Final Report

Strengthening and enabling effective pollination for Australia

Project leader:

Lisa Evans

Delivery partner:

Plant and Food Research NZ

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Strengthening and enabling effective pollination for Australia (PH15000)

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Level 7
141 Walker Street
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Telephone: (02) 8295 2300

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Public summary

For many crops, limited information exists on best practices for pollination and there is a particular gap in understanding how reliant crop production is on honey bees versus other pollinators. Honey bee pests and diseases, including the *Varroa* mite (*Varroa destructor*), have potential to dramatically alter 'passive' crop pollination. Our research program aimed to identify key pollinators across various Australian crops and produce recommendations for pollination management to optimise sustainable yields and minimise the risk of pollination failure.

The project focused on three main research areas:

1. Establishing and disseminating current best practice information for crop pollination.
2. Identifying crop-specific pollinators, understanding pollination requirements, and developing diverse pollination strategies.
3. Assessing innovations to enhance honey bee health.

We successfully identified effective pollinating insects for several different crops including: avocado, macadamia, watermelon, blueberry and lychee, and flower visitors to papaya and almond. The relative abundance of these insects varied across crops, regions, and individual farms.

Honey bees were the most abundant pollinators in five out of the seven crops studied. Notably, honey bees were the predominant flower visitor to watermelon grown in the Queensland region during spring and early summer. They were found to be highly effective pollinators of melons, likely contributing to the majority of fruit set during this period. Considering the recent arrival of *Varroa* in Australia and its potential to impact the supply of managed honey bee colonies for pollination, it is critical that growers continue to deploy honey bees as managed pollinators, and use their colonies as efficiently as possible. Based on results from manipulation trials, we provide recommendations for honey bee hive placement, stocking rates, and colony feeding regimes, to standardise and enhance their pollination potential for crops such as watermelon and macadamia.

Many crops studied (e.g., lychee, avocado, and macadamia) were visited by a diverse assemblage of non-honey bee insects. The abundance of these insects varied between regions and farms within a region – a relatively dominant insect at one site might be represented by only a few individuals at another site, underscoring the role of site and temporal variation in pollination ecology, and the need for practical on-farm monitoring and assessment systems. We demonstrate that some of these insects, including native bees (e.g., *Tetragonula* sp.), are effective crop pollinators, and that certain crops and production areas may be reliant on wild, non-honey bee pollinators, such as moths (papaya) and flies (for fruit set in avocado grown in Sunraysia). Considering a broader range of managed species (e.g., *Tetragonula*) alongside honey bees and encouraging wild pollinators (such as other species of native bees and flies) could positively impact crop yields and bolster the resilience of pollination services. We have compiled best practice pollination information for our focal crops, presented in concise, crop-specific manuals available on the Hort Innovation and Plant Health Australia websites.

To support the growth of the Australian beekeeping industry and ensure adequate pollination services, it is necessary to manage honey bee health issues that hinder colony survival and growth. Our research program also explored new monitoring methods for American foulbrood (*Paenibacillus larvae*) and behaviours in honey bees conferring resistance to *Varroa* mite, such as *Varroa* Sensitive Hygiene (VSH). We identified a promising genetic marker (SNP 9-9224292) that has potential to be used to select for VSH traits in queen breeding programs in Australia and New Zealand and as part of individual beekeepers' queen management.

Keywords

Crop pollinators, pollinator efficiency, pollination requirements, alternative pollinators, honey bee management, honey bee health, *Varroa* Sensitive Hygiene, VSH, watermelon, macadamia, blueberry, lychee, avocado, almond, and papaya.

Introduction

Pollination-dependent crops in Australia have been estimated to be worth over $AUD4.3 billion per annum based on 2005–2006 data, with a direct contribution by honey bees (*Apis mellifera*) estimated to be over $1.6 billion (Hafi et al. 2012). If honey bee pollination was catastrophically lost, the economic impact would be significantly greater than $1.6 billion as even relatively small declines in pollination could make it uneconomic to grow many crops. While a total loss of honey bee pollination may seem an unlikely scenario, Australia is particularly vulnerable to some losses because of its reliance on incidental honey bee pollination rather than managed pollination (Keogh et al. 2010), because, until very recently it held the status as the world's last continent that is free of the parasitic mite *Varroa destructor* (Cunningham et al. 2002, Hafi et al. 2012). A significant proportion of Australia's pollination-dependent industries may not have sufficient access to managed honey bee colonies following the spread of *Varroa*.

Australia has widespread feral colonies of honey bees, although density estimates vary widely from as high as 150 colonies/km^2 (Oldroyd et al. 1997) to as low as 0.1 colonies/km^2 (Garibaldi et al. 2013). This discrepancy in estimates probably reflects a patchy distribution of colonies that is dependent on intra-habitat distribution of nest, water, and floral resources as well as varied pressure from pests and disease (e.g., small hive beetle). As a result, the contribution of these feral colonies to crop pollination is likely to vary between sites and years. Whatever their contribution, these feral colonies are likely to be almost entirely eradicated within a few years following *Varroa* arrival (Goodwin & Van Eaton 2001, Hafi et al. 2012).

Honey bee hives managed for honey production are another source of incidental pollination. With a colony foraging radius of up to 5 km, the ~600,000 hives managed for honey in Australia make a significant contribution to horticultural productivity (Keogh et al. 2010). These hives are currently broadly distributed across much of the growing regions of Australia. Following the arrival of *Varroa* it is to be expected that a large proportion of small beekeeping operations and hobbyists will disappear due to the cost of managing *Varroa* (Hafi et al. 2012). The resultant aggregation of managed hives in a smaller number of large operations has the potential to significantly shift the distribution patterns of these managed hives and could lead to unexpected loss of pollination services in some regions.

In addition to introduced honey bees, a diverse assemblage of native bees, flies, and other pollinators make a contribution to pollination (Keogh et al. 2010; Goodwin 2012; Howlett et al. 2015; Rader et al. 2016). The level of this contribution is poorly understood for most crops, and is likely to be highly variable based on regional and seasonal differences, and within regions because of landscape management practices and wider habitat and resource availability (e.g., Blanche et al. 2006). Potential alternative managed pollinators already exist, such as native stingless bee colonies currently deployed in orchards in Queensland (Heard 1999). By quantifying the current contribution of honey bees versus other pollinators we will be able to assess the degree to which alternative managed and unmanaged species could substitute for incidental honey bee pollination.

To ensure consistent, optimised pollination, growers need access to crop-specific recommendations for pollinator management that take into account the pollination requirements of the crop and the behaviour of pollinators (Rollin & Garibaldi 2019). For most pollinator dependent industries, the development of crop specific pollinator management strategies can increase the volume, quality, and reliability of harvested crops (Keogh et al. 2010, Goodwin 2012).

Following the arrival of *Varroa*, direct chemical control of the mite is required to ensure managed honey bee colony survival (Goodwin & Van Eaton 2001). Despite Australia's much larger landmass, both Australia and New Zealand have similar numbers of managed hives (about 600,000). It is unlikely that current numbers of managed hives can meet Australia's crop pollination requirements in the absence of feral honey bees (Keogh et al. 2010). To ensure that the Australian beekeeping industry can grow to a sufficient size to provide required pollination service, is it critical to address honey bee health issues that impede colony survival and growth.

A proportion of feral honey bee colonies are likely to be infected with the brood disease American Foulbrood ('AFB',

Paenibacillus larvae) and are likely to be an important source of infection for managed hives (Goodwin et al. 1994). The presence of this disease in a managed hive on mainland Australia requires the colony to be destroyed, so control of this disease is important for the viability of beekeeping operations (Oldroyd et al. 1989). New Zealand has a world leading AFB disease control program, which our team has had a major part in designing (Goodwin et al. 2005; Goodwin 2006). *Varroa* incursions can affect the prevalence of AFB in managed colonies – when *Varroa* spread through NZ, there was a resultant doubling in the incidence of AFB in managed colonies due to robbing of dead feral colonies by managed hives (Goodwin et al. 1994; Goodwin 2005). If the same were to occur with the much larger feral bee population in Australia, it may prove to be a significant limiting factor in hive availability for pollination.

A key limitation in the control of AFB is the lack of a rapid diagnostic tool. Current methods rely on culturing bacterial colonies in vitro (Goodwin et al. 2005). Polymerase Chain Reaction (PCR) assays have been developed to detect and quantify AFB DNA, but these have not yet been assessed as a surveillance tool (Han et al. 2008; Martinez et al. 2010).

A naturally occurring genetic trait, *Varroa* Sensitive Hygiene (VSH), confers an advantage to colonies by increasing behaviours that limit the survival and reproduction of *Varroa* mites (Danka et al. 2008). This trait can be incorporated into queen breeding programs to offer beekeepers another option for controlling *Varroa* (Danka et al. 2011). However, the presence of the trait in a queen can only be detected by assessing bee behaviour in a functioning colony, a lengthy and expensive procedure with limited practical application in breeding programs (Villa et al. 2009). Genetic polymorphisms have been proposed as putative markers for VSH in global bee populations (Zakar et al. 2014), but this has not been tested as a tool to assist breeding programs. The identification and assessment of this strategy could provide a significant advantage to Australian beekeepers in fighting *Varroa* and other health threats.

To help ensure Australia's pollinator-dependent industries remain competitive post-*Varroa*, it is essential to develop crop-specific strategies that optimise pollination. Our program aimed to improve pollination in Australia through:

i) The development of crop-specific resources to help growers understand the pollination requirements of their crop.

ii) Identification of crop-specific pollinators and the development of practical pollination management recommendations for growers.

iii) Targeted management practices for two critical honey bee health issues: AFB and *Varroa*.

General methodology

To increase industry understanding of the importance of crop pollination and to help safeguard pollination practices, the current project activities focused on the following three objectives:

1. Establishing and disseminating current best practice information.
2. Identifying crop specific pollinators and developing diverse pollination strategies.
3. Assessing innovations to improve honey bee health.

Establishing and disseminating current best practice information

The New Zealand Institute for Plant and Food Research Limited (PFR) partnered with Plant Health Australia (PHA) to collate, design and disseminate resources describing best-practice pollination management for six of Australia's pollination dependent industries (watermelon, lychee, macadamia, blueberry, papaya, and avocado). These included four-page manuals for each selected industry. The information covered in the manuals includes various topics relevant to pollination, including:

- Flower biology and pollination requirements
- Growing systems
- Potential pollinators
- Assessing pollination/fruit set
- Honey bee hive stocking rates, timing of introduction, and placement.

These crop-specific manuals build on the general pollination guide written by Mark Goodwin of PFR, funded by Hort Innovation and Agrifutures (Goodwin, M., 2012. Pollination of crops in Australia and New Zealand. RIRDC).

Identifying pollinators and developing diverse pollination strategies

To identify crop-visiting insects and measure their abundances we surveyed/filmed/collected insect flower visitors using standardised methods for each crop. These data were collected for the following crops: watermelon, lychee, macadamia, blueberry, papaya, avocado, and also almond. The efficacy of the most abundant pollinators/taxonomic groups was determined for melon, blueberry, lychee and macadamia. For some of these crops, we determined whether there is a pollination deficit and the value of cross pollination (blueberry, lychee, avocado, and almond). For other crops we assessed management techniques for honey bees and native stingless bees and provide recommendations for improved pollination service delivery (watermelon and macadamia).

Assessing innovations to improve honey bee health

This project made progress towards the development of diagnostic tools to facilitate effective control of two key threats to honey bee health; AFB and *Varroa*. Our team assessed the surveillance potential of PCR assays that have been developed to detect and quantify AFB DNA. We also determined whether identified genetic markers correlate with VSH in local populations, which would potentially enable queen breeders to genetically select for VSH traits as well as enhance the queen management of individual beekeepers.

Crops and locations

Research into pollinators, their pollination efficacy, behaviour, and management where applicable, was conducted across seven different cropping industries in 19 different growing regions, in five Australian states (Figure 1). Six of these crops were our focus in this research program including: melon, blueberry, lychee, macadamia, papaya, and avocado. Two additional trials were conducted in a 7th crop – almond.

