

Final Report

Development and implementation of protocols to enable importation of improved honey bee genetics to Australia

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Development and implementation of protocols to enable importation of improved honey bee genetics to Australia (MT18019)

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Summary

The Australian honey bee industry provides valuable pollination services across the horticultural sector and securing these services relies on having healthy and productive honey bee colonies. High priority exotic pests like Varroa mites and the harmful viruses they transmit will have a major impact on hive health and pollination services. One way we can prepare for these threats is through genetic improvement of honey bee stocks to be more Varroa resistant. However, the honey bee industry needs a reliable importation process to access the valuable progress made in honey bee breeding programs around the world without introducing harmful pests and pathogens.

New honey bee genetics can be introduced to Australia under permit through live queen bees contained in the Post Entry Quarantine (PEQ) facility at Mickleham VIC or more directly through bee semen. However, these pathways have not been successfully demonstrated and industry has had low confidence to attempt importation. There are also concerns about the effectiveness of testing requirements for bee viruses. This project set out to test both importation pathways to introduce honey bee genetics selected for Varroa resistance, better understand the risk of virus transmission and increase capability to support future importations.

The project team, led by the CSIRO with the support of industry and government partners, achieved the first importation of bee semen into Australia, the first importation of live queen bees through the Mickleham PEQ facility, and the first introduction of Varroa resistant genetics into Australia. Through this activity we developed a logistical framework to guide future honey bee importations and demonstrated that this can be done safely without introducing exotic bee viruses. The imported stock continues to be evaluated for performance and paves the way for establishing Varroa resistance in Australia's honey bee population.

This project has improved pollination security for industry levy payers by removing significant barriers for Australia accessing Varroa resistant and other improved honey bee stock from overseas breeding programs. Whether through pre-emptive introductions of resistant genetics or rapid importation in response to a Varroa incursion, the Australian honey bee industry and its vital pollination services are better prepared to reduce the impact of these serious biosecurity threats.

Keywords

Pollination security, Varroa resistance, honey bee importation

Introduction

The Australian honey bee industry provides valuable pollination services to the horticultural sector and securing these services relies on having healthy and productive honey bee colonies. One way to achieve this is through genetic improvement of honey bee stocks. However, the honey bee industry needs a reliable importation process to access the valuable progress made in honey bee breeding programs around the world without introducing harmful pests and pathogens. These advances include traits such as improved production and pollination, resistance to pests and pathogens (e.g. Varroa mites), and climatic adaptation. Currently two pathways exist in Australia for the introduction of new bee germplasm: grafted larvae from live queens contained in the Post Entry Quarantine (PEQ) facility at Mickleham VIC and through drone bee semen.

Importation of honey bee germplasm through live queens has not occurred since the Mickleham PEQ facility was commissioned in 2015. The most recent importations were in 2012 via the former PEQ facility at Eastern Creek NSW and were not successful, although successful importations have occurred through this facility in the past. Industry beekeepers are not confident in the suitability of the Mickleham facility in both its climate and staff capacity to enable successful queen importations.

Similarly, no bee semen importation has been conducted since the release of importation protocols in 2016. After much industry consultation, the importation protocols for bee semen (which does not involve the PEQ facility) were finalised to include testing requirements for specific viruses that are globally associated with bee colony mortality. One virus in particular, deformed wing virus (DWV), is highly prevalent in overseas honey bee stocks and it is unknown if this would prevent a successful semen importation. The risk of introducing exotic viruses is also currently unmanaged through live queen importations.

Consequently, there was a strong need to trial queen importations through the new quarantine facility and to evaluate the bee semen importation protocol for practicality and biosecurity integrity. This current project emerged from conversations between multiple industry participants, the Australian Honey Bee Industry Council (AHBIC), the Australian Queen Bee Breeders Association (AQBBA), the Commonwealth Scientific and Industrial Research Organisation (CSIRO), the Department of Agriculture, Water and Environment (DAWE), the When Bee Foundation and Hort Innovation. This project aimed to assess the importation protocols and procedures for both queens and semen to successfully introduce honey bee genetics selected for resistance to the Varroa mite. Establishing Varroa mite resistance in Australia's honey bee population can help prepare for a future invasion of this pest and minimize its impact on pollination services for horticulture.

Methodology

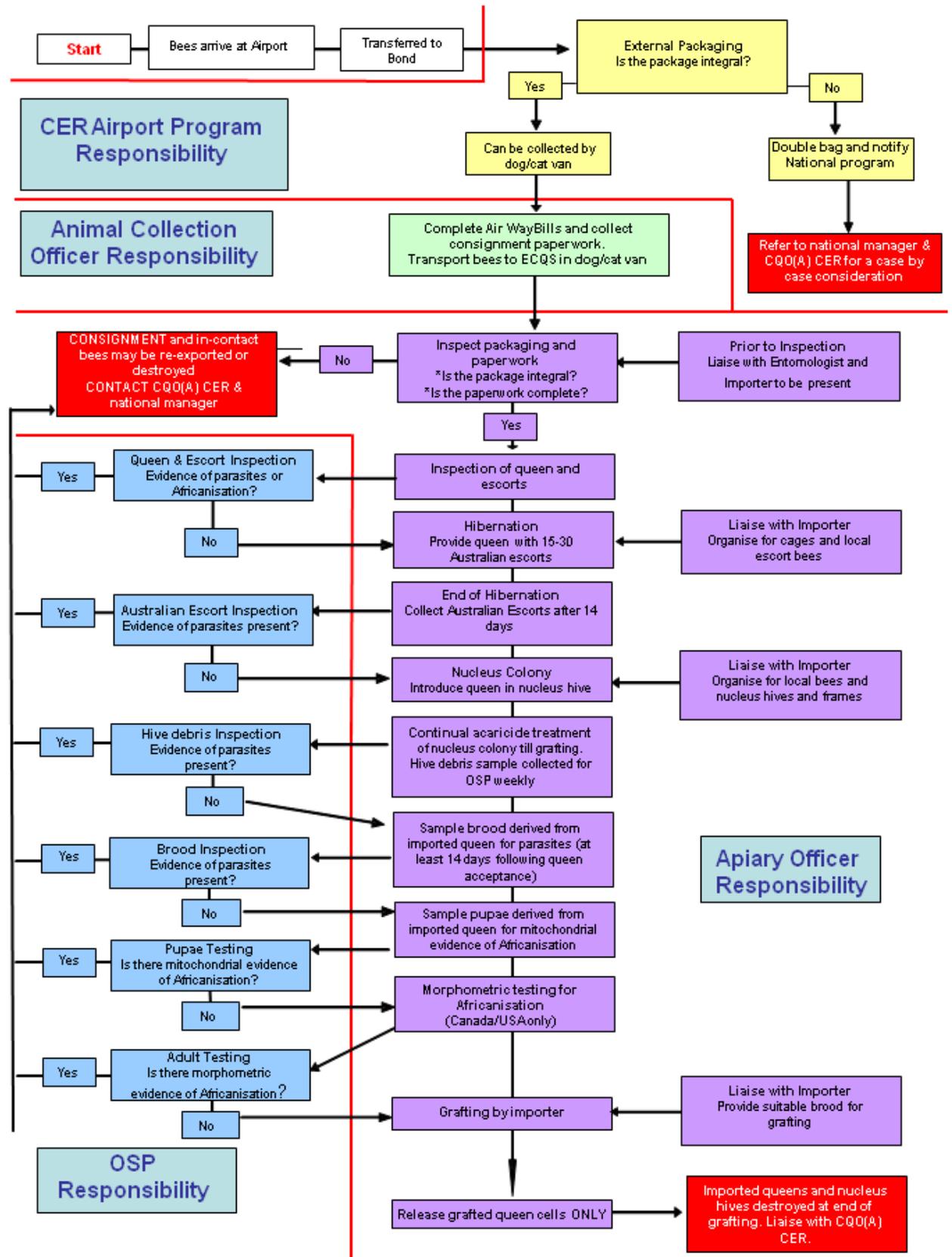
The two-year project started in July 2019 and involved four main stages.

Domestic Trial of the Mickleham PEQ facility

A trial run of a live queen importation was conducted between September and December 2019 by introducing local Australian queens into the Mickleham PEQ facility. The goals of this activity were to better understand the PEQ workflow, facilitate greater industry involvement in the process, and build knowledge and capacity for managing queens and nucleus colonies in the PEQ flight cages.

