations in the surveillance frame, based on a nominated risk for each location.

This approach can be extended in a general way to accommodate different points of entry and a variety of surveillance components³².

An overall feature of a surveillance strategy that is appropriate for this approach is the overall power: the probability of detecting varroa, given that it has arrived at one of the points of entry.

The surveillance plan is then developed as follows:

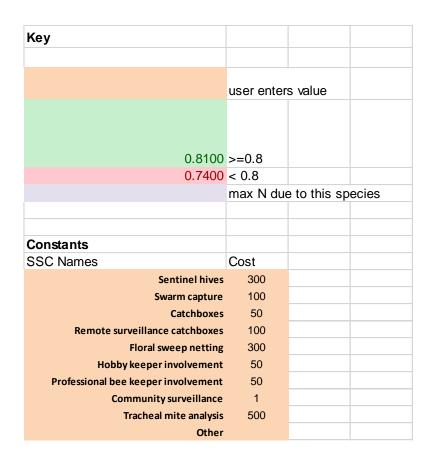
- Specify the desired overall power of the surveillance strategy. A suggested value is 0.80. This means that there is an 80% chance of detecting *Varroa* within an acceptable timeframe if it arrives and establishes at one of the entry points in the surveillance frame.
- 2. Define a set of surveillance activities.
- 3. Specify a set of entry points to be included in the surveillance frame. Note that these do not necessarily have to be ports. Moreover, they could be aggregated locations, for example at a State or Territory level, if the surveillance strategy is to be administered at this level with more autonomous (less designed) decision-making about surveillance design with these regions.
- 4. Ascribe the following risk values to each entry point:
 - (i) the desired area to be covered in the surveillance
 - (ii) a relative measure of hazard of entry and detection.
- 5. Allocate the overall power determined in Step 1 to the different activities, based on their surveillance attributes determined in Step 3.
- 6. For each type of activity, determine the number of items required to achieve the ascribed power determined in Step 6.
- 7. Allocate the number of surveillance items to the different locations, based on their risk attributes determined in Step 5.

A snapshot of a prototype spreadsheet is given below. Note that all input (and hence output) values are hypothetical at this stage.

³² This approach is based on the methodology used for biosecurity surveillance on Barrow Island, WA.

Hypothetical example spreadsheet for power-based surveillance strategy

Specify Surveillance System Components (SSC), denoted as activities in the report.



The plan allows for multiple pests that can be included or excluded. Here, only *Varroa* is included. For each SSC, the user elects to use that SSC (Utilise?) and specifies sigma (sensitivity = probability of detection of K pests with a single unit of the SSC), footprint (surveillance area covered by a single unit of the SSC), and cost (per unit SSC; this can be monetary or conceptual, including practicality and manpower considerations, etc). The spreadsheet then allocates a proportion of the overall power to each SSC, and determines the total number of SSCs required to meet the allocated power.

	Final Power					
	Power (varroa)	0.8				
	Final Power (all pests)	0.8				
Include			Sigma	Footprint	Cost (\$	Calculated
pest?		Utilise?	values	(m ²)	equivalent)	N
Y	Varroa (A. varroa)	K=	100			
	Sentinel hives	Y	1	100	300	132
	Swarm capture	Y	0.2	50	100	395
	Catchboxes	Y	0.6	100	100	395
	Remote surveillance catchboxes	Ν	0.6	100	100	0
	Floral sweep netting	Y	0.8	50	300	132
	Hobby keeper involvement	Ν	0.8	1,000	50	0
	Professional bee keeper involvement	N	0.8	1,000	50	0
	Professional bee keeper involvement Community surveillance	N	0.8 0.9	1,000 10	50 1	-
						0 0 0

The user is required to specify locations and, for each location, the area over which surveillance is to be undertaken. The spreadsheet then allocates the SSCs to these lo/cations. Note that in this example, for exposition the locations are states and territories. This can be changed to individual points of entry.

Rows refer to numbers of items of SSCs: sentinel hives, swarm capture, catchboxes, floral sweep netting, tracheal mite analysis.

E.g., 27 sentinel hives, 84 swarm capture, 85 catchboxes, 33 floral sweep netting activities allocated to NSW; 25 sentinel hives etc. to NT, etc.

A final cost for the SSCs and overall strategy can then be calculated.