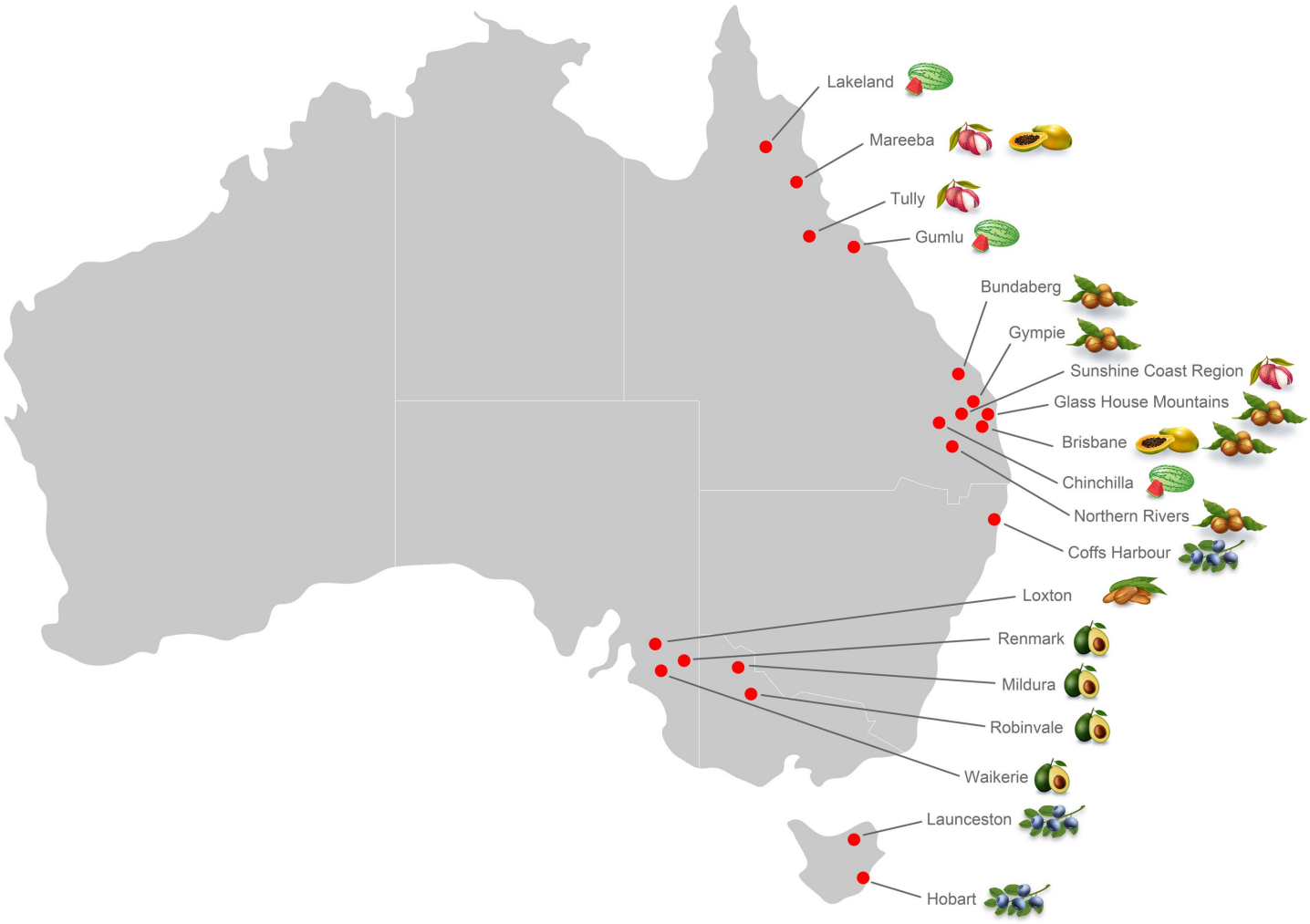


Figure 1. Map showing the crops and location of field trials conducted as part of PH15000.

Floral biology and standard pollination management

Watermelon

Watermelon are grown in all Australian states, with Queensland (QLD), Western Australia (WA), and New South Wales (NSW) being the biggest producers. Watermelon are produced on annual vines, which are planted in rotation for a longer growing season. In northern parts of Australia, multiple plantings per year are possible. Most production is of 'seedless' watermelon, which are grown on triploid plants – these require pollen from diploid 'seeded' varieties for pollination. Most growers plant either three or four seedless plants per single seeded plant. All plants produce both male and female flowers.

The farms visited in the current program were variable in their surroundings – some were surrounded by bush/scrubland, while others were in heavily modified environments with production of sugar cane and melons across wide areas. Most growers use managed honey bees for pollination, but the methods observed were varied. Some growers rented hives, which were deployed throughout the crop, while others kept their own hives permanently on site and do not intersperse them among the crop.

Lychee

Lychee is a mass-flowering tropical/subtropical crop in the family Sapindaceae. The trees produce three types of flowers, borne in sequence on panicles containing hundreds of flowers each. The first flowers to open are functionally male ('M1') and they produce both pollen and nectar, but cannot set fruit. M1 flowers are followed by female flowers ('F') which produce nectar but no pollen – these are the flowers that set fruit. The last flowers to open are again functionally male ('M2') producing both nectar and pollen, but these flowers produce greater volumes of pollen more viable than M1 flowers – as such M2 flowers are especially important for pollination. Typically there is no overlap between each floral stage within a panicle. Sometimes different panicles within a tree may have overlap in types of flowers produced, but generally it is important to plant varieties with slightly different flowering periods to ensure good overlap between M2 and F flowers.

Most lychee farms in Australia are surrounded by bushland and likely benefit from feral honey bees and native pollinators. Approaches to managed pollination vary greatly, with some growers introducing honey bees and/or stingless bees for pollination and others taking no steps for increasing pollinator abundance in the growing environment.

Macadamia

Macadamia is a mass-flowering tropical tree native to Australia. The majority of macadamia farms are located in northern NSW and south-eastern QLD. Flowers are borne on brush-like racemes with dozens to hundreds of individual flowers that typically open within a few days of each other. Like many other plants in the Proteaceae, each flower releases its pollen onto the end of the style (in this sense, referred to as a 'pollen presenter'). Thus, the nectar-producing glands at the base of the flower are somewhat physically separated from the pollen. The receptive stigma is at the very distal end of the style. Most macadamia varieties are only partially self-compatible and plants getting adequate cross pollination produce more nuts. It is believed that the self-pollen on the pollen presenter must be physically removed (e.g., by insect visits) before cross-pollen can reach the stigma.

Grower approaches to pollination have historically varied, with many growers not relying on managed pollinators. With increasing awareness in Australia about the importance of pollination, many growers now introduce honey bees, and in some cases cultivate or rent colonies of stingless bees for pollination. Unmanaged pollination of macadamia is likely to vary between regions, as some growing regions (e.g., Bundaberg) are intensively farmed, whereas other regions contain substantial surrounding bushland that may represent habitat for feral honey bees and other flower visitors.

Blueberry

Cultivars of blueberry produced in Australia consist of three species/types: rabbiteye (*Vaccinium virgatum*), northern highbush (*V. corymbosum*), and southern highbush (*V. corymbosum* hybrid). Rabbiteye are obligate outcrossers, requiring pollen from a different variety to set fruit, while highbush varieties are at least partially self-compatible.

Australian blueberries are grown from Tasmania to northern NSW, with northern highbush varieties grown in the cooler climates and rabbiteye and southern highbush varieties typically grown in the warmer climates. There are substantial differences in flower-visiting insect communities across their growing range: Tasmania has an introduced population of *Bombus terrestris* bumblebees – these are important pollinators of blueberries elsewhere in the world. Blueberry production in northern NSW overlaps with a range of Australian stingless bees, social bees that include some species that are managed for pollination. Blueberries are also grown in varied environments in Australia, while some are open-field grown, many growers are now producing blueberries under bird/hail netting or within plastic poly-tunnels, which can be problematic for pollination.

Papaya
Papaya is a fast-growing herbaceous plant with a tree-like structure. It is produced primarily in warmer parts of Australia, predominately northern QLD and the Mareeba district, as time from planting to cropping is shortened in warmer climates. 'Papaya' refers to the red-fleshed varieties. These are most typically clonal varieties with primarily perfect flowers, with both male and female parts and are believed to be self-fertile. Yellow-fleshed varieties are referred to as 'pawpaw', and are dioecious plants, which are either male or female. Female-only flowers do not produce pollen and lack nectar rewards. They attract pollinators by way of glands, which produce volatile compounds thought to mimic insect pheromones.

The papaya farms visited as part of this program did not rely on managed honey bees for pollination, but all were surrounded by bushland that likely contained feral honey bee colonies, as well as habitat for many other flower visiting animals.

Avocado
Avocado is a mass-flowering sub-tropical tree. The flowering system of avocado is complex – each individual flower first opens as a female flower, producing nectar but no pollen. After being open for several hours, these flowers close, to reopen later as male flowers, producing both nectar and pollen. Certain conditions may lead to overlap of male and female flowers being open within a single tree, but generally this does not happen and the trees depend on cross pollination from other varieties. Varieties of avocado fit into two groups (type A and type B) – the flowering times of these varieties are such that type A normally has female flowers open during times when type B trees have open male flowers – the reverse is also true.

The farms visited in the current program all deployed honey bees for pollination.

Almond
Almond is a temperate mass-flowering rosaceous tree, in Australia it is primarily produced in South Australia and Victoria. Most production is of the variety 'Nonpareil' which is grown for its consistent cropping, shape, and flavour. This variety strongly benefits from cross pollination – as such the requirement for honey bee colonies for pollination is high. Almonds are the principal driver of migratory beekeeping in the USA and Australia. New varieties that are self-compatible are in development, however the degree to which these varieties depend on insect visits to move self-pollen is not yet determined.

Project Outcomes

1. Establishing and disseminating current best practice information

Four-page pollination manuals were produced for the following crops: avocado, macadamia, watermelon, blueberry, lychee, and papaya. The manuals and the links to their locations online are provided below.

Melon pollination manual:

1. https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/melon-pollination-brochure/
2. https://www.planthealthaustralia.com.au/industries/melon/
3. https://beeaware.org.au/pollination/pollinator-reliant-crops/melon/https://beeaware.org.au/pollination/pollinator-reliant-crops/melons/

Maximise your melon crop with better pollination

1 THE BASICS OF MELON POLLINATION

Watermelon, rockmelon and honeydew melon are commonly grown in Australia, and all are dependent on insect pollinators to produce large, evenly shaped fruit. Although their flowers look the same, each melon type has different pollination requirements.

Rockmelon and honeydew represent about 29 per cent of the melon industry in Australia. Each vine contains a mix of male flowers and fruit-producing flowers that have both male and female reproductive parts. These flowers don't need other plants to cross-pollinate, but they do need insect pollinators to dislodge pollen and move it onto the stigma for seed set and fruit development. Flowers that are cross-pollinated have been shown to produce heavier fruit than those pollinated from flowers on the same plant.

Watermelon makes up 70 per cent of melon production in Australia. Two types of watermelon are commonly grown: seeded (diploid) and seedless (triploid). Each type produces separate male and female flowers on one plant (Figure 1), and pollination requirements differ between types.

Seeded melons are self-fertile: like rockmelon and honeydew they require visits by pollinating insects, but do not require cross-pollination between plants.

Seedless melon plants have to be cross-pollinated to produce fruit, so pollen from seeded varieties is needed. Although no seeds are produced, pollen on female flowers initiates fruit development.

Typically, seeded 'pollinisers' are planted in the same row as the seedless plants at a ratio of 1:3 or 1:4. Interplanting like this (not in separate rows) gives even pollination throughout the block and allows for the maximum area to be planted with seedless varieties.

Choose polliniser varieties that are a good match for the growing conditions, and that flower at the same time as the seedless variety. In some cases, 'special pollinisers' may be used: these make a lot of pollen but have low space and nutrient requirements.

As seedless watermelon require cross-pollination, pollinating insects are especially important in these crops.

Figure 1 Watermelon flowers. Male flowers (bottom) have bright yellow, pollen-producing anthers, whereas female flowers (top) have greenish yellow stigmas and an expanded ovary beneath the flower.