The domestic trial was initiated on October 8th 2019 with the introduction of three Australian queens into the Mickleham PEQ facility. We engaged industry expert Bruce White for this initial stage to provide practical advice on managing queens in quarantine based on his experience with the previous NSW Eastern Creek Facility. The PEQ workflow shown in Figure 1 was followed for each queen, with some testing steps omitted. After initial examination for parasites, queens must undergo a 14-day holding period as a mitigation step for tracheal mites. After this each queen was introduced to a nucleus colony containing food stores and some capped brood. Nucleus colonies must also be treated with miticides (e.g. Bayvarol®) during quarantine for risk mitigation of Varroa mites and given supplementary pollen and sugar syrup. Once successfully established queens produce brood, additional biosecurity tests for parasitic mites and Africanised bee genetics are completed. After these biosecurity steps are completed, grafting age larvae can be removed from PEQ and developed into new queens.

During the trial, we experienced challenges with queen survival during the holding period, introduction to nucleus colonies and colony productivity. However, a small number of larvae were produced for grafting to complete the trial. We decided to run a second trial, introducing three new queens on November 20th 2019 to implement several changes to improve queen survival and brood production. These included changes to the handling of the bees during the 14-day holding period and changes to the methods of feeding the nucleus colonies. These changes were successful, achieving better queen health and brood production. Both trials were also successful in building knowledge and capacity for the project team and PEQ staff to support live queen importation.



Importation of honey bee germplasm bee semen

Our objectives were to determine the feasibility of the import protocols for bee semen and live queens and target stock selected for Varroa resistance. For this we established a relationship with the Dutch breeding program Arista Bee Research (<https://aristabeereseearch.org>) to provide stock selected for Varroa Sensitive Hygiene (VSH). Colonies selected for this specialized form of hygienic behaviour produce honey bees that are better at detecting brood infested with Varroa mites and 'hygienically' remove these brood. This behaviour disrupts the Varroa mite life-cycle and results in low mite populations for colonies. Arista agreed to allocate three VSH colonies from their program to our project to provide semen and the queens for importation

Bee semen importation

The importation workflow for bee semen is shown in Figure 2. We began the process for importing bee semen by working with the DAWE to obtain an import permit for bee semen (Appendix 1) and discuss the practicalities of meeting the permit requirements. We also gave the import permit to Arista to discuss with Dutch authorities the requirements that need to be met on a signed health certificate. Dutch authorities identified issues with the original health certificate requiring statements of country freedom for Africanised bees (*Apis mellifera scutellata*) or Cape honey bees (*A. m. capensis*) when the Netherlands (and most other countries) have no active surveillance program for these pests. The project team and Arista negotiated revised statements from DAWE with the Dutch authorities, which included additional pre-export testing of ten bees from each donor colony for Africanised bee genetics.



Figure 2. Flowchart of bee semen importation process

Bee semen imports are also required to be tested for two bee viruses not present in Australia – deformed wing virus (DWV) and slow bee paralysis virus (SBPV). There are three options available to importers; test 60 bees from the donor colony within 30 days of semen collection, test the collected semen directly pre-export, or test the semen on arrival in Australia. We chose to test the donor colony pre-export to meet the import requirements and conducted additional virus testing at CSIRO post import to further mitigate the risk of unwanted viruses and to assess the robustness of the current importation protocol.

Testing for Africanised genetics and viruses by the exporting country was investigated and possibly available through a university laboratory in the Netherlands, but this was interrupted by COVID-19 closures. Instead, we engaged AgriBio in Victoria to conduct the Africanised genetics and virus testing. Twin samples were sent to CSIRO for testing confirmation. Africanised mitochondrial DNA testing followed the protocol of Pinto et al. (2003) and virus testing was consistent with CSIRO protocols based on published methods (de Miranda et al. 2010, Kevill et al. 2017) used for the National Bee Pest Surveillance Program.

Although the life history of honey bees is polyandrous where drones from many colonies are reflected in propagated colony genetics, we chose to import semen from only one colony to better manage the threat of virus introduction. Although not prescribed by the importation protocol, the team decided to apply the chemical treatment Bayergate® to each of the three potential drone donor colonies to ensure low mite levels and reduce the likelihood of virus transmission.

Our first attempt to import semen in early 2020 was unsuccessful because of delays with finalising health certificates and import permit variations. Arista had produced mature drones for semen collection and Africanised DNA and virus testing was completed for the three donor colonies to show no evidence of these pests. However, we had to abandon this attempt and replan for August 2020.

Arista encountered difficulties with drone production due to local conditions. Only one colony produced sufficient drones for semen collection and this colony also experienced a large die-off of drones confined to the hive before semen was collected. On August 7th semen was collected from approx. 25 drones and prepared for shipment. This

small volume of semen (~25 μ L) limited the number of queens we could inseminate and did not allow direct testing of semen for viruses to compare with the colony test results. Nevertheless, we proceeded with the import and following health certification from Dutch authorities, the shipment of semen was sent on August 19th and cleared customs in Melbourne on August 25th (Figure 3).

Imported semen was artificially inseminated into four Australian virgin queens produced from two breeding lines from queen mothers with high hygienic behaviour, as measured by the freeze-killed brood test (Reuter & Spivak 1998). Additional queens from these lines (sister queens) were inseminated with local drone semen to create comparison colonies. Three of the four Arista-semen queens were successfully inseminated and accepted into colonies. The fourth Arista-semen queen failed to lay and was euthanized. The three successful Arista-semen queens headed colonies until August 2021 when two of the queens were superseded. The last remaining colony continues to head a strong and productive colony.

To determine that the Arista-semen queens were not infected and transmitting exotic viruses to their offspring, we tested 20 pupae and a pooled worker bee sample from each colony monthly from October to December 2020 and confirmed these colonies are not infected by DWV or SBPV.



Figure 3. Imported Arista bee semen used to artificially inseminate Australian queens.

Live queens importation

Following the lessons of the semen import, we negotiated with DAWE and Dutch authorities agreed variations to the import permit requirements and health certificate for live queen bees, which included pre-export testing for Africanised genetics (Appendix 2). The live queen importation was now planned for early 2021. Arista prepared the three VSH colonies over the Netherlands winter to comply with import conditions by applying chemical treatments for parasitic mites in January and February 2021 and sent bee samples from each colony to AgriBio for Africanised DNA testing. Bee samples were also sent to CSIRO for virus testing, although not required by policy.

Unfortunately, one VSH queen died over winter so only two live queens were shipped to Melbourne and entered Mickleham PEQ facility on March 1st 2021. The VSH queens were examined following the workflow described in Figure 1. The two queens and escort bees were visually inspected for external mites and escorts were dissected for internal tracheal mites (Figure 4). VSH queens were then transferred to fresh cages with young local escort bees for 14 days before dissection of local escort bees for tracheal mite inspection. No tracheal mites were found in any workers.



Figure 4. PEQ examination of queens and escort bees for parasitic mites

One queen died during this incubation period leaving only one queen for introduction to a nucleus colony into a PEQ flight cage. After several days and laying approximately 100 eggs, this final queen (DB12) died. However, the colony had created several queen cells from the small number of eggs laid, which provided an opportunity to recover some genetic stock. With permission from DAWE, we hatched three virgin queens into nucleus colonies into flight cages and artificially inseminated them with Australian semen in April 2021 (Figure 5).



Figure 5. Artificial insemination of daughter Arista queens in PEQ after original imported queens died.

Two queens survived but only one queen began laying to continue the quarantine process. This queen (B3) was fully tested for mites and Africanisation following PEQ protocols and was additionally tested for viruses by AgriBio to meet Victorian requirements to import bees from the PEQ facility into the state of Victoria. On May 10th 2021 larvae were grafted into queen cells and released from PEQ and those mature cells were hatched into nucleus

colonies. Emerged queens were artificially inseminated and established in colonies to overwinter. The surviving daughter queen, B3 was over-wintered in the PEQ facility and more larvae were grafted in September and October 2021 to establish more queens from this import derived stock (Figure 6).



Figure 6. Grafted larvae from the imported stock were removed from PEQ and raised into queens.

An important adaptation of the established workflow for importation of live queens was the augmentation of support colonies located at Mickleham PEQ facility with colonies further north in Central and North Eastern Victoria. The location of Mickleham PEQ being south of the Great Dividing Range means that colonies on site have to contend with frequent cold fronts with high winds and showery weather. The established practices of creating flight cage nucleus colonies from the support hives and after grafting, using support hives to rear queen cells were not feasible. The project adapted to this situation by supplying nucleus colonies already established in home apiaries and bringing cell starter colonies to Mickleham and then transporting started cells back to NE or Central Victoria to continue the cell rearing process.