Area	No. major/mino	or ports per loca	tion									Area (m ²) =	# major er
100,000	5	4	4	2	2	2	4						
20,000	6	4	1	4	2	1	10						
			Locatio	n SSC allo	cation - ca	n exclude	sites with	Yes/No					
	NSW	NT	Qld	SA	Tas	Vic	WA						
Include?	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N (sum locations)	Final Cost (\$)
Area	620,000	480,000	420,000	280,000	240,000	220,000	600,000	0	0	190,000	0		
	27	25	21	14	9	9	27	0	0	0	0	132	3960
	84	58	58	33	43	33	86	0	0	0	0	395	3950
	86	64	51	42	34	31	87	0	0	0	0	395	3950
	0	0	0	0	0	0	0	0	0	0	0	0	
	33	26	24	11	10	6	22	0	0	0	0	132	39600
	0	0	0	0	0	0	0	0	0	0	0	0	(
	0	0	0	0	0	0	0	0	0	0	0	0	(
	0	0	0	0	0	0	0	0	0	0	0	0	(
	0	0	0	0	0	0	0	0	0	0	0	0	
		-	-				-				-	-	

A6.7 Conclusion

This document has provided some summaries and evaluations of possible designs for the detection of *Varroa* in Australia.

Overall, it appears that the following designs are preferred:

- 6 sentinel hives at 3km spacing, inspected at 2 month intervals
- 3 sentinel hives at 2km spacing, inspected at 1 month intervals, and preferably other surveillance activities
- at least 3 sentinel hives, less than 5km spacing and inspection interval 1 or 2 months, and other surveillance activities.

The type and number of other surveillance activities depend on the acceptable level of hazard (months to first detection, area of infection) and the characteristics of the site.

The lack of information and/or agreement about the potential efficacy of the available surveillance components in detecting varroa, and their relative contribution to the surveillance effort in general, motivates further discussion. This information could be used to increase the predicted probability of detecting *Varroa* under designs that are currently based only on sentinel hives. These other surveillance components are obviously important, but without more details it is difficult to quantify this important and translate it to improvement of site-based hazard or the development of a multi-site or national biosecurity design.

Appendix 7: Design options and costings for the sentinel hive component of the NBPSP

Table A7.1 Current sentinel hive arrangement vs. proposed options (displayed with port risk rating, port number, sentinel hive number and cost). Table displays (purple) current sentinel hive arrangements per jurisdiction as outlined in contract (displayed with: port, hive number and cost), the actual cost of this contracted work is given. As most jurisdictions have added and gone beyond what is contracted in the current payment allocation, the cost of this is also given (contracted NBPSP + additional/optional hives), this hive and port arrangement is what is used to develop the three NBPSP proposals. There are three proposals provided (orange and green) in two categories: 1. all ports (all risk rating) where currently contracted and non-contracted hives are located, and 2. Only high and medium risk ports where contracted and non-contracted hives are located hives are located and non-contracted hives allocated), **proposal #2 (green)**: deployment of 6 hives at high and medium risk ports where activity is currently occurring, and **proposal #3 (orange)**: deployment of 6 hives at high and medium risk ports at low and unknown risk ports. Totals for port number, hive number and national annual cost of sentinel hive component is tabulated at the bottom.

			Curren	nt sentinel	hive arran	gements	;		Ports where sentinel hives are currently in place					
ion	E Contracted in the NRPSP					itracted NB onal/optio	-	All ports All high + medium risk p						
Jurisdiction	Port risk ra	Contract payment	# ports used	Number of hives in total	Actual cost (\$)	# ports used	Number of hives in total	cost (\$)	# ports used	Proposal #1 6hives/port cost (\$)	Proposal #3 6h (H&M) & 4h (L&U)/port cost (\$)	# ports used	Proposal #2 6hives/port cost (\$)	
QLD	H M	28,000	3	24	60000	3 1	24	60,000	4	60000	60000	3	60000	
NS W	H M L U	23,000	3 0 0 0	22	55000	3 0 0 6	26	65,000	9	135000	45000 0 0 60000	3	45000	
WA	H M L U	18,000	2 0 0 2	22	55000	2 3 0 3	22	55,000	8	120000	75000 0 30000	2 3 0 0	75000	
VIC	H M U	21,000	1 2 2	28	70000	1 2 2	32	80,000	5	75000	45000 20000	1 2 0	45000	

Tot senti hive o	inel	132,000 Contract payment	# ports used	Number of hives in total	284,00 0 Actual cost (\$)	# ports used	Number of hives in total	377,50 0 cost (\$)	# ports used	525,000 Proposal #1 6hives/port	450,000 Proposal #3 6h (H&M) & 4h (L&U)/port	# ports used	300,000 Proposal #2 6hives/port cost (\$)
-				122			151			210	180		120
Tot por			20			35			35			20	
	U		0			0		-,			0 10000	0	
NT	M	14,000	1	6	15000	1	6	15,000	2	30000	15000	1	15000
	U H		0			0					0	0	
SA	L	14,000	0	6	15000	2	18	45,000	3	45000	20000	0	15000
_	H	4.4.000	1 0		45000	1 0	10	45.000	-	45000	<u> </u>	1 0	45000
	L		0			1					10000	0	
TAS	H M	14,000	1 1	14	14,000	1 2	23	57,500	4	60000	45000	1 2	45000