What you need to know

- Pollinators need to transfer pollen from male to female flowers for fruit production.
- Flowers open once, some for just three to six hours in the morning. Hot weather often shortens the flowering time.
- At least twice as many pollinators are needed to produce seedless watermelons as for other melons.
- Optimal insect pollination leads to better fruit quality and desirable shape.
- A diversity of insects visiting flowers will ensure good rates of pollination no matter when the flowers open.
- Honey bees may visit other preferred crops or plants if they are also in flower.
- The number of pollinators working in your crop can differ from year to year depending on land management, weather and the health of local honey bees.

2 INSECT POLLINATORS

Insect pollinators are essential for the production of marketable fruit. The flowers are attractive to a diverse range of insect visitors such as solitary bees (Figure 2), many of which can contribute to crop pollination. In some parts of the world, several different species of wild or managed bees are the main melon pollinators, but most melon farms in Australia rely on managed honey bees (*Apis mellifera*).

Figure 2 A solitary bee in the family *Halictidae* visiting a female watermelon flower for nectar.

Honey bees

The ideal hive stocking rates for honey bees can vary depending on environmental conditions, the local population of unmanaged pollinators (i.e. feral honey bees and other wild insects), and flowers in the area surrounding the farm. Recommended rates for watermelon range from two to 7.5 hives per hectare, while for rockmelon and honeydew at least two colonies per hectare is suggested.

At least six honey bee visits are needed for optimal pollination of a single flower on seed producing watermelons. For seedless varieties, double the number of visits (twelve or more) is needed, so having more hives per hectare is recommended for a crop of seedless fruit.

Colonies should be spread evenly throughout the block, in groups of 2–4 hives. Placing hives within the field rather than on the edge can increase the number of visits to melon flowers. By monitoring pollination during flowering, you can decide on the best honey bee stocking rates for your farm.

In dry areas, bees may need a supply of water. Place buckets near hives, with rags draped over the edge to allow bees to drink from the water wicked up the fabric.

Watermelon nectar and pollen alone do not provide good nutrition for bees over long periods. While sugar syrup or protein cakes may enhance bee activity, a recent study showed that feeding bees sugar syrup within the crop had no effect on bee visits to flowers and pollination.

Always use strong hives for melon pollination. A hive should contain at least five frames covered with bees and 4000 cm² of brood prior to being moved into the crop.

Ordering hives early in the season allows beekeepers to supply the required numbers at the appropriate strength. A formal pollination agreement will help to ensure that good quality hives are provided.

Honey bees can potentially spread some plant diseases, including cucumber green mottle mosaic virus (CGMMV). Work with your beekeeper to minimise biosecurity risks associated with the introduction of hives.

Figure 3 A honey bee visiting a male melon flower can collect both pollen and nectar.

Top tip

Honey bees are usually critical for pollination of melons in Australia, particularly for seedless watermelons

Work with your beekeeper to ensure strong hives are placed appropriately on the farm.

Other pollinators

A wide range of insects – including stingless bees (Figure 4), other non-managed solitary native bees (Figure 2), flies and beetles – can pollinate melons, boosting yields above what can be achieved by honey bees alone. Other pollinators can complement honey bees because they are active at times when honey bees are not.

While small insects like stingless bees can move pollen between male and female flowers, they do not distribute pollen as evenly on the stigma as larger bees like honey bees. Because of this, more visits may be needed to get evenly shaped fruit.

If you see large numbers of wild insects (other than honey bees) visiting your crop, they are likely to be contributing to pollination. Try to avoid using pesticides during the flowering period, as this may affect their numbers. Some pollinators such as flies and beetles shelter within the crop during periods of inactivity, so if you have to treat your crop, use pest control products that are listed as being safe for beneficial insects to help protect them.

Figure 4 A stingless bee (*Tetragonula hockingsi*) collects pollen from a male watermelon flower.

How to boost pollination

- For seedless watermelon, make sure seeded and seedless plants flower at the same time. Maximise cross-pollination by planting at least one seeded to four seedless plants within a row.
- Identify and count insects that visit flowers in your field. At most times, you should see a minimum of one foraging bee per plant on a fine sunny day.
- Check to see if honey bee foraging is consistent throughout your crop. Rearrange or bring in more beehives if bees are not evenly distributed.
- Protect unmanaged pollinators. Learn what they look like, and their preferred habitat, and protect their nesting or shelter sites.
- Avoid the use of pesticides during flowering. If unavoidable, choose those labelled less toxic to bees and apply only after flowers have closed.
- Agree to a pollination contract with your beekeeper and ensure you receive strong hives.

3 CHECKLIST

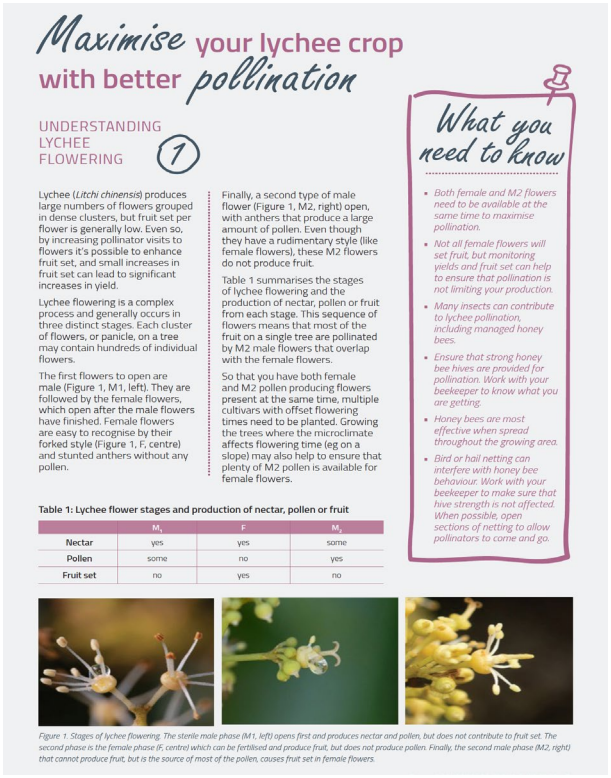
DEVELOP A POLLINATION PLAN FOR YOUR FARM			
ACTION	YES	NO	COMMENT
If multiple melon varieties are required for cross pollination, planting ratio and spacing is appropriate (one polliniser for every four seedless plants).			
Crop loads (e.g. number and quality) are recorded over multiple years, providing a benchmark to assess changes in pollination.			
Number of flowers and flowering times of different varieties planted on farm are recorded and adjusted as required.			
Staff can identify common insects visiting flowers.			
Bees and other insects on flowers are counted along several 10 metre stretches of the crop distributed around the farm, for about 10 minutes during peak activity times, usually mid-morning.			
If pollinator activity is low on parts of the farm, nearby honey bee hives are checked for activity, and additional honey bee hives placed in the area if required.			
Managed and unmanaged pollinators are protected by limiting sprays, not spraying while flowers are open, and conserving areas where unmanaged bees live and breed (undisturbed soils and bush with diverse year-round flowers).			

HIVE MANAGEMENT			
ACTION	YES	NO	COMMENT
Pollination agreements are drawn up with beekeepers, detailing respective responsibilities.			
Beekeeper has provided evidence of compliance with the Australian Honey Bee Industry Biosecurity Code of Practice.			
Honey bee hives are placed in small groups that are evenly spaced in the field, at an overall stocking rate of between two and 7.5 hives per hectare.			

Goodwin (2012) *Pollination of Crops in Australia and New Zealand* 121 p.
Images courtesy of Brian Cutting of Plant & Food Research Australia, unless otherwise stated.

Lychee pollination manual:

1. https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/lychee-pollination-brochure/
2. https://www.planthealthaustralia.com.au/industries/lychee/
3. https://beeaware.org.au/pollination/pollinator-reliant-crops/lychees/



Maximise your lychee crop with better pollination

UNDERSTANDING LYCHEE FLOWERING 1

Lychee (*Litchi chinensis*) produces large numbers of flowers grouped in dense clusters, but fruit set per flower is generally low. Even so, by increasing pollinator visits to flowers it's possible to enhance fruit set, and small increases in fruit set can lead to significant increases in yield.

Lychee flowering is a complex process and generally occurs in three distinct stages. Each cluster of flowers, or panicle, on a tree may contain hundreds of individual flowers.

The first flowers to open are male (Figure 1, M1, left). They are followed by the female flowers, which open after the male flowers have finished. Female flowers are easy to recognise by their forked style (Figure 1, F, centre) and stunted anthers without any pollen.

Finally, a second type of male flower (Figure 1, M2, right) open, with anthers that produce a large amount of pollen. Even though they have a rudimentary style (like female flowers), these M2 flowers do not produce fruit.

Table 1 summarises the stages of lychee flowering and the production of nectar, pollen or fruit from each stage. This sequence of flowers means that most of the fruit on a single tree are pollinated by M2 male flowers that overlap with the female flowers.

So that you have both female and M2 pollen producing flowers present at the same time, multiple cultivars with offset flowering times need to be planted. Growing the trees where the microclimate affects flowering time (eg on a slope) may also help to ensure that plenty of M2 pollen is available for female flowers.

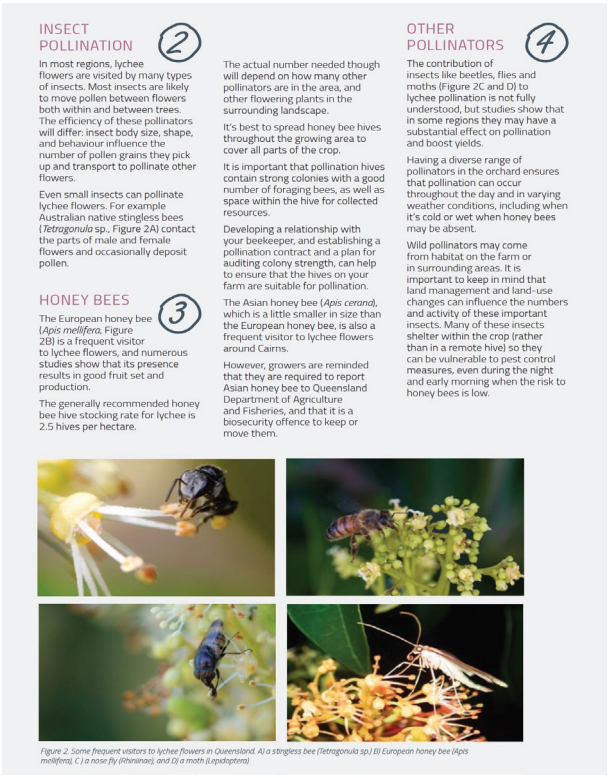
Table 1: Lychee flower stages and production of nectar, pollen or fruit

	M_1	F	M_2
Nectar	yes	yes	some
Pollen	some	no	yes
Fruit set	no	yes	no

What you need to know

- *Both female and M2 flowers need to be available at the same time to maximise pollination.*
- *Not all female flowers will set fruit, but monitoring yields and fruit set can help to ensure that pollination is not limiting your production.*
- *Many insects can contribute to lychee pollination, including managed honey bees.*
- *Ensure that strong honey bee hives are provided for pollination. Work with your beekeeper to know what you are getting.*
- *Honey bees are most effective when spread throughout the growing area.*
- *Bird or hail netting can interfere with honey bee behaviour. Work with your beekeeper to make sure that hive strength is not affected. When possible, open sections of netting to allow pollinators to come and go.*

Figure 1. Stages of lychee flowering. The sterile male phase (M1, left) opens first and produces nectar and pollen, but does not contribute to fruit set. The second phase is the female phase (F, centre) which can be fertilised and produce fruit, but does not produce pollen. Finally, the second male phase (M2, right) that cannot produce fruit, but is the source of most of the pollen, causes fruit set in female flowers.



INSECT POLLINATION 2

In most regions, lychee flowers are visited by many types of insects. Most insects are likely to move pollen between flowers both within and between trees. The efficiency of these pollinators will differ: insect body size, shape, and behaviour influence the number of pollen grains they pick up and transport to pollinate other flowers.