Evaluation of imported stock before release to industry

Stock from the imported bee semen and live queen import were crossed with Australian stock and established for evaluation of general performance and hygienic behaviour.

The three Arista-semen queens showed a high degree of hybrid vigour, with excellent colony build-up and productivity. These colonies also showed a high hygienic behaviour response when tested by the freeze-kill brood test. Fifteen open mated and ten artificially inseminated daughters from the three Arista-semen queens have been produced and managed in apiaries in central and northeast VIC and alongside local stock. The performance of these colonies has generally been consistent with local honey bee stocks with regard to commercial traits such as temperament, honey gathering and swarming inclination. Hygienic behaviour testing of open mated daughter colonies was completed in November 2021 with nearly half of the colonies scoring extremely hygienic (above 95% on the liberal test) (Figure 7).



Figure 7. Hygienic behaviour testing of imported stock used a freeze-kill brood method.

Open mated daughters from queen B3 in PEQ have also shown strong hybrid vigour having very fecund queens laying exceptional brood patterns as a result of mating between completely unrelated stock. Their performance in terms of honey production, temperament, hygienic behaviour and general management will continue to be evaluated over the 2021/22 season.

Artificially inseminated daughters from the October 2021 graft had not yet become sufficient sized colonies for evaluation. Some of these daughters were inseminated with drones reared from Arista-semen daughter queens. As the bee semen and the live queen imports both originated from the same Dutch colony (DB12), this backcross will enable a consolidation of the DB12 genetics. Several of these queens will also be established in the CSIRO research apiary for continued investigation of the Varroa resistant genetics.

Outputs

1. Logistical framework for importing honey bee germplasm into Australia developed from the experience of the domestic trial, bee semen import and live queen import (Figure 8).

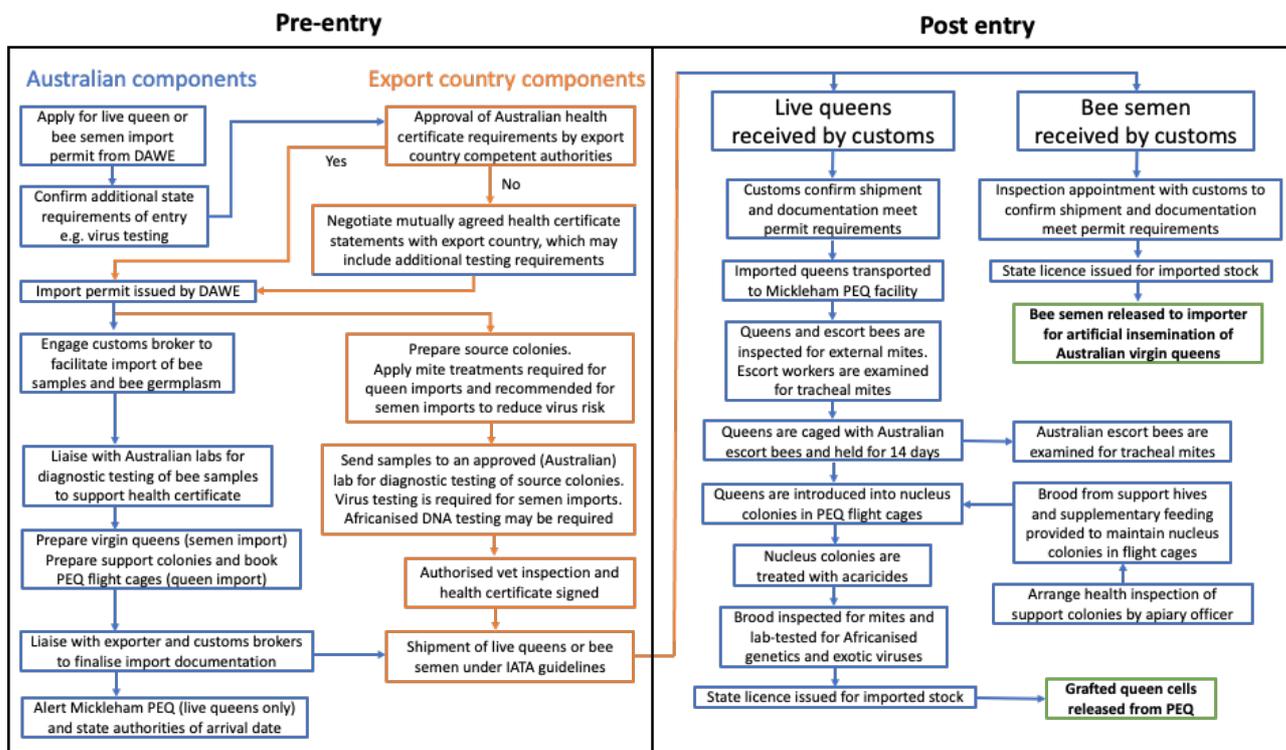


Figure 8. Logistical framework for importation of honey bee germplasm.

2. Varroa resistance honey bee stock successfully imported.
 - Bee semen sourced from a colony selectively bred for Varroa resistance imported.
 - Three Australian queens were inseminated with this imported bee semen.
 - A total of 33 open mated and 25 artificially inseminated queens were produced from the original three Arista-inseminated Australian queens and a further 5 artificially inseminated queens were produced forming a third generation. All of this stock is currently in Victoria.
 - Live queens sourced from colonies selectively bred for Varroa resistance imported into PEQ.
 - Two original imported queens died in PEQ but daughter queens were reared from the eggs laid. A single daughter queen (B3) was successfully reared and artificially inseminated with Australian bee semen. A total of 100 daughter queens were grafted from B3 over four occasions
 - The virgins arising from B3 were distributed to industry through two channels: Eight virgin queens reared from B3 in September were sent to an Australian Queen Bee Breeders' Association representative in QLD and a further five virgins were sent to the National Bee Breeding Project (Plan Bee) in NSW. These virgins were inseminated with selected drone stock from those regions to provide diversity in the stock released from this project. The balance of the virgins remained in Victoria with approximately 17 open mated and 35 artificially inseminated queens.
 - Hybrid 'Arista-Australian' queens from the bee semen and live queen imports are under evaluation for general productivity and temperament and have undergone hygienic testing using the freeze-kill brood method. Current evaluations have shown commercial performance consistent with local stock

and as expected return high hygienic behaviour response from freeze-kill brood testing.

3. Industry extension material for the biosecure importation framework

- Project update, Melon News February 2021 <https://www.melonsaustralia.org.au/industry-news/>
- Project update, In a Nutshell Autumn 2021 <https://australialmonds-com-au.cld.bz/In-A-Nutshell-Autumn-2021/14/>
- ABC Landline, <https://www.abc.net.au/landline/bee-prepared:-importing-bees-to-help-protect/13450032>
- ABC news article, <https://www.abc.net.au/news/2021-07-16/bee-imports-to-protect-against-varroa-mite/100289356>
- Presentation at Australasian Bee Conference Perth July 2021
- Presentations to Melbourne Bee Club and Victorian Apiarists Association Melbourne section
- Briefing report to AHBIC and AQBBA
- Preparation of recommendations for DAWE seeking consideration of some improvements to PEQ workflow

4. New knowledge on vertical transmission of DWV through imported germplasm

- Demonstrated that virus-free semen and queens as determined by sensitive quantitative PCR testing can be successfully sourced and imported.
- Five Arista-VSH colonies were tested for viruses in May 2020. One of these colonies had known significant mite levels whereas the other four colonies had low mite levels and were receiving acaricide treatment. Twin samples of 60 worker bees from these colonies were sent to AgriBio and CSIRO for diagnostic virus testing. Only the colony with significant mite numbers tested positive for DWV-B and all colonies were SBPV-negative. These samples were additionally tested by next-generation sequencing by AgriBio which confirmed the qPCR results. Testing was repeated in August 2020 for three Arista colonies with twin samples of 60 workers sent to AgriBio and CSIRO, which again found these three colonies negative for DWV and SBPV.
- Source colonies for the live queen import were also pre-screened by CSIRO and found negative for DWV and SBPV. This diagnosis was confirmed by testing brood from the imported live queen daughter in PEQ. Testing by AgriBio, as required for the Victorian government licence, found the live queen stock was negative for these viruses and three additional viruses - Acute bee paralysis virus and Apis rhabdovirus 1 and 2.

Outcomes

Importation framework developed that mitigates the risk of exotic bee viruses and improves bee industry confidence to undertake importation of desirable germplasm.

Through road testing the importation protocols for bee semen and live queens we were able to address multiple challenges and uncertainties in the process and demonstrate a successful path for importing honey bee germplasm into Australia. Within the current policy framework we have suggested a workflow that mitigates the risk of exotic bee viruses and gives the bee industry confidence to undertake future importations.