Even small insects can pollinate lychee flowers. For example Australian native stingless bees (*Tetragonula* sp., Figure 2A) contact the parts of male and female flowers and occasionally deposit pollen.

HONEY BEES 3

The European honey bee (*Apis mellifera*, Figure 2B) is a frequent visitor to lychee flowers, and numerous studies show that its presence results in good fruit set and production.

The generally recommended honey bee hive stocking rate for lychee is 2.5 hives per hectare.

The actual number needed though will depend on how many other pollinators are in the area, and other flowering plants in the surrounding landscape.

It's best to spread honey bee hives throughout the growing area to cover all parts of the crop.

It is important that pollination hives contain strong colonies with a good number of foraging bees, as well as space within the hive for collected resources.

Developing a relationship with your beekeeper, and establishing a pollination contract and a plan for auditing colony strength, can help to ensure that the hives on your farm are suitable for pollination.

The Asian honey bee (*Apis cerana*), which is a little smaller in size than the European honey bee, is also a frequent visitor to lychee flowers around Cairns.

However, growers are reminded that they are required to report Asian honey bee to Queensland Department of Agriculture and Fisheries, and that it is a biosecurity offence to keep or move them.

OTHER POLLINATORS 4

The contribution of insects like beetles, flies and moths (Figure 2C and D) to lychee pollination is not fully understood, but studies show that in some regions they may have a substantial effect on pollination and boost yields.

Having a diverse range of pollinators in the orchard ensures that pollination can occur throughout the day and in varying weather conditions, including when it's cold or wet when honey bees may be absent.

Wild pollinators may come from habitat on the farm or in surrounding areas. It is important to keep in mind that land management and land-use changes can influence the numbers and activity of these important insects. Many of these insects shelter within the crop (rather than in a remote hive) so they can be vulnerable to pest control measures, even during the night and early morning when the risk to honey bees is low.

Figure 2. Some frequent visitors to lychee flowers in Queensland. A) a stingless bee (Tetragonula sp.) B) European honey bee (Apis mellifera), C) a nose fly (Rhinidae), and D) a moth (Lepidoptera)

Top tip

Lychee flowers are visited by many types of insects.

Keep tabs on what's happening on your farm to better understand which pollinators are in your orchard, so you will know if something changes.

PROTECTED CROPPING 5

It is common for farms to have protective netting covers to prevent damage from storms and pest animals. These covers can disrupt normal pollinator behaviour and beekeepers may be reluctant to move hives into covered blocks.

Where possible, it is recommended to leave the sides of protected areas open during flowering to allow natural foraging of honey bees and wild pollinators.

Some unmanaged pollinators and Australian stingless bees appear to be less affected by covers and may be particularly useful as pollinators in these situations.

Hail and bird netting (left and above) has potential to alter behaviour of both managed and unmanaged pollinators. Raising the sides of some netting to allow pollinator movement is a strategy for getting good pollination and keeping honey bee hives strong.

6 CHECKLIST

DEVELOP A POLLINATION PLAN FOR YOUR CROP			
ACTION	YES	NO	COMMENT
Both female and M2 flowers are available at the same time in each block.			
Plant cultivars with slightly offset flowering times are used to ensure overlap in flowering.			
Crop loads (e.g. number and quality) are recorded over multiple years, providing a benchmark to assess changes in pollination.			
Crops with multiple varieties are regularly checked to compare flowering intensity and weather records, to understand patterns of flowering.			
Orchard staff can identify common insects visiting flowers.			
The number of insect pollinators active on four, 10 m transects located diagonally across the flowering crop are counted (recommended time is 10 am – noon).			
Staff are aware that land use changes can influence the populations and activity patterns of unmanaged pollinators.			

HIVE MANAGEMENT			
ACTION	YES	NO	COMMENT
Pollination agreements are drawn up with beekeepers, detailing respective responsibilities.			
Beekeeper has provided evidence of compliance with the Australian Honey Bee Industry Biosecurity Code of Practice.			
When or where pollinator activity is lower than usual, additional managed honey bee hives are brought in to maintain pollination rates.			
Honey bee hives are placed in small groups that are evenly spaced in the orchard, at an overall stocking rate of 2.5 hives per hectare.			
Where possible, openings are created in covers and enclosures during flowering to promote honey bee health and to allow access for other pollinators.			

Goodwin (2012) *Pollination of Crops in Australia and New Zealand* 121 p.

Images courtesy of Brian Cutting of Plant & Food Research Australia, unless otherwise stated.

Macadamia pollination manual:

1. https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/macadamia-pollination-brochure/
2. https://www.planthealthaustralia.com.au/industries/macadamia/
3. https://beeaware.org.au/pollination/pollinator-reliant-crops/macadamias/

Maximise your macadamia crop with better pollination

1 THE BASICS OF MACADAMIA POLLINATION

Macadamia flowers are borne on racemes containing 100–300 flowers. It takes about 6 to 12 days for all of the flowers on a raceme to open and the flowers produce both pollen and nectar. Importantly, the flowers can only be pollinated when they are fully open.

Macadamia raemes.

Many varieties of macadamias produce more nuts when cross pollinated with another variety.

Top Tip

Diversity is the key to consistent macadamia production

A mix of varieties and a range of pollinators will help you obtain a consistently high yield every season.

2 MIX & MATCH YOUR MACADAMIA VARIETIES

Experiments where the pollen from the flowers of one variety are crossed with the flowers on a raceme of another variety produce more and larger nuts than from pollination within a variety (see table over page).

Because cross pollination has been shown to increase final nut set in trees, growing multiple varieties within a block will improve the rate of cross pollination when pollinators are present.

Nuts of different varieties are usually harvested together. This provides opportunity to spread multiple varieties within a block to improve cross pollination.

To improve pollinator performance, it's best to grow a mix of different varieties within the same row in alternating sequence.

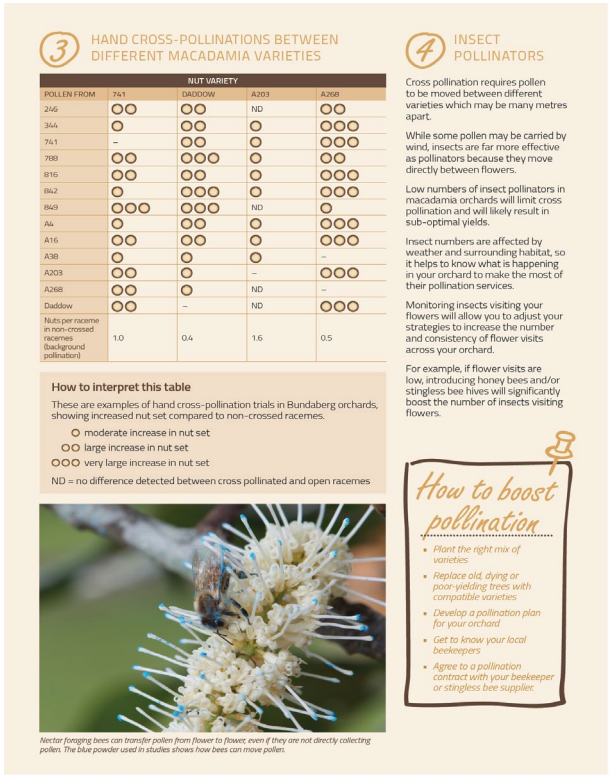
Honey bees in particular prefer to move along a row of trees rather than from one row to another, so having different varieties in the same row will increase the amount of pollen moving between varieties.

Choose varieties for planting carefully because cross pollination can only occur between varieties that flower at the same time.

What you need to know

- *Cross pollination between compatible macadamia varieties increases nut set*
- *The right orchard plantings can promote cross pollination*
- *Insects are the key to good, consistent pollination*
- *Knowing what is pollinating your crop can protect and enhance pollination*
- *Having the right number of hives evenly spaced in an orchard is the easiest way to boost pollinator numbers quickly*
- *Honey bee hives need to be strong and evenly spaced in an orchard so that bees visit all the flowering trees*
- *Stingless bees can be great pollinators for macadamias if hives are spaced close enough to ensure good flower visitation*
- *Other beetles and birds can help with pollination*

You can improve the pollination rate in an existing orchard by replacing unproductive or incompatible trees with another variety that is compatible.



3 HAND CROSS-POLLINATIONS BETWEEN DIFFERENT MACADAMIA VARIETIES

	NUT VARIETY			
POLLEN FROM	741	DADDOW	A203	A268
246	○○	○○	ND	○○
344	○	○○	○	○○○
741	–	○○	○	○○○
788	○○	○○○	○	○○
816	○○	○○	○	○○○
842	○	○○○	○	○○○
849	○○○	○○○	ND	○
A4	○	○○	○	○○○
A16	○○	○○	○	○○○
A38	○	○	○	–
A203	○○	○	–	○○○
A268	○○	○	ND	–
Daddow	○○	–	ND	○○○
Nuts per raceme in non-crossed racemes (background pollination)	1.0	0.4	1.6	0.5

How to interpret this table

These are examples of hand cross-pollination trials in Bundaberg orchards, showing increased nut set compared to non-crossed racemes.

- ○ moderate increase in nut set
- ○○ large increase in nut set
- ○○○ very large increase in nut set

ND = no difference detected between cross pollinated and open racemes

Nectar foraging bees can transfer pollen from flower to flower, even if they are not directly collecting pollen. The blue powder used in studies shows how bees can move pollen.

4 INSECT POLLINATORS

Cross pollination requires pollen to be moved between different varieties which may be many metres apart.

While some pollen may be carried by wind, insects are far more effective as pollinators because they move directly between flowers.

Low numbers of insect pollinators in macadamia orchards will limit cross pollination and will likely result in sub-optimal yields.

Insect numbers are affected by weather and surrounding habitat, so it helps to know what is happening in your orchard to make the most of their pollination services.

Monitoring insects visiting your flowers will allow you to adjust your strategies to increase the number and consistency of flower visits across your orchard.

For example, if flower visits are low, introducing honey bees and/or stingless bee hives will significantly boost the number of insects visiting flowers.

How to boost pollination

- *Plant the right mix of varieties*
- *Replace old, dying or poor-yielding trees with compatible varieties*
- *Develop a pollination plan for your orchard*
- *Get to know your local beekeepers*
- *Agree to a pollination contract with your beekeeper or stingless bee supplier.*

Honey bees

Although pollen gathering honey bees are more likely to contact the stigma directly and fertilise the flower, both pollen and nectar foragers will pollinate macadamia flowers and improve nut set.

The suggested stocking rate for macadamia orchards is five to eight hives per hectare, but you should check to make sure that honey bees are actively foraging on flowers across your blocks. More hives might be needed if there are other crops or native plants in the area that are more attractive to the bees.

It is important to order hives early enough in season to ensure an adequate supply when trees are flowering, as beekeepers need time to prepare colonies suitable for pollination. To be sure of what you are getting and avoid misunderstandings about what is being supplied, develop a pollination agreement with your beekeeper.

Honey bee pollinating a macadamia flower.

The placement of hives is important. Colonies should be spread evenly throughout the orchard in groups of 2–4 hives, to ensure bees visit flowers across the entire orchard. Ask your beekeeper to provide strong colonies managed to encourage more pollen collection. Honey bees also need a supply of water, so you need to agree on how this will be provided.

Bees prefer to forage on racemes that are in the sunlight, so careful pruning of trees to open the canopy can increase the number of flowers they visit.

Look for these pollinators on your flowers

- Net winged beetles
- Soldier beetles
- Stingless bees

A stingless bee (Tetrogonula) pollinating a macadamia flower.

Stingless bees

Individual stingless bees are more efficient pollinators than nectar collecting honey bees, but their contribution to pollination in orchards is often limited by the numbers of foraging bees and where the hives are placed.

Preserving local native plants in which stingless bees nest can boost the amount of pollination you can get for free. But if you rely on wild bees you will have less control over the number of bees present. To overcome this natural variability, some beekeepers manage stingless bees in purpose-built hives that can be hired or purchased when needed.

Stingless bee (Tetrogonula)

Stingless bees don't fly as far from their hives as honey bees, so their hives need to be put closer together than honey bee colonies. You should look for foraging bees and note where they are throughout the block to decide on the best stocking rate and placement of hives for your orchard.

Soldier beetles pollinating a macadamia flower.

Other pollinators

Beetles, flies and even birds can also contribute to pollination in macadamia orchards.

Net winged beetle pollinating a macadamia flower

If you are lucky enough to have large populations of wild pollinators, make sure that your orchard management practices don't jeopardise pollination.

If you decide to use broad spectrum pesticides just before or during flowering, you may need to add some hives of honey bees afterwards to maintain pollination levels and nut set.

There are no specific management strategies for non-bee pollinators at present, but giving them the right habitat will encourage healthy populations in orchards.

Did you know

Improved cross pollination can increase tree nut set by more than 50 per cent

5 CHECKLIST

DEVELOP A POLLINATION PLAN FOR YOUR ORCHARD			
ACTION	YES	NO	COMMENT
Nut set is counted on 10 marked racemes on each of 10 trees and yearly records are maintained to keep track of your levels of pollination			
Hand-crosses are conducted to provide a benchmark of optimum pollination for your orchard's mix of varieties			
New orchards or blocks are established with an appropriate mix and spacing of compatible varieties			
Unproductive trees in single variety blocks are replaced with compatible cross varieties			
Orchard staff can identify common flower-visiting insects (between 10 am and 2 pm when warm and sunny)			
Weekly counts of pollinator numbers are conducted during flowering: walk around 10 fully-flowering trees and count all visible pollinators			
Decide whether the introduction of more honey bees or stingless bees to your orchard is required (if your pollinator activity on flowers is lower than four honey bee equivalents per tree per minute).			
Where dependence on unmanaged pollinators (>50% of flower visitors) is identified, plans are developed to protect or enhance these populations			
Canopies are pruned to increase light penetration to racemes to promote flowering and increase insect pollinator activity			

HIVE MANAGEMENT			
ACTION	YES	NO	COMMENT
Pollination agreements are drawn up with beekeepers to detail responsibilities and hive strengths (more than 30,000 bees per hive)			
Honey bee hives are placed in small groups evenly spaced through the orchard, at an initial stocking rate of between 5–8 hives per hectare			
Stingless bee colonies are distributed evenly at closer distances than honey bee colonies, and numbers are increased if required based on pollinator counts			
Beekeeper has provided evidence of compliance with the Australian Honey Bee Industry Biosecurity Code of Practice			

Howlett et al (2017) *Optimising pollination of macadamia & avocado in Australia* 125 p.
Goodwin (2012) *Pollination of Crops in Australia and New Zealand* 121 p.
Department of Agriculture and Food (2006) *Bee pollination benefits for nut crops*

Images courtesy of Brian Cutting and Lisa Evans of Plant & Food Research NZ, unless otherwise stated.
Funded by the Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from pollination reliant industries and contributions from the Australian Government.

PH17-027

Blueberry pollination manual:

1. https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/blueberry-pollination-brochure/
2. https://www.planthealthaustralia.com.au/industries/blueberries/
   https://beeaware.org.au/pollination/pollinator-reliant-crops/blueberries/

Maximise your blueberry crop with better pollination

THE BASICS OF BLUEBERRY POLLINATION 1

Blueberry plants form clusters of bell-shaped flowers, which contain a central style surrounded by shorter pollen-producing anthers (Figure 1).

While flower shape varies, the stigma that receives pollen is usually just at the opening of the flower. The pollen-producing anthers are further inside the flower and surround the stigma. Nectar is produced at the base of the flower.

For fruit to develop, pollen must be moved from the anthers to the stigma. Blueberry pollen does not readily travel by wind, so pollinators are needed for adequate fruit set to occur.

Top tip

Insects other than honey bees may play a major role in pollination on some farms

Monitor pollinator numbers and work with your beekeeper to make sure you have sufficient activity on flowers at all times.

Most blueberry cultivars grown in Australia are either 'highbush' (*Vaccinium corymbosum*) or 'rabbiteye' (*Vaccinium virgatum*) varieties.

Rabbiteye blueberries are mostly self-infertile, and highly dependent on cross-pollination for adequate fruit set: pollen moved to the stigma must be from plants of a different variety.

Highbush varieties are generally less dependent on cross-pollination, but most have improved fruit set with cross-pollination.

Some popular highbush varieties like 'Brigitta' require a high degree of cross-pollination. In addition to increasing fruit set, adequate cross-pollination can increase seed counts and fruit size, and the speed of berry development.

Figure 1 A blueberry flower with part of the corolla removed, showing the central style and stigma surrounded by pollen producing anthers.

What you need to know

- *Fruit set in all blueberry varieties is increased by insects moving pollen between cultivars.*
- *Rabbiteye varieties need to be cross-pollinated for fruit to set, so pollinators are vital.*
- *Improved pollination can add value by increasing fruit weight and shortening fruit development times.*
- *Honey bees are essential pollinators on many Australian blueberry farms.*
- *Pollination hives need to be of adequate strength and distributed through the farm to improve pollination.*
- *Unmanaged pollinators, such as native bees and bumble bees (in Tasmania) contribute to pollination.*
- *Bumble bees and carpenter bees may chew holes at the base of flowers to get to nectar, but these 'nectar-robbing' visits still contribute to pollination.*
- *Honey bee behaviour can be disrupted by protective covers (e.g. tunnel houses, hail netting) so ensure flower visiting insects are present if covers are used.*
- *The number of pollinator visits to flowers should be assessed and hives added if needed during flowering.*

BLUEBERRY POLLINATORS 2

A diversity of pollinators including bees, flies and moths visit blueberry flowers, many of which can contribute to crop pollination. Visits by unmanaged pollinators are likely to vary from farm to farm, and between growing regions.

Honey bees

European honey bees (*Apis mellifera*) are important pollinators of blueberry because their populations can be easily manipulated via the addition of managed hives (Figure 2). Current guidelines for hive stocking density for blueberry pollination vary widely, from 1.25 to 10 hives per hectare, and will depend on factors such as flower density and the number of feral honey bees or other pollinators.

Monitoring pollinator activity on your crop can help guide decisions about pollinator management. To assess honey bee activity, count bee numbers mid-morning on a fine day. When there are only a few other pollinators, you should see about two honey bees foraging per bush as you stroll down a row.

Figure 2 Honey bees are the most important pollinator of blueberries on many farms, and can be moved in large numbers to boost pollinator numbers.

Other bees

There are about 1500 species of bees in Australia. Research is ongoing to determine which of these are most important for blueberry pollination. Several species – like carpenter bees, blue-banded bees and teddy bear bees – are capable of 'buzz pollination' that vibrates pollen loose from flower anthers. Because this releases large amounts of pollen, some of these bees have been shown to be more efficient (per individual) than honey bees (Figure 3).

Figure 3 Many species of bees visit blueberry flowers, including bumble bees in Tasmania (top) and peacock carpenter bees in Queensland and NSW (bottom). Both are capable of 'buzz pollination' and 'nectar robbing'

The bumble bee, *Bombus terrestris*, is an introduced species to Tasmania, and on some farms it can be the primary pollinator of blueberry flowers. While bumble bees are known to be good pollinators of blueberries, the environmental consequences of bumble bee introduction in Tasmania are not fully understood, and these bees are not present in other parts of Australia.

Stingless bees are frequent visitors to blueberry flowers in mainland Australia. These small bodied native bees make good contact with stigmas while foraging and are likely to be efficient pollinators for blueberries. Stingless bees are social and in warmer regions managed hives are available for hire.

Other pollinators

Various flies, butterflies, beetles and even birds may all contribute to blueberry pollination (Figure 4). Understanding the life histories of these species is key to preserving their populations on farms. Some unmanaged pollinators move between farms and surrounding natural or low-disturbance areas. Others take shelter in the crop and can be more susceptible to non-target effects of pest control measures.

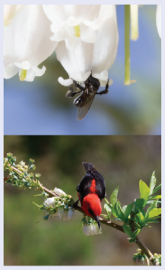


Figure 4 Blueberry flowers are attractive to a wide variety of pollinators, including stingless bees (top) and the scarlet honey eater (bottom).

Nectar robbing

Large bees like bumble bees and carpenter bees can sometimes struggle to reach pollen at the base of blueberry flowers, but they can chew holes at the base of the flower to reach nectar. This is called 'nectar-robbing' or 'side-working' and you'll know it's happening if you see damage to the flower petals (Figure 5).

Once a hole is made, other visitors to the flower, including honey bees, may use this rather than the flower opening. Many growers worry that nectar-robbing will decrease pollination and fruit development, but research has shown that even robbing visits usually add to pollination and fruit set, although more visits are required for full pollination.

Figure 5 Bumble bees and carpenter bees may chew holes at the base of flowers to feed on nectar. Honey bees may then use these holes. 'Nectar robbing' visits still contribute to pollination, although they are not as efficient as other visits.

POLLINATION UNDER COVERS 3

Many farms use protective covers such as plastic poly-tunnels and woven hail or bird netting to enhance blueberry growing conditions and protect the crop from damage. These covers may disrupt normal pollinator behaviour. Honey bees forage less under netted covers, and can lose hive strength under such conditions (Figure 6).

Figure 6 Honey bee pollination may be less efficient in some covered environments.

Other pollinators may be less sensitive to the environmental changes faced under covers. Pollination in these conditions is still achievable, but you'll need to monitor pollinator activity to be sure it is sufficient. If you can, delay covering the crop completely until the pollination period is over. Since foraging bees may travel less under covers, hives may help to ensure pollination throughout the farm.

ENSURING POLLINATION OF YOUR CROP 4

On most farms, pollination occurs thanks to a mix of different pollinating species. Managed honey bees should be used to raise pollinator activity in places where other pollinators are not very active in the crop.

In colder regions, pollination of early flowering varieties may be a challenge, as populations of pollinators (including honey bees) may not have fully rebounded from winter, and some are less active in cooler conditions. Work with your beekeeper to ensure that colonies supplied for pollination are of adequate strength, and that these are distributed in a way to maximise pollination coverage across the farm.

Develop a pollination contract with your beekeeper to ensure that responsibility for pollination and hive management is clear. Consult your beekeeper prior to applying agrichemicals, as even non-insecticidal sprays can affect pollinators if applied at the wrong time. Also, provide water for bees and shelter for hives in exposed locations.

How to measure fruit set

You can easily monitor your pollination rates by tagging individual flowers, or clusters of flowers, with small 'jewellers' tags' or other robust markers. Count the number of tagged flower buds, and go back later to count the number of berries that develop. This will give you an estimate of fruit set rate. Doing this at several locations across the farm can tell you if pollination is occurring in a consistent way. By noting weather conditions and pollination management actions, you can work out which strategies are best for your farm from year to year.

5 CHECKLIST

FARM MANAGEMENT			
ACTION	YES	NO	COMMENT
Unless growing a known self-compatible cultivar, rows of compatible cultivars are planted next to each other to maximise cross-pollination.			
Peak flowering times of cultivars overlap in your region.			
Flowers (or flower clusters) are tagged each year and the fruit set rate is recorded to monitor changes in pollination from year to year, or in different parts of the farm.			
Shrubs are pruned annually to maintain a range of canes of different ages, including new fruiting canes.			
Options to mitigate the effects on honey bees of protective covers are discussed with your beekeepers.			

POLLINATOR MANAGEMENT			
ACTION	YES	NO	COMMENT
Staff can identify common insects visiting flowers.			
Checks are done for adequate pollinator activity on fine days – about two honey bees per large bush in mid-morning.			
When or where pollinator activity is lower than usual, managed honey bee hives are brought in to maintain pollination rates (at 5 per cent flowering).			
If hives are not usually brought in for pollination, the degree of reliance on local honey bees is known, and plans made to supplement this service when needed.			
Pollination agreements are drawn up with beekeepers, detailing respective responsibilities.			
Beekeeper has provided evidence of compliance with the Australian Honey Bee Industry Biosecurity Code of Practice.			
Honey bee hives are placed in small groups that are evenly spaced in the farm, at an overall stocking rate of between 1.25 and 10 hives per hectare.			
Where dependence on unmanaged pollinators is known to occur (i.e. more than half of flower visitors), management plans are developed to protect or enhance their numbers.			