Prior to this project, there was concern by the bee industry that biosecurity measures aimed at reducing the risk of introducing unwanted bee viruses were ineffective. Many believed that the ubiquitous nature of viruses like DWV would make 'virus-free' bee semen and queens impossible. Other industry stakeholders were concerned that the mitigation measures were untested and may not sufficiently reduce the risk from viruses. We showed that with careful selection of colonies, isolation of those colonies and use of acaricides to ensure low mite infestation levels it was possible to meet the bee semen import requirements to have non-detectable levels of DWV and SBPV in pooled samples of 60 worker bees. Virus testing of semen on arrival was not possible for our importation because of low volumes, and remains an untested option of the import policy. Our discussion with DAWE identified that a separate 'representative' semen sample (approximately 20 μ l) could be prepared by the exporter that can be sent for virus testing on arrival. This approach increases the holding time of the imported semen with customs until virus testing is completed, which could take several days. We would still recommend the donor colony be pre-screened for viruses to reduce the risk for the importer. An additional safeguard for industry is to arrange virus testing of the first brood cycle from any inseminated queens. We also recommend that testing be done in Australia by approved labs to ensure that testing is consistent with current surveillance protocols. However, there can be benefits for testing turnaround times and transport logistics if diagnostic services are available in the exporting country. Many countries also do not conduct routine diagnostics for bee viruses and identifying suitable diagnostics labs overseas can be challenging for industry importers.

While the live queen import policy has no current requirements for virus testing, we also showed that biosecurity measures could be included to reduce the risk of this pathway. Similar to the semen importation, pooled samples of 60 worker bees from the source colonies for the live queen import were pre-screened by CSIRO and found negative for DWV and SBPV. This diagnosis was confirmed by testing brood from the imported live queen daughter in PEQ, which was required for a Victorian government import licence to take grafted larvae out of PEQ (Appendix 3). This state level requirement created a challenge to arrange diagnostic testing for additional viruses not currently part of the national program (Apis rhabdovirus 1 & 2), this was also an effective mechanism to enhance biosecurity against viruses for live queen imports.

Another key outcome of this project was the development of import permit and health certificate variations that meet Australian and export country authority expectations. The Australian government has put considerable thought into the wording of the import permit requirements and accompanying health certificate statements, but we found that these have not been vetted by overseas counterparts. In our case, the Netherlands (and other EU countries) is considered an 'approved country' by Australia because they do not have Africanised bees, however the Netherlands were not able to state country freedom because they do not have active surveillance for Africanised bees. In fact, we are not aware of any countries including Australia who undertake routine surveillance for Africanised genetics in their honey bee population. The Netherlands authorities also found the wording for other parts of the health certificate difficult to sign off on. Agreement between Australian and export country authorities on the health certificate statements is a key part of the process that should not be under-estimated. We hope that the lessons learned here will assist with future imports.

Improved capacity at PEQ Mickleham to support bee germplasm importations.

This project provided staff at the Mickleham PEQ facility the opportunity to grow their experience and capability to support live queen importation into Australia. The domestic trial was extremely valuable for PEQ staff and the project team to better understand the various elements of the quarantine workflow and identify where optimisation was needed. Survivability of queens during the initial 14-day holding period was improved through better temperature and humidity maintenance, although this continues to be a challenging step on queen survival. Conditions for nucleus hives in the PEQ flight cages are also not ideal for colony productivity. The facility will be

most used in spring and autumn when there are frequent cool conditions, which keeps the flight cage temperatures low. Spot heating of nucleus colonies was not available for this project but could be implemented in future to support hives. Light levels are also very low due to building design, making colony inspections and colony function more challenging. Additional artificial lighting is also something the PEQ facility would like to implement for future imports.

Increased industry participation during the PEQ process was also a key outcome of this project. It is critical that strict biosecurity protocols are in place, however maintaining queen and colony health within the PEQ facility requires specialist beekeeping knowledge beyond what can be expected from PEQ staff. We showed that the collaborative approach taken here is highly beneficial to the success of importations but also encourages knowledge exchange between PEQ staff and experienced beekeepers. The failures of queen survival in PEQ were also extremely informative and created opportunities to test what could be done with the facility, which would be less likely to occur outside of this project. We have been able to show queen supersedure can be used to allow some recovery of imported stock in the event of queen failure. We have also shown that queens can be maintained within the PEQ flight cages over winter to allow additional grafting opportunities. Continuing to have sensible industry participation to support PEQ staff in managing queen health will ensure strong industry confidence to undertake future importations.

Increased diagnostic capability for bee viruses and Africanised bees at AgriBio Victoria was another significant outcome of this project. Bee virus testing was initially established earlier in collaboration with CSIRO and through this project was developed into a diagnostic service to support industry needs. Testing for Africanised genetics was not available from any Australian labs as a diagnostic service and was provided specifically by AgriBio for this project. Maintaining this diagnostic capacity at AgriBio and CSIRO and inclusion of additional labs will be important for having robust diagnostic support for future bee importations.

Insights on the general performance, virus transmission and trait retention of Varroa resistant stock in Australian conditions

This was an ambitious project that had to overcome multiple challenges in order to deliver the first importation of Varroa resistant stock into Australia. Delays throughout the project reduced the time available for stock evaluation, however we have been able to gain useful insights into the general performance, virus transmission and potential retention of the VSH trait under Australian conditions.

Hybrid stock from the semen importation in August 2020 has been evaluated over approximately one year for general performance through comparison with quality Australian stock under the same apiary conditions. This stock has shown comparably strong brood and honey production and demonstrated good temperament.

Hybrid stock from the queen importation in March 2021 has had little opportunity for performance evaluation, with the first grafted queens establishing colonies in late autumn and subsequent grafts occurring in spring in the final months of the project. Initial evaluation of the first grafted queens have shown good productivity and temperament.

Virus transmission is a key biosecurity risk for honey bee importation. Through virus testing we can minimise this risk, although we still need to better understand the vertical transmission pathway (queen to egg) to ensure measures are effective. Unfortunately, the amount of imported stock provided limited opportunity to explore vertical transmission of viruses like DWV. Our results from testing multiple brood generations from the hybrid stock does give confidence in the biosecurity measures and is consistent with overseas studies that show DWV is not effectively transmitted by queens if virus levels are low.

Evaluation for retention of the VSH trait in the imported stock is very difficult without direct challenge with Varroa mites. The only method available for this project was a freeze-kill brood test to determine the level of general hygienic behaviour of hybrid colonies, which can correlate with VSH. Our testing results showed that hybrid colonies displayed high general hygienic behaviour. Further evaluation for VSH trait retention will require improved field bioassays that more closely replicate Varroa infestation or require imported stock to be returned overseas for direct assessment against Varroa mites.

Monitoring and evaluation

KEQ1. To what extent has the project informed the feasibility of biosecure importation of Varroa resistant honey bee germplasm?

The complexity of this project created many challenges for the project team but has still been highly successful for informing industry and its stakeholders on the feasibility of biosecure honey bee importations into Australia. This project has delivered the first importation of bee semen into Australia, the first importation of live queen bees into the Mickleham PEQ facility and the first live queen import since the early 2000's, and the first introduction of Varroa resistance genetics into Australia. These successes took significant effort from the project team and its collaborators but ultimately provide valuable practical experience and knowledge to facilitate future imports of bee semen and live queens.

Our experience found that bee semen may be the more feasible importation pathway in some respects, as all biosecurity checks can be completed before arrival and then stock is immediately available to the importer. However, this does require more expertise and effort from the exporter to provide bee semen that meets the health certificate requirements. In comparison, live queens are likely a preferred option for many exporters because it requires more standard hive management before export. The PEQ process for live queens is more logistically challenging for the importer but as we have demonstrated, is still feasible and likely to further improve with each new importation.

Importation of honey bee germplasm is not a trivial exercise and each activity will experience different challenges. However, we expect the lessons learnt here will give industry the confidence to pursue importation to strengthen Australia's honey bee population and vital pollination services.

KEQ2. Has the project met the needs of industry levy payers of improved pollination security?

This project was supported by levies from the avocado, almond and melon industries for its value in having secure pollination for horticulture. We believe this project has met the needs of industry for improved pollination security by removing significant barriers for Australia accessing Varroa resistant (or otherwise improved) honey bee stock from overseas breeding programs.