Goodwin (2012) *Pollination of Crops in Australia and New Zealand* 121 p.

Papaya pollination manual:

1. https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/papaya-pollination-brochure/
2. https://www.planthealthaustralia.com.au/industries/papaya/
3. https://beeaware.org.au/pollination/pollinator-reliant-crops/papaya/

Maximise your papaya crop with better pollination

PAPAYA AND PAWPAW POLLINATION BASICS (1)

The fruit of *Carica papaya* is known as papaya if it has red flesh or pawpaw if it has yellow flesh. Historically, red papaya orchards consisted of plants that were either male or female, but production has shifted to using plants with flowers that contain both male and female parts. Yellow pawpaw orchards are still mostly planted with separate male and female plants.

Papaya with both male and female flower parts

Papaya plants do sometimes set fruit without pollination, but the fruit is generally seedless and small. To consistently produce marketable fruit, some pollen must move from the male to the female parts within the flower.

Most papaya varieties that have both male and female flower parts (hermaphrodites or mixed sex flowers) are self-fertile to some degree.

The female stigmas can be receptive to pollination several days before the flower opens, and they stay receptive until several days after flowering begins, so they are able to self-pollinate before the flower even opens.

It's not known how much the yield from this type of papaya plant benefits from cross-pollination.

Pawpaw with separate male and female plants

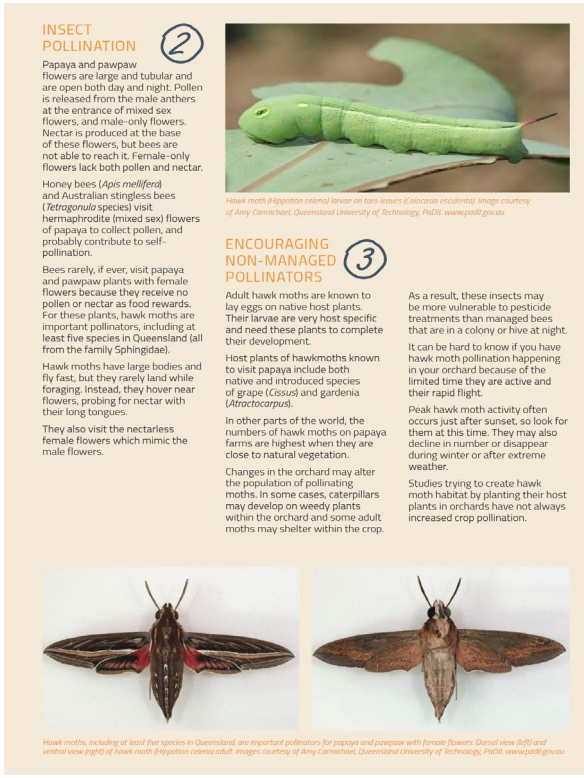
In pawpaw orchards where you have separate male and female plants, pollen must be moved between the male and female plants for pollination to occur. While wind pollination does occur, insects are a more important way to pollinate these flowers.

It's usual to have about 10 per cent of the orchard as male plants for pollination, but planting ratios and arrangements vary from one male plant for every eight female plants, to one male for every 25 female plants.

What you need to know

- *Papaya and pawpaw pollination depends on the cultivar.*
- *Orchards with separate male and female trees (generally pawpaw) need male plants inter-planted among female plants to ensure enough pollen is available.*
- *Papaya and pawpaw pollen can be moved by wind, but generally not enough to ensure pollination.*
- *For plantings with both male and female plants, hawk moths are probably the most important pollinators.*
- *Hawk moths depend on particular native host plants for their development. Changes to land use on or around the farm may affect populations of pollinating hawk moths.*
- *For mixed sex flowers of papaya, the role of honey bees and stingless bees in strengthening pollination is not yet fully understood.*
- *Weather extremes and plant management can affect the formation of female or mixed sex flowers, or pollen viability.*

Papaya (Carica papaya) L. fruit and flowers of a female plant (left) and a male plant (right). Image courtesy of Whitney Cranshaw, Colorado State University, Bugwood.org



INSECT POLLINATION (2)

Papaya and pawpaw flowers are large and tubular and are open both day and night. Pollen is released from the male anthers at the entrance of mixed sex flowers, and male-only flowers. Nectar is produced at the base of these flowers, but bees are not able to reach it. Female-only flowers lack both pollen and nectar.

Honey bees (*Apis mellifera*) and Australian stingless bees (*Tetragonula* species) visit hermaphrodite (mixed sex) flowers of papaya to collect pollen, and probably contribute to self-pollination.

Bees rarely, if ever, visit papaya and pawpaw plants with female flowers because they receive no pollen or nectar as food rewards. For these plants, hawk moths are important pollinators, including at least five species in Queensland (all from the family Sphingidae).

Hawk moths have large bodies and fly fast, but they rarely land while foraging. Instead, they hover near flowers, probing for nectar with their long tongues.

They also visit the nectarless female flowers which mimic the male flowers.

Hawk moth (Hippotion celerio) larvae on taro leaves (Colocasia esculenta). Image courtesy of Amy Carmichael, Queensland University of Technology, PaDIL www.padil.gov.au

ENCOURAGING NON-MANAGED POLLINATORS (3)

Adult hawk moths are known to lay eggs on native host plants. Their larvae are very host specific and need these plants to complete their development.

Host plants of hawkmoths known to visit papaya include both native and introduced species of grape (*Cissus*) and gardenia (*Atractocarpus*).

In other parts of the world, the numbers of hawk moths on papaya farms are highest when they are close to natural vegetation.

Changes in the orchard may alter the population of pollinating moths. In some cases, caterpillars may develop on weedy plants within the orchard and some adult moths may shelter within the crop.

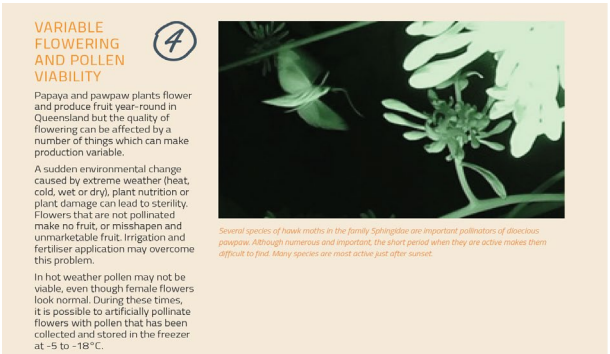
As a result, these insects may be more vulnerable to pesticide treatments than managed bees that are in a colony or hive at night.

It can be hard to know if you have hawk moth pollination happening in your orchard because of the limited time they are active and their rapid flight.

Peak hawk moth activity often occurs just after sunset, so look for them at this time. They may also decline in number or disappear during winter or after extreme weather.

Studies trying to create hawk moth habitat by planting their host plants in orchards have not always increased crop pollination.

Hawk moths, including at least five species in Queensland, are important pollinators for papaya and pawpaw with female flowers. Dorsal view (left) and ventral view (right) of hawk moth (Hippotion celerio) adult. Images courtesy of Amy Carmichael, Queensland University of Technology, PaDIL www.padil.gov.au



VARIABLE FLOWERING AND POLLEN VIABILITY (4)

Papaya and pawpaw plants flower and produce fruit year-round in Queensland but the quality of flowering can be affected by a number of things which can make production variable.

A sudden environmental change caused by extreme weather (heat, cold, wet or dry), plant nutrition or plant damage can lead to sterility. Flowers that are not pollinated make no fruit, or misshapen and unmarketable fruit. Irrigation and fertiliser application may overcome this problem.

In hot weather pollen may not be viable, even though female flowers look normal. During these times, it is possible to artificially pollinate flowers with pollen that has been collected and stored in the freezer at -5 to -18 °C.

Several species of hawk moths in the family Sphingidae are important pollinators of dioecious pawpaw. Although numerous and important, the short period when they are active makes them difficult to find. Many species are most active just after sunset.

Top tip

Pawpaw (male and female plants) are highly dependent on native moths as pollinators.

Mixed sex papaya flowers may self-pollinate but benefit from visiting bees.

5 CHECKLIST

DEVELOP A POLLINATION PLAN FOR YOUR CROP			
ACTION	YES	NO	COMMENT
An adequate ratio of pollen-providing and fruiting plants is available (ratios are cultivar-specific).			
Crop yields (e.g. number and quality) are recorded over multiple years, providing a benchmark to assess changes in pollination.			
Orchards with multiple varieties are regularly checked, with flowering intensity, weather conditions and yield recorded to understand flowering patterns and to make adjustments if required.			
Presence and number of hawk moths confirmed by looking at flowers with a torch just after sunset.			
Staff are aware that changes to surrounding vegetation may affect populations of hawk moths.			

HIVE MANAGEMENT			
ACTION	YES	NO	COMMENT
Managed colonies of honey bees or stingless bees are introduced to papaya orchards with male and female plants to boost pollination.			
Pollination agreements are drawn up with beekeepers, detailing respective responsibilities.			

Goodwin (2012) *Pollination of Crops in Australia and New Zealand* 121 p.
Images courtesy of Brian Cutting of Plant & Food Research Australia, unless otherwise stated.

PH400-009

Avocado pollination manual:

1. https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/avocado-pollination-brochure/
2. https://www.planthealthaustralia.com.au/industries/avocados/
3. https://beeaware.org.au/pollination/pollinator-reliant-crops/avocados/

Maximise your avocado crop with better pollination

1 THE BASICS OF AVOCADO POLLINATION

Avocado flowers open first as females that are receptive to pollen, then close before reopening the next day as male pollen donors. Fertilisation only occurs when pollen is transferred to a female flower from a male flower.

Avocado plant cultivars are grouped into two types based on when the flowers are in the male or female phases. Type B (eg Bacon, Edranol, Ettinger, Fuerte) will generally be in the male phase and release pollen when Type A (eg Hass) is in the receptive female phase. Inter-planting cultivars of the different types is therefore not only good for promoting cross-pollination, it also increases the chances that enough pollen will be available when the female flowers are ready to be fertilised and produce fruit.

Male Hass avocado flower releasing pollen

It's typical for about three fruit to be produced from one thousand flowers. This very low fruit set rate means that even slight increases in pollination can significantly increase fruit set and yield.

Female Hass avocado flower ready to be pollinated

Although avocado flowers are self-fertile, the entire breeding system has evolved to reduce the amount of self-pollination that occurs. Growing a mix of cultivars increases the chances of successful pollination and improves final yields because cross-pollinated fruit are less likely to be dropped early during periods of stress.

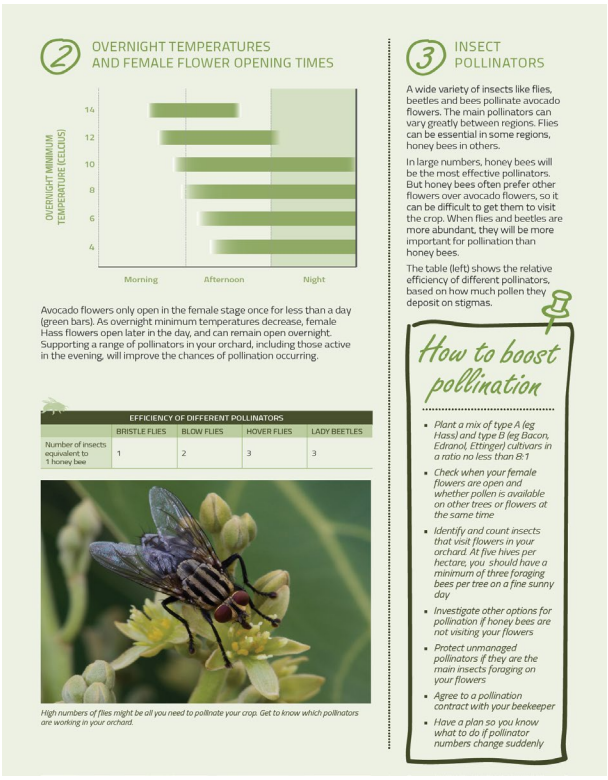
Planting in the ratio of at least one pollen donor tree for eight main cultivar trees will reduce the negative effect of distance from pollen donor trees on fruit set in low flowering years.

Self-pollination within a single cultivar can occur when there is overlap between male and female phases. When it's warm, the overlap is usually for a few hours in the middle of the day. If it's colder, the overlap may be in the late afternoon or evening, or even overnight.

The time at which female Hass flowers open changes with temperature. As overnight temperatures drop, flowering is delayed until later in the day. When overnight temperatures drop below about 7°C, most female flowers open in the late afternoon, stay open overnight, and close the next morning. It's not known how much night active insects like moths contribute to pollination.

What you need to know

- *Pollination of avocados is less predictable than for other crops*
- *Flowers open first as females, then open again as males*
- *Flowers open once as females, usually for only 4-6 hours*
- *Plants grown as pollen providers need to have their male flowers open when the main crop has female flowers open to make sure you have a good supply of pollen*
- *While self-pollination is possible, cross pollinated fruit are more likely to stay on the tree until harvest*
- *When overnight temperatures are low, female flowers don't open until the afternoon or evening*
- *Having a wide range of insects visiting flowers will ensure you get good levels of pollination no matter when the flowers open*
- *Honey bees often prefer other flowers over avocado*
- *High numbers of flies might be all you need to pollinate your crop, depending on where you are*
- *The number of pollinators can differ from year to year depending on land management and the health of local honey bees*



2 OVERNIGHT TEMPERATURES AND FEMALE FLOWER OPENING TIMES

Avocado flowers only open in the female stage once for less than a day (green bars). As overnight minimum temperatures decrease, female Hass flowers open later in the day, and can remain open overnight. Supporting a range of pollinators in your orchard, including those active in the evening, will improve the chances of pollination occurring.

EFFICIENCY OF DIFFERENT POLLINATORS

	BRISTLE FLIES	BLOW FLIES	HOVER FLIES	LADY BEETLES
Number of insects equivalent to 1 honey bee	1	2	3	3

High numbers of flies might be all you need to pollinate your crop. Get to know which pollinators are working in your orchard.

3 INSECT POLLINATORS

A wide variety of insects like flies, beetles and bees pollinate avocado flowers. The main pollinators can vary greatly between regions. Flies can be essential in some regions, honey bees in others.

In large numbers, honey bees will be the most effective pollinators. But honey bees often prefer other flowers over avocado flowers, so it can be difficult to get them to visit the crop. When flies and beetles are more abundant, they will be more important for pollination than honey bees.

The table (left) shows the relative efficiency of different pollinators, based on how much pollen they deposit on stigmas.

How to boost pollination

- *Plant a mix of type A (eg Hass) and type B (eg Bacon, Edranol, Ettinger) cultivars in a ratio no less than 8:1*
- *Check when your female flowers are open and whether pollen is available on other trees or flowers at the same time*
- *Identify and count insects that visit flowers in your orchard. At five hives per hectare, you should have a minimum of three foraging bees per tree on a fine sunny day*
- *Investigate other options for pollination if honey bees are not visiting your flowers*
- *Protect unmanaged pollinators if they are the main insects foraging on your flowers*
- *Agree to a pollination contract with your beekeeper*
- *Have a plan so you know what to do if pollinator numbers change suddenly*

Honey bees

Honey bees might only pollinate one flower per minute, so generally the more you have on flowers the better. Counting bees and other insects on trees will help you to work out the amount and evenness of pollination in your orchard.

Even if you do not currently bring in hives, it is important to know how reliant you are on local honey bees to pollinate your crop.

Recommended hive stocking rate for avocados is 5-8 per hectare, but you should check bee activity in your orchard after hive placement. If you see few bees, and few other insect pollinators, then you need more hives.

The presence of other crops that are flowering at the same time can greatly reduce the numbers of bees foraging on your avocado. For this reason, new avocado orchards should not be sited next to other crops such as citrus that flower at the same time.

Honey bees that are gathering nectar can visit both female and male flowers while foraging.

It is important to order hives early enough in the season to ensure an adequate supply when trees are flowering, as beekeepers need time to prepare colonies suitable for pollination. To be sure of what you are getting, and there are no misunderstandings about what is being supplied, develop a pollination agreement with your beekeeper.

The placement of hives is important. Colonies should be spread evenly throughout the orchard in groups of 2-4 hives, to ensure bees visit flowers across the entire orchard.

Honey bees need water, so growers and beekeepers should agree on how this will be provided.

Flies

Flies can be better at cross-pollinating avocado than honey bees because they move randomly through an orchard between different cultivars, visiting male and female flowers.

The effectiveness of flies as pollinators varies between species, but there can often be more than 20 types in an orchard. This means it's likely that some good pollinators will be present, and that pollination can occur whenever the female flowers are open.

Green blow fly pollinating an avocado flower

When they are visiting in large numbers, flies can be the most important pollinators.

If you think flies are important pollinators in your orchard, they might be breeding in damp leaf litter (eg some hoverflies) or on other organic matter (eg blow flies, flesh flies). Although it's easy to encourage flies to breed in your orchard, you should consider their potential negative impacts on you and your neighbours. Breeding sites may pose a biosecurity risk because they can attract disease carrying insects (eg mosquitoes), vermin and feral or wild animals.

Other types of flies can be beneficial because they are predators of plant pests. Aphids for example are preyed upon by the larvae of hoverflies, which may be breeding on long grass.

Orange hover fly pollinating an avocado flower

Lady beetle pollinating an avocado flower

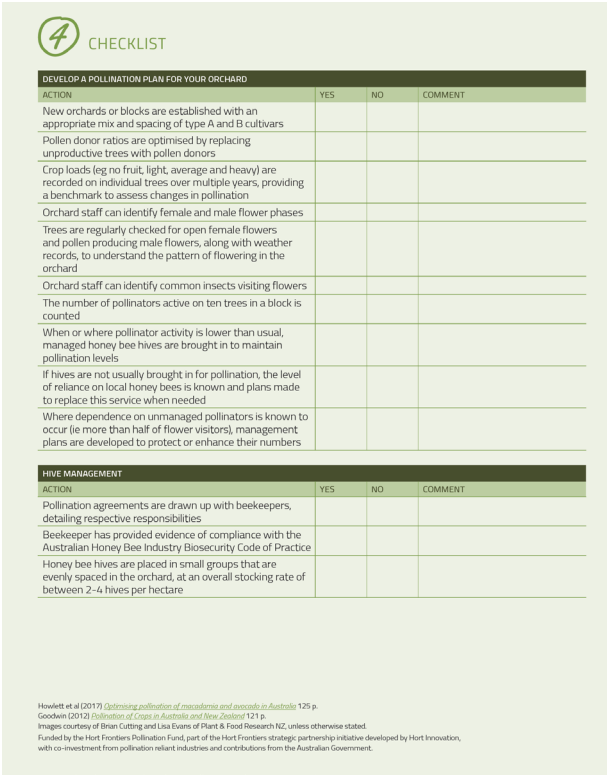
It is particularly important to avoid the use of pesticides during flowering. Non-bee pollinators are likely to be present in orchards both day and night and strategically applying pesticides to minimise honey bee losses is still likely to cause large losses of non-bee pollinators.

Top Tip

Having a wide range of abundant pollinators in your orchard is the key to good avocado yield.

Other pollinators

Beetles, moths and other small insects can also boost pollination in avocado orchards. Just like the benefits of fly diversity, having a range of other insects in your orchard can improve pollination and crop yields.



4 CHECKLIST

DEVELOP A POLLINATION PLAN FOR YOUR ORCHARD			
ACTION	YES	NO	COMMENT
New orchards or blocks are established with an appropriate mix and spacing of type A and B cultivars			
Pollen donor ratios are optimised by replacing unproductive trees with pollen donors			
Crop loads (eg no fruit, light, average and heavy) are recorded on individual trees over multiple years, providing a benchmark to assess changes in pollination			
Orchard staff can identify female and male flower phases			
Trees are regularly checked for open female flowers and pollen producing male flowers, along with weather records, to understand the pattern of flowering in the orchard			
Orchard staff can identify common insects visiting flowers			
The number of pollinators active on ten trees in a block is counted			
When or where pollinator activity is lower than usual, managed honey bee hives are brought in to maintain pollination levels			
If hives are not usually brought in for pollination, the level of reliance on local honey bees is known and plans made to replace this service when needed			
Where dependence on unmanaged pollinators is known to occur (ie more than half of flower visitors), management plans are developed to protect or enhance their numbers			

HIVE MANAGEMENT			
ACTION	YES	NO	COMMENT
Pollination agreements are drawn up with beekeepers, detailing respective responsibilities			
Beekeeper has provided evidence of compliance with the Australian Honey Bee Industry Biosecurity Code of Practice			
Honey bee hives are placed in small groups that are evenly spaced in the orchard, at an overall stocking rate of between 2-4 hives per hectare			

Howlett et al (2017) *Optimising pollination of macadamia and avocado in Australia* 125 p.
Goodwin (2012) *Pollination of Crops in Australia and New Zealand* 121 p.
Images courtesy of Brian Cutting and Lisa Evans of Plant & Food Research NZ, unless otherwise stated.
Funded by the Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from pollination reliant industries and contributions from the Australian Government.

2. Identifying pollinators and developing diverse pollination strategies

Here we report which insect species/taxonomic groups visit and pollinate our focal crops. We also provide some crop-specific recommendations for how to manipulate honey bee and stingless bee colonies to achieve better pollination. Each of the following topics in this section covers methods, results, and a summary:

2.1 Identity of insects visiting the crop and their abundance.
2.2 Pollination efficacy and behaviour of the most abundant insect groups.
2.3 Pollination deficits.
2.4 Management practices for key pollinators.

2.1 Visiting insects and their abundance

Methods

We visited farms across different growing regions to identify the flower visitors for seven crop species, including watermelon, blueberry, macadamia, lychee, papaya, avocado, and almonds. Flower visiting insects were captured in sweep nets and preserved for identification purposes. To determine their abundance we conducted surveys of insects on the crop flowers throughout the day and across multiple blocks/farms. The survey method used was tailored to each crop/growing system but all surveys and subsequent analysis controlled for number of flowers observed, the observation period, and the location within the crop (distance from edge). The following publications provide the precise survey method employed for a given crop:

- **Watermelon**: Subasinghe Arachchige EC, Rader R, Cutting BT, Keir M, van Noort T, Fale G, Howlett BG, Samnegård U, Evans LJ 2022. Honey bees are the most abundant visitors to Australian watermelon but native stingless bees are equally effective as pollinators. Ecological Solutions and Evidence, 3(4): e12189.
- **Blueberry**: Kendall LK, Gagic V, Evans LJ, Cutting BT, Scalzo J, Hanusch Y, Jones J, Rocchetti M, Sonter C, Keir M, Rader R 2020. Self-compatible blueberry cultivars require fewer floral visits to maximize fruit production than a partially self-incompatible cultivar. Journal of Applied Ecology 57(12): 2454-2462.
- **Macadamia**: Evans LJ, Jesson L, Read SFJ, Jochym M, Cutting BT, Gayrard T, Jammes MAS, Roumier R, Howlett BG 2021. Key factors influencing forager distribution across macadamia orchards differ among species of managed bees. Basic and Applied Ecology 53: 74-85.
- **Lychee:** Wilson RS, Evans L, Cutting B, Elliott B, Fuller C, Heard T, Keir M, Nathan T, Searle C, Wallace HM (submitted to Scientia Horticulturae Feb 2024). Insect visitors and their behaviour on *Litchi chinensis* (lychee) flowers in different growing regions.
- **Avocado**: Howlett BG, Evans LJ, Kendall LK, Rader R, McBrydie HM, Read SF, Cutting BT, Robson A, Pattemore DE, Willcox BK 2018. Surveying insect flower visitors to crops in New Zealand and Australia. BioRxiv: 373126.

Results

Watermelon

Locations and number of farms: Lakeland (QLD) 2 farms, Gumlu (QLD) 3 farms, Chinchilla (QLD) 3 farms. Each farm was surveyed on 2–3 fine weather days.