This increased opportunity for importation can be utilised in two ways to improve pollination security. The first is to pre-emptively introduce Varroa resistant stock into Australia to prepare our bee population for the impact of a Varroa mite incursion. Importantly, the Varroa resistant stock obtained through this project is not an endpoint for securing pollination services. Establishing the Varroa resistance trait in Australian honey bees before the arrival of Varroa mites has challenges that go beyond importation. Retaining Varroa resistance without exposure to the parasite will need innovative tools to support ongoing selective breeding and further introductions of desirable stock.

The second approach is to prepare the honey bee industry for a rapid importation response of resistant stock in a future Varroa mite incursion. Maintaining capability in the system will be a challenge that will need regular imports or training scenarios, similar to our trial import in PEQ. However, the demand for new genetic material from the honey bee industry goes beyond Varroa resistance and following from this project we can expect further importations for commercial production traits.

It is likely that the combination of both approaches will be needed to have the best outcomes for improving pollination securing against future mite incursions.

KEQ3. Have regular project updates been provided to industry levy payers? Have regular project updates been provided to the honey bee industry?

In addition to the six-monthly project progress reports, we have provided project summaries and updates to industry levy payers for dissemination to their members (see Outputs). Unfortunately, delays with importations during the project reduced our opportunity to provide more regular updates to industry.

The honey bee industry has received project updates through a conference presentation, industry article on importation (extensionAUS Professional Beekeepers) and a summary report to AHBIC and AQBBA.

KEQ4. How appropriate was extension material to industry levy payers? How appropriate was extension material to the honey bee industry?

Extension material was provided in formats consistent with other projects e.g. industry articles, progress reports, and are likely appropriate for providing effective project updates to industry levy payers and the honey bee industry.

Project personnel are available and engaged with industry associations at national and state levels and will continue to play a role in industry discussions around the importation of honey bee germplasm. The donation of some stock from this project to the National Bee Breeding Program and the engagement of project personnel in this program is an important point of connection between this project and future bee breeding activity in Australia

We were fortunate to have the project featured on ABC Landline, which provided valuable visibility for the project and its outcomes to a wide public and industry audience.

KEQ5. What efforts did the project make to improve efficiency?

The nature of this project did not provide much opportunity to improve efficiency, as each activity was new ground and did not involve repeated practices to further optimise, e.g. field collection, sample analysis. However, the project aimed to have improved efficiency through the domestic trial of the PEQ facility and implementation of changes to support queen success.

The logistical framework published above, coupled with future publishing of the outcomes and learnings from this project should considerably improve the efficiency of future importers navigating the import permit and health certificate requirements for both semen and live queen importations. Many of the challenges in this area were un-foreseen prior to this project.

Working with Arista Bee Research was also an important part of ensuring project efficiency. Before starting the project, we underestimated the level of input needed from the exporting country collaborator providing the bee stock. Arista Bee Research brought vital experience to the project to help identify and navigate the logistical challenges in preparing and safely importing bee semen and live queens.

Recommendations

Recommendations to Department of Agriculture, Water and Environment

1. Implementation of changes to the PEQ process to improve the success of future live queen imports. This includes
 1. updating PEQ work instructions to enable greater beekeeper involvement in bee handling procedures
 2. simple practical improvements to the heating and lighting within flight cages to support nucleus hive management.
 3. ensuring clear approval for the use of nucleus and cell starter colonies brought to PEQ during the PEQ consignment
2. Consideration of reviewing the 14-day incubation of imported queens for tracheal mite risk mitigation. This step greatly increases the risk of queen death in PEQ. There are alternative risk mitigation measures that could be adopted that can reduce the impact on queen bees while maintaining effective biosecurity. For instance, source colonies could be tested pre-export and nucleus colonies could be tested in PEQ. Further, DNA testing of pooled bee samples for tracheal would also greatly improve efficiency.
3. Utilisation of state licensing requirements to provide additional safeguards against introducing unwanted viruses. This has greater flexibility than the Commonwealth import policy to allow virus testing to occur for queen imports, enable longer-term stock monitoring, and can adapt to new virus threats.

Recommendations to the honey bee Industry:

1. Consideration of forming a collaborative approach between interested parties in sourcing imported stock. The scale and complexities of germplasm importation are likely beyond the capacity of individual commercial operations and would benefit from collaboration.
2. Investigation of novel tools/techniques to assess the level of Varroa resistance without direct exposure to mites. This is a unique challenge for Australia that needs to be addressed in order to effectively pre-emptively establish Varroa resistance in Australia.

Refereed scientific publications

No scientific publications currently published from this project.

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Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

Acknowledgements

We are greatly appreciative of the assistance, expertise and effort provided by our project collaborators to achieve the outcomes of this challenging project.

BartJan Fernhout of Arista Bee Research was critical to the success of this project. BartJan and his team provided invaluable experience and expertise as well as considerable effort to help the project overcome numerous challenges to successfully import Arista honey bee stock into Australia.

DAWE were very supportive of the project in facilitating the import process and PEQ staff working with us to ensure the best outcomes for the live queen import.

AgriBio in VIC (Linda Zheng, Lea Rako, Mark Blackett, Fiona Constable) were fantastic providing the diagnostic testing for viruses and Africanised bees for both imports, all while navigating COVID-19 lockdowns and restrictions.

The AQBBA, AHBIC and When Bee Foundation were also vital to getting this project off the ground to be supported by Hort Innovation from avocado, almonds and melons industry levies.

Appendices

Appendix 1. Bee semen import conditions.

Permit conditions

It is the importer's responsibility to ensure that the following permit conditions are met in relation to each consignment. Where more than one set of permit conditions is shown for a good please read each set of conditions to determine which applies to a specific consignment.

1. Bee semen

This section contains permit conditions for the following commodity (or commodities):

1. Animal reproductive material

1.1. Biosecurity Pathway

The import conditions can be found in the official health certificate requirements. Approved countries of origin:

Austria, Belgium, Bulgaria, Canada, Croatia, Cyprus, Czechia (Czech Republic), Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Japan, Latvia, Lithuania, Luxemburg, Malta, Netherlands, New Zealand, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and the United Kingdom.

a. Variation to standard import conditions

The standard the Department of Agriculture, Water and the Environment import conditions state that:

Official health certification requirements for honey bee (*Apis mellifera*) semen:

The country of export is free from Cape honey bees (*A. mellifera capensis*). The country of export meets ONE of the following two conditions (Strike through and initial the option that does not apply):

Please note: if selecting option 2, all three conditions within this option must be met.

1. The country of export is free from Africanised honey bees (*A. mellifera scutellata* and its hybrids) and the country does not share a land border with a country where Africanised honey bees are known to be present.

OR

2. a) The country of export is free from Africanised honey bees but the country shares a land border with a country where Africanised honey bees are known to be present OR the semen for export has been sourced from colonies in a zone recognised by Australia to be free from Africanised honey bees within a country where Africanised honey bees are known to be present.

AND

b) At least 10 worker or drone honey bees from the colonies from which the semen was sourced were randomly sampled and tested within three months prior to export. The results of mitochondrial DNA testing of this selection has shown that that colonies were not Africanised.

AND

c) The exporter has confirmed that the drones producing the semen in the consignment have been confined to the colonies of origin by a queen excluder and have not been permitted free flight prior to semen collection.

Having considered the information provided, the Department of Agriculture, Water and the Environment has determined that:

The following are acceptable alternative statements for this permit only, relating to certification of Cape honey bee and Africanised honey bee status in the Netherlands:

Cape honey bees:

At the time of certification there is no indication that Cape honey bees (*A. mellifera capensis*) do occur in The Netherlands.

Africanised honey bees:

1. At the time of certification there is no indication that Africanised honey bees (*A. mellifera scutellata* and its hybrids) do occur in The Netherlands.

AND

2. Within the last three months prior to certification at least 10 worker or drone honey bees from the colonies from which the semen is collected were randomly sampled and tested for mitochondrial DNA. The result of this test shows that the sampled bees are not Africanised.

AND

3. The drones producing the semen for this consignment have been confined by a queen excluder and have not been flying free prior to semen collection.

All other health certification requirements in Appendix 1 must be present on the health certificate. All other import conditions must be met in full. The dispensation/equivalence outlined above must be included on the official health certification that accompanies the animal(s) to Australia (or the certifying official must sign and endorse the conditions outlined under this permit case).

b. The inspecting biosecurity officer must be advised of the entry number prior to inspection.

c. It is the responsibility of the person in charge of the goods to identify and to ensure they have complied with requirements of any other regulatory and advisory bodies prior to and after importation. It is the responsibility of the person in charge of the goods to arrange for any additional testing for genetic and endemic infectious diseases, or for movement of animals or genetic material into certain animal health zones within Australian territory.

d. One person in charge of the goods as listed on the import permit, or a nominated agent, must be accessible to biosecurity officers and accept responsibility for ensuring that all import conditions are met, including the inspection by the department.

e. Consignments must be addressed and sent to the Australian Government Department of Agriculture, Water and the Environment at the port of arrival. Each consignment must be accompanied by a valid import permit or by means to allow the identification of the import permit and the Official health certificate as required by these conditions.

f. Consignments must not be sent through international mail.

g. The department must be given at least three (3) working days notice of the intended arrival of the honey bee semen into Australian territory. This should be done by emailing/faxing the department's office (Appendix 2) located in the Australian state in which the bee semen will arrive.

h. The person in charge of the goods or agent must make an appointment for an inspection of the goods and documentation by the department.

i. The person in charge of the goods, as listed on the import permit, or a nominated agent will be required to be present at the inspection.

j. The department will hold the consignment until the inspection is complete.

k. Certification must be provided to verify freedom from disease(s) and other import condition requirements.

To demonstrate compliance with this requirement you must present the following on a Health certificate:

Evidence that all of the requirements on the Official Health Certificate have been met. In addition to meeting the department's Minimum documentary and import declaration requirements policy, the Official Health Certificate must comply with the following:

1. The document must be written in a language understood by the Official Government Veterinarian or Government Apiary Officer of the exporting country.

2. The document must contain the Department of Agriculture, Water and the Environment import permit number.

3. The document must name the country of export (country of residence of all donors).

4. The document must contain the name of the certifying government Ministry or Department.

5. The document must meet all requirements of the Official Health Certification conditions.

6. The document must contain the signature, date and stamp of the Official Government Veterinarian or Government Apiary Officer on each page (with the stamp of the government veterinary administration). The date must conform to the day/month/year format. Other documents e.g. laboratory reports and tables which form part of the extended health certification must also contain this information. Certification must comply with Chapter 5.2 of the OIE Terrestrial Animal Health Code, including that any manual deletions to the health certificate must be signed and stamped by the Official Government Veterinarian or Government Apiary Officer.
7. The document must contain the name of the Official Veterinarian or Apiary Officer of the government of the exporting country.
8. The document must include the title of the Official Government Veterinarian or Government Apiary Officer.
9. The document must contain the email contact details of the Official Veterinarian or Apiary Officer of the government of the exporting country.
10. The document must contain the fax number of the Official Veterinarian or Apiary Officer of the government of the exporting country.
11. The document must contain the telephone number of the Official Veterinarian or Apiary Officer of the government of the exporting country.
12. The document must contain the address of the Official Veterinarian or Apiary Officer of the government of the exporting country.
13. The Department of Agriculture will accept copies of documents where each page bears the original signature, date and stamp of the Official Government Veterinarian or Government Apiary Officer.
14. The health certificate must conform to the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code 2009.
15. Any corrections made to the Official Health Certificate must be struck through, remain legible and be signed and stamped by the Official Government Veterinarian or Government Apiary Officer (Note: Correction fluid must not be used).

l. Arrival in Australian territory

On arrival in Australian territory your goods will be subject to biosecurity control where they will remain until a biosecurity officer has completed a physical inspection of the goods and all required documentation. If any conditions on this permit are not fully met, the goods must remain under biosecurity control until all issues are resolved, or until the goods are exported from Australia. The person in charge is responsible for any additional costs related to resolving any non-compliance issues associated with this permit. Goods will not be released from biosecurity control until all conditions for importation have been fulfilled.

- m. Consignments that do not meet the import conditions of the department will either remain under biosecurity control, be exported or disposed of without recompense.
- n. Under the Biosecurity Charges Imposition (General) Regulation 2016 and Chapter 9, Part 2 of the Biosecurity Regulation 2016, fees are payable to the Department of Agriculture, Water and the Environment for all services. Detail on how the department applies fees and levies may be found in the Charging guidelines.
- o. In addition to the conditions for the goods being imported, non-commodity concerns must be assessed including container cleanliness, packaging and destination concerns, and may be subject to inspection and treatment on arrival. Please refer to the Non-Commodity Cargo Clearance BICON case for further information.

Appendix 2.

Permit conditions

It is the importer's responsibility to ensure that the following permit conditions are met in relation to each consignment. Where more than one set of permit conditions is shown for a good please read each set of conditions to determine which applies to a specific consignment.

1. Live honey bees

This section contains permit conditions for the following commodity (or commodities):

1. Bees

1.1. Biosecurity Pathway

a. This import permit is granted subject to conditions.

Contravening a condition of the permit may constitute a criminal offence or a contravention of a civil penalty provision. You may be liable to a civil penalty for giving false or misleading information. Giving false or misleading information is a serious offence.

b. If the permit holder, their agent or any person in charge of the goods becomes aware that information provided to a biosecurity official for the purpose of any of the below conditions was incomplete or incorrect, they must as soon as practicable:

1. notify the Director of Biosecurity,
2. give the Director of Biosecurity the additional or corrected information.

c. Import permit scope

This permit allows for the importation of multiple consignments of live queen European honey bees (*Apis mellifera*) each with 6–12 escort worker bees. Drones are not permitted to be imported. A separate import permit is required for each apiary¹ from which bees for export originate. An apiary is considered to be all hives at the one location owned by the same person. Hives at different locations but owned by the same person are separate apiaries. Each consignment of queen bees and escorts from a single apiary must be accompanied by a valid import permit, or by means to allow the identification of the import permit.

d. It is the responsibility of the permit holder, their agent or any person in charge of the goods, to ensure compliance with all requirements of any other regulatory and advisory bodies prior to and after importation. Consignments that do not meet the department's import conditions will remain under biosecurity control, and may be exported or disposed of without recompense.

e. Prior to arrival in Australian territory

The permit holder, their agent or person in charge of the goods will need to make a booking at the Australian government quarantine facility prior to importing their animal. Bookings can only be made once an import permit has been granted by the department. Further details about Australian quarantine facilities and contact details are available on the department's website.

f. Certification requirements

The permit holder, their agent or person in charge of the goods must provide evidence about the location and health status of the apiary from which the bees originated. To demonstrate compliance with this requirement you must present the following on an Owner declaration:

1. Identification of the animals in the consignment (species, number of queen bees and number of escorts)
2. The name and address of the apiary from which the bees originated
3. The name and address of the person in Australia that will be in charge of the imported bees
4. The following declarations:

4.1. "The queen bees and escorts in this consignment originated from progeny that were bred and reared in (country of origin) and are from an apiary located at (address) of which I am the registered owner".

4.2. “I have knowledge of the Australian import requirements”.

4.3. “The queen bees and escorts in this consignment are from colonies that are clinically healthy and have no visible evidence of the following hazards:

- a. Acarapisosis (tracheal mite) (*Acarapis woodi*)
- b. Africanised bees (*Apis mellifera scutellata* and its hybrids)
- c. Braula fly (*Braula coeca*)
- d. Cape bees (*A. m. capensis*)
- e. Tropilaelaps (*Tropilaelaps* spp.)
- f. Varroosis (*Varroa destructor* and *V. jacobsoni*)”.

4.4. “The queen bees and escorts in this consignment are themselves free of physical and behavioural abnormalities and show no visible evidence of the hazards listed above”.

4.5. “The country or region of export is free from tracheal mite (*Acarapis woodi*)”; or “The colonies from which the bees for export to Australia originated were treated according to manufacturer’s directions with a commercially available formic acid product within 7 days prior to export for a minimum period of 24 hours”. The product name, manufacturer, date/s of application and dose must be included.

4.6. “Before export, the colonies from which the bees for export to Australia originated were continuously treated, according to the manufacturer’s directions, with a product of demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp”. The product name, manufacturer, date/s of application and dose must be included.

4.7. “The escort bees accompanying each queen bee originated from the same hive/colony as the queen bee in each instance”. The owner’s declaration must be provided by the owner of the apiary from which the queen bees and escorts originate. The original owner’s declaration must accompany the consignment and be signed and dated by the owner and witnessed by the government apiary officer or official veterinarian. A separate owner’s declaration is required for each apiary from which bees for export are sourced. The owner’s declaration must be written in English and a language understood by the official veterinarian or government apiary officer of the country of export.

g. The permit holder, their agent or person in charge of the goods must provide evidence that the consignment has been certified by the official veterinarian, or a government apiary officer on behalf of the official veterinarian, of the veterinary administration of the exporting country. Certification must be provided to verify freedom from disease(s) and specify the health status of each animal. To demonstrate compliance with this requirement you must present the following on a Health certificate:

1. Identification of the animals in the consignment (breed, number of queen bees and number of escorts)
2. The name and address of the apiary from which the bees and escorts originated
3. The name and address of the person in Australia that will be in charge of the imported bees
4. The following declarations:

4.1. “After due enquiry, I have no reason to doubt the owner’s declaration providing assurances that the owner has knowledge of the Australian import requirements and has complied with the requirements in the owner’s declaration.”

4.2. “The bees in the export consignment originate from the apiary described in the owner’s declaration and were bred and raised in (country of origin)”.

4.3. “Within 7 days before export, I examined the source colony of the bees for export and found it to be clinically healthy and free from visible evidence of the following hazards:

- a. Acarapisosis (tracheal mite) (*Acarapis woodi*)
- b. Africanised bees (*Apis mellifera scutellata* and its hybrids)
- c. Braula fly (*Braula coeca*)
- d. Cape bees (*A. m. capensis*)

e. *Tropilaelaps* (*Tropilaelaps* spp.)

f. Varroosis (*Varroa destructor* and *V. jacobsoni*)”.

The date of examination must be included on the health certificate.

4.4. “The country or region of export is free from tracheal mite (*Acarapis woodi*)”; or “It has been declared to me, and I have no reason to doubt, that the colonies, from which the queen bees and their escorts originated were treated according to the manufacturer’s directions with a commercially available formic acid product within 7 days prior to export for a minimum period of 24 hours”. The product name, manufacturer, date/s of application and dose must be included.

4.5. “It has been declared to me, and I have no reason to doubt, that the colonies, from which the queen bees and their escorts originated, were continuously treated with a product of demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp. immediately prior to export and for a duration according to the manufacturer’s directions”. The product name, manufacturer, date/s of application and dose must be included.

4.6. “The country of export is free from Cape bees (*A. mellifera capensis*)”.

4.7. “The country of export is free from Africanised bees (*A. mellifera scutellata* and its hybrids) and the country does not share a land border with a country where Africanised bees are known to be present; or The bees for export have been sourced from zones or countries that have been approved by the Department of Agriculture, Water and the Environment as being free from Africanised bees but are within or share at least one land border with a country that has Africanised bees; and Within 7 days before export, I visually inspected the source colonies for characteristics, including behavioural, of Africanisation and no evidence of Africanisation was observed (date of inspection must be included); and Testing of mitochondrial lineage has been performed within 30 days prior to export on a randomly selected sample of at least 10 worker bees representing the progeny of each of the queen bees for export and no evidence of Africanisation was present”. Date of test must be included. An original health certificate must accompany the consignment of bees. A separate health certificate must be provided for each apiary from which queens and associated escorts are sourced. The health certificate must be written in English and a language understood by the official veterinarian or government apiary officer of the country of export. An official veterinarian or a government apiary officer must sign, date and stamp the health certificate and all associated documents, and mark every page with the stamp of the veterinary administration of the exporting country. The department will only accept copies of documents where each page bears the original signature, date and stamp of the official veterinarian or government apiary officer.

h. Variation to standard import conditions

To demonstrate compliance with this requirement you must present the following on a Veterinary Certificate:

Having considered the information provided, the department has determined that:

Consignments of bees that originate in Belgium and are exported from the Netherlands will be accepted, provided all import conditions are met and the official health certificate must state the country of origin as Belgium and country of export as Netherlands. The following is an acceptable alternate statement for the official health certificate concerning treatment of the colony from which the bees for export originate with a product with demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp.:

'It has been declared to me, and I have no reason to doubt, that before export, the colonies from which the bees for export to Australia originated were treated, according to the manufacturer's directions and for a duration recommended by the manufacturer, with a product of demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp. The manufacturer's recommended duration of treatment concluded at the time the bees were removed from the colony and prepared for export.' The product name, manufacturer, date/s of application and dose must be included.

The following are acceptable statements as alternatives to the standard statements for Cape and Africanised bee status in the Netherlands:

Cape honey bees:

At the time of certification there is no indication that Cape honey bees (*A. mellifera capensis*) do occur in The Netherlands.

Africanised honey bees:

1. At the time of certification there is no indication that Africanised honey bees (*A. mellifera scutellata* and its hybrids) do occur in The Netherlands.

AND

2. Within the last three months prior to certification at least 10 worker or drone honey bees from the colony from which the Queen and escort bees for export originate were randomly sampled and tested for mitochondrial DNA. The result of this test shows that the sampled bees are not Africanised.

All other standard declarations must also be present on the health certificate. All other import conditions must be met in full. The dispensation/approved variation to the import conditions outlined above must either be included on the veterinary health certification that accompanies the animal(s) to Australian territory or the certifying official must sign and endorse the conditions outlined under this permit.

i. Variation to standard import conditions

Having considered the information provided, the department has determined that: Standard import conditions state that a minimum of 6, up to a maximum of 12, escort worker bees may be imported with each imported queen bee. For this permit only, up to 20 escort bees per queen bee may be imported. All applicable post-entry quarantine requirements will apply to all escorts.

For the Owner Declaration for European honey bees exported to Australia:

Consignments of bees that originate in Belgium and are exported from the Netherlands will be accepted, provided all import conditions are met and the owner's declaration must state the country of origin as Belgium and country of export as Netherlands. The following is an acceptable alternate statement for the owner declaration concerning treatment of the colony from which the bees for export originate with a product with demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp.:

Before export, the colonies from which the bees for export to Australia originated were treated, according to the manufacturer's directions and for a duration recommended by the manufacturer, with a product of demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp. The manufacturer's recommended duration of treatment concluded at the time the bees were removed from the colony and prepared for export. The product name, manufacturer, date/s of application and dose must be included. All other import conditions must be met in full. The dispensation/approved variation to the import conditions outlined above must either be included on the veterinary health certification that accompanies the animal(s) to Australian territory or the certifying official must sign and endorse the conditions outlined under this permit.

j. Travel to Australia

Queen bees must be imported as manifested air freight in packaging that prevents the escape of hazards of biosecurity concern and meets the International Air Transport Association (IATA) regulations for transport of live bees. The import permit number or health certificate number and the words 'live bees' must be clearly visible on the outside of the packaging.

k. Consignments must be addressed to:

Department of Agriculture, Water and the Environment, care of Mickleham PEQ facility, 135 Donnybrook Rd
Mickleham VIC 3064

l. The permit holder, their agent or person in charge of the goods must make an appointment for the department to inspect the bees and associated documentation at Mickleham post entry quarantine facility. The person in charge of the goods may be present at this inspection.

m. At least seven (7) days prior to the expected arrival of the bees, the department must be provided with written notification of the flight number and expected arrival time. This notification must include:

1. the Department of Agriculture, Water and the Environment import permit number
2. the entry number
3. a phone number for the permit holder, their agent or person in charge of the goods
4. the flight number
5. the date and estimated time of arrival

6. the air waybill number

7. the number of queen bees and accompanying escorts being imported.

n. Arrival in Australian territory

On arrival in Australian territory, imported bees will be taken by the department to the bee facility at Mickleham post entry quarantine facility.

o. On arrival in Australian territory, or any stage of post-entry quarantine, if the certification or the imported bees are found not to meet Australia's biosecurity requirements, the entire consignment may be returned to the country of origin at the expense of the permit holder, their agent or person in charge of the goods or be disposed of without recompense. Any progeny from that consignment of imported bees, and other colonies and equipment that the department considers to have come into contact with affected bees may be disposed of without recompense.

p. No liability will be accepted by the Director of Biosecurity for deaths of imported bees subject to biosecurity control or the failure of establishment of imported queens in nucleus hives, or the failure of any larval graft.

q. Post-entry quarantine requirements

1. During the post-entry quarantine period, the permit holder, their agent or person in charge of the goods is responsible for providing beekeeping equipment (frames, boxes etc), local bees as escorts for imported queen bees, nucleus colonies, and other resources as required.

2. All routine maintenance procedures associated with the imported bees are carried out by biosecurity officers familiar with bee management. Permit holders, their agents or person in charge of the goods may be permitted to be present, or in some instances assist with post-entry quarantine procedures. Approval must be requested, in writing, to officers at the post-entry quarantine facility at least 2 business days prior to attendance. For all bee management procedures within the post-entry quarantine facility, personnel (including the nominated grafter) must use protective clothing provided by the department which must remain in the post-entry quarantine facility.

3. Removal from post-entry quarantine will be restricted to only grafted queen cells produced from the nucleus colony. At the end of the quarantine period, the imported queen, adult bees, brood and all other components comprising the nucleus colony will be disposed of.

4. If, at any stage during the post-entry quarantine period, hazards of biosecurity concern are identified, the entire consignment may be returned to the country of origin at the expense of the permit holder, their agent or person in charge of the goods or be disposed of without recompense.

5. The permit holder, their agent or any person in charge of the goods must comply with state legislation regarding the registration and movement of bees and associated equipment within Australian territory. This includes the movement and keeping of hives at the quarantine facility, and release of the grafted queen cells from quarantine.

r. All consignments will be held under biosecurity control at the post-entry quarantine facility for a minimum isolation period. During that time, the consignment must comply with each stage of the post-entry quarantine process as follows:

1. Document assessment and initial inspection of all imported bees on arrival. Imported escort bees will also be euthanised for internal inspection by department entomologists for the presence of parasitic mites (tracheal mite, *Tropilaelaps* spp. and *Varroa* spp.).

2. Minimum 14 day hibernation of imported queen with new Australian escorts (escorts must be sourced from local support hives and must not be older than 4 days).

3. Examination of Australian escorts, both internally and externally, by a department entomologist for the presence of parasitic mites (tracheal mites, *Tropilaelaps* spp. and *Varroa* spp.).

4. Imported queen introduced to nucleus colony (nucleus colony must be sourced from a colony that has been inspected by a biosecurity officer and found to be free of visible evidence of disease. Nucleus colony must include a frame of comb containing uncapped larvae).

5. Treatment of the nucleus colony with a product with demonstrated efficacy for the control of *Varroa* spp and *Tropilaelaps* spp.

6. Inspection of hive debris for the presence of *Varroa* spp. and *Tropilaelaps* spp.

7. At least 14 days after acceptance of the imported queen, inspection of all larvae/pupae from a frame containing brood from the nucleus colony.
8. Testing of at least 10 pupae from the imported queen at a government approved laboratory for the presence of mitochondrial DNA of Africanised bees. Additional Africanisation testing may be required.
9. If all requirements are met and no issues of biosecurity concern arise, permission may be granted to graft eggs/larvae from the nucleus colony. Eggs and larvae grafted into queen cells are the only live materials permitted to be removed from the post-entry quarantine facility unless prior written approval has been provided by the department.
10. The permit holder, their agent or person in charge of the goods must provide the name and address of the owner/s of all apiaries where bee material released from post-entry quarantine is sent.
11. The permit holder, their agent or person in charge of the goods must notify the department in writing when all grafting is completed. When notification is received, the imported queen bees, all remaining adult bees, brood, frames, boxes and other components comprising the nucleus colony will be disposed of. Applicable fees for an imported queen bee cease with the disposal of that queen bee and the associated colony.
- s. Under the Biosecurity Charges Imposition (General) Regulation 2016 and Chapter 9, Part 2 of the Biosecurity Regulation 2016, fees are payable to the Department of Agriculture, Water and the Environment for all services. Detail on how the department applies fees and levies may be found in the Charging guidelines.
- t. In addition to the conditions for the goods being imported, non-commodity concerns must be assessed including container cleanliness, packaging and destination concerns, and may be subject to inspection and treatment on arrival. Please refer to the Non-Commodity Cargo Clearance BICON case for further information.

Appendix 3. Licence Authorising the bringing of Bees into Victoria from livestock imported from the Netherlands

Pursuant to section 10(4) of the *Livestock Disease Control Act 1994* and on reasonable suspicion of infection with an exotic disease, as European honey bees that are produced from livestock that has travelled on any land, being the Netherlands, in accordance with s 135(3) of the *Livestock Disease Control Act 1994*, the Secretary to the Department of Jobs, Precincts and Regions issues this licence:

This licence authorises the import of live grafted larvae and daughter queens into Victoria bred from a European honeybee (*Apis mellifera*) queen (source queen) imported from the Netherlands, subject to the following conditions:

Grafted larvae and daughter queens may only be imported into Victoria via the Department of Agriculture, Water and Environment post entry quarantine facility in Mickleham. Drones and worker bees are not permitted to be imported.

This licence allows for the import of live grafted larvae and the daughter queens produced only from the source queen specified on this licence during the approved period.

The daughter queens have been inseminated with semen obtained from drones bred in Victoria.

The person responsible for the consignment of the source queen must advise the Department of Jobs, Precincts and Regions (DJPR) at the earliest opportunity if there are any anticipated or actual variations to the consignment including changes to the entry period.

Grafted larvae and daughter queens can only be brought into Victoria after the following samples have been tested; the source queen abdomen and a minimum of 10 larvae from the daughter queen placed in the source queen colony and the escort bees that accompanied the source queen from the Netherlands and from the Victorian support colony during the quarantine period

AND

are found to be free from the following viruses; Deformed Wing Virus (variant A and variant B) and Slow bee paralysis virus and Acute bee paralysis virus and *Apis* rhabdovirus 1 and 2.

Testing of mitochondrial lineage has been performed within 30 days prior to export to Australia on a randomly selected sample of at least 10 worker bees representing the progeny of each of the source queens and no evidence

of Africanisation was present. The test result has been provided to DJPR.

In relation to the consignment of the source queen, the following declarations must be made via a completed Appendix 1 and 2 or via equivalent documentation approved by DJPR;

- 7.1. “The queen bees and escorts in this consignment originated from progeny that were bred and reared in (country of origin) and are from an apiary located at (address) of which I am the registered owner”.
- 7.2. “I have knowledge of the Australian and Victorian import requirements”.
- 7.3. “The queen bees and escorts in this consignment are from colonies that are clinically healthy and have no visible evidence of the following hazards:
 - a. Acarapisosis (tracheal mite) (*Acarapis woodi*)
 - b. Africanised bees (*Apis mellifera scutellata* and its hybrids)
 - c. Braula fly (*Braula coeca*)
 - d. Cape bees (*A. m. capensis*)
 - e. Tropilaelaps (*Tropilaelaps* spp.)
 - f. Varroosis (*Varroa destructor* and *V. jacobsoni*)”.
- 7.4. “The queen bees and escorts in this consignment are themselves free of physical and behavioural abnormalities and show no visible evidence of the hazards listed above”.
- 7.5. “The country or region of export is free from tracheal mite (*Acarapis woodi*)”; or “The colonies from which the bees for export to Australia originated were treated according to manufacturer’s directions with a commercially available formic acid product within 7 days prior to export for a minimum period of 24 hours”. The product name, manufacturer, date/s of application and dose must be included.
- 7.6. “Before export, the colonies from which the bees for export to Australia originated were continuously treated, according to the manufacturer’s directions, with a product of demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp”. The product name, manufacturer, date/s of application and dose must be included.
- 7.7. “The escort bees accompanying each queen bee originated from the same hive/colony as the queen bee in each instance”.

The following variations to the import conditions in section 7 will be accepted and must be present on the Veterinary Certificate.

- 8.1 Consignments of bees that originate in Belgium and are exported from the Netherlands will be accepted, provided all import conditions are met and the official health certificate must state the country of origin as Belgium and country of export as Netherlands.
- 8.2 The following is an acceptable alternate statement for the official health certificate concerning treatment of the colony from which the bees for export originate with a product with demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp.: 'It has been declared to me, and I have no reason to doubt, that before export, the colonies from which the bees for export to Australia originated were treated, according to the manufacturer's directions and for a duration recommended by the manufacturer, with a product of demonstrated efficacy for the control of *Varroa* spp. and *Tropilaelaps* spp. The manufacturer's recommended duration of treatment concluded at the time the bees were removed from the colony and prepared for export.' The product name, manufacturer, date/s of application and dose must be included.
- 8.3 The following are acceptable statements as alternatives to the standard statements for Cape and Africanised bee status in the Netherlands:

Cape honey bees:

at the time of certification there is no indication that Cape honey bees (*A. mellifera capensis*) do occur in The Netherlands.

Africanised honey bees:

at the time of certification there is no indication that Africanised honey bees (*A. mellifera scutellata* and its

hybrids) do occur in The Netherlands and

within the last three months prior to certification at least 10 worker or drone honey bees from the colony from which the source queen and escort bees for export originate were randomly sampled and tested for mitochondrial DNA. The result of this test shows that the sampled bees are not Africanised.

8.4 Standard import conditions state that a minimum of 6, up to a maximum of 12, escort worker bees may be imported with each imported queen bee. For this licence only, up to 20 escort bees per queen bee may be imported.

The results of all tests undertaken for any exotic pest or disease pre and post entry must be provided to DJPR.

Any suspicion of the presence of an exotic pest or disease pre or post entry must be immediately reported to the Emergency Plant Pest Hotline on 1800 084 881.