Note: PFR collaborated with the University of New England (UNE) to survey watermelon farms more broadly across Australia. The same type of data were collected in Katherine (Northern Territory) 3 farms, and Riverina (NSW) 4 farms. Data from all 15 farms are presented in this report only where specified, but are presented together in the following publication: Subasinghe Arachchige EC, Rader R, Cutting BT, Keir M, van Noort T, Fale G, Howlett BG, Samnegård U, Evans LJ 2022. Honey bees are the most abundant visitors to Australian watermelon but native stingless bees are equally effective as pollinators. Ecological Solutions and Evidence, 3(4): e12189.

Visiting insects in Queensland watermelon – There were representatives from five taxonomic orders: Hymenoptera, Diptera, Lepidoptera, Hemiptera and Coleoptera, on watermelon flowers in all QLD growing regions. We were able to identify 14 different insect families and 11 different confirmed genera. Some examples are shown in Figure 2.
Overall the number of insects on watermelon flowers was greatest in the Lakeland region, with our standardised point-count surveys showing 25% more insect activity compared with Chinchilla and 15% more than Gumlu. Honey bees (*A.*

mellifera) were the predominant flower visitors in all three growing regions, constituting 74% to 86% of visitors (Figure 3). The prevalence of native bees was higher in our southernmost sampling region, Chinchilla (21%), and included stingless bees (*Tetragonula* sp.), leaf-cutting bees in the family Megachilidae, and sweat bees from the family Halictidae such as *Lasioglossum*, *Homalictus*, and *Lipotriches*. Dipteran flies, primarily from the family Syrphidae, were present in limited numbers across all regions, although their abundance was relatively higher in Lakeland (constituting 4.6% of the total observed insects) than the other regions. Other insect taxa, including beetles (e.g., Coccinellidae and Chrysomelidae), butterflies and moths (e.g., Hesperiidae), true bugs (Miridae), and wasps (e.g., Ichneumonidae) were also recorded in small numbers in all regions. The relative abundance of these other insect taxa was most pronounced in Chinchilla (5.8%). Our surveys were conducted between August and December (spring to early summer), which is common period for growing watermelon in QLD. However in these regions, watermelon can be grown year-round and the insect visitors/pollinators may differ throughout the year.

Figure 2. Examples of flower visitors of watermelon in Queensland: a) honey bee (*Apis mellifera*) feeding on nectar, b) pollen foraging stingless bee (*Tetragonula* sp.), c) sweat bee (*Homalictus* sp.), d) *Homalictus* sp, e) a dipteran and lepidopteran, f) skipper (Lepidoptera: Hesperiidae). Insects in panels (a,b,d,e,f) are on male flowers and panel (c) on female flowers. Photos: Brian Cutting.

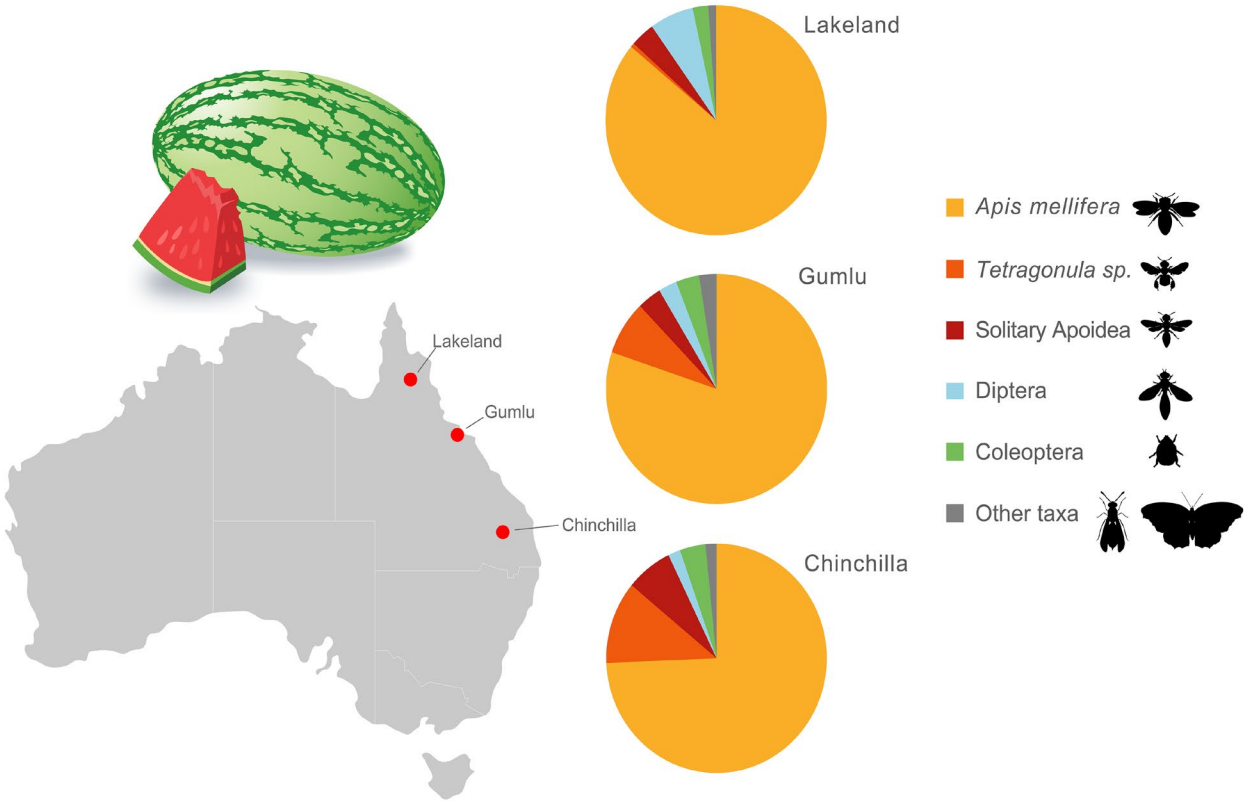


Figure 3. Relative abundance of insect visitors within three Queensland watermelon growing regions (Lakeland, Chinchilla, and Gumlu). Insect visitors have been categorised as honey bees (*Apis mellifera*), stingless bees (*Tetragonula* sp.), native bees (solitary Apoidea), flies (Diptera), beetles (Coleoptera), and other taxa, within each region.

Figure 3. Is sourced from the below publication under license: https://creativecommons.org/licenses/by/4.0/. Modifications have been made to the categorisation of insects, sampling regions shown, and insect images. Subasinghe Arachchige EC, Rader R, Cutting BT, Keir M, van Noort T, Fale G, Howlett BG, Samnegård U, Evans LJ 2022. Honey bees are the most abundant visitors to Australian watermelon but native stingless bees are equally effective as pollinators. Ecological Solutions and Evidence, 3(4): e12189.

Flower visitation rates to watermelon flowers were also assessed for the most abundant flower visitors, taking into account the number of flowers being observed. These results include data from all 15 farms across Australia and flower visitors were grouped as honey bees (*A. mellifera*), native bees, and flies (Diptera). Watermelon flowers received 7 times more visits from honey bees compared with native bees (honey bee mean visits ± SE = 36 ± 0.8 flowers/h; native bee mean visits ± SE = 5.3 ± 0.4 flowers/h; model estimate (Est.) = 2.07, SE = 0.07, t. ratio = 27.8, $p < 0.001$) and 36 times more visits compared with flies (flies mean visits ± SE = 1 ± 0.2 flower/h: Est. = 3.72, SE = 0.18, t. ratio = 20.4, $p < 0.001$; Figure 4).

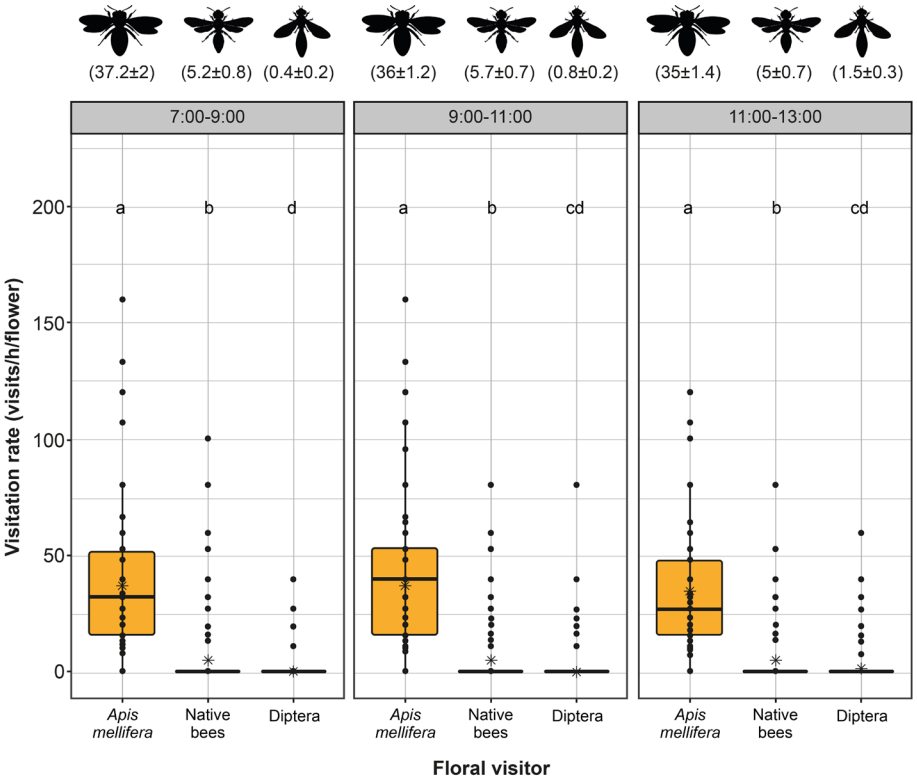


Figure 4. Visitation rate (number of visits per flower per hour) by honey bees (*Apis mellifera*), native bees and flies to watermelon flowers over the course of the daily flowering period. In each box, the bold horizontal line is the median, and means are shown with an asterisk (*). The lower and upper edges of the box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (circular data points). Different letters indicate significant difference of floral visitors across time periods (interaction between floral visitors × time period interaction) (EMMeans pairwise comparisons at α = 0.05 and Confidence Level = 0.95).

Figure 4. Is sourced from the below publication under license: https://creativecommons.org/licenses/by/4.0/. Modifications have been made to the colour scheme, insect images, and axes labels. Subasinghe Arachchige EC, Rader R, Cutting BT, Keir M, van Noort T, Fale G, Howlett BG, Samnegård U, Evans LJ 2022. Honey bees are the most abundant visitors to Australian watermelon but native stingless bees are equally effective as pollinators. Ecological Solutions and Evidence, 3(4): e12189.

Lychee

Locations and number of farms: Tully (QLD) 2 farms, Mareeba (QLD) 2 farms; Sunshine Coast Region (QLD) 2 farms. All farms were surveyed on 3–5 fine weather days.

Note: PFR collaborated with Griffith University to survey a larger number of lychee farms. The same type of data were collected in 3 further farms in Wide Bay Burnett/Sunshine Coast Region (QLD). These data are reported on together in the following paper: Wilson RS, Evans L, Cutting B, Elliott B, Fuller C, Heard T, Keir M, Nathan T, Searle C, Wallace HM (submitted to Scientia Horticulturae Feb 2024). Insect visitors and their behaviour on *Litchi chinensis* (lychee) flowers in different growing regions.

Visiting insects in Queensland lychee – We observed representatives from five orders: Hymenoptera, Diptera, Coleoptera, Lepidoptera and Hemiptera visiting lychee flowers on farms in Tully and on the Sunshine Coast. Farms surveyed in Mareeba were less diverse, with representatives sighted only from Hymenoptera and Diptera. Overall we identified 20 morphospecies of hymenopterans, including European honey bees (*A. mellifera*), Asian honey bees (*A. cerana*), and stingless bees (*Tetragonula* sp.), 11 morphospecies of Diptera (e.g., Rhiniidae, Calliphoridae, and Syrphidae), eight morphospecies of Lepidoptera (e.g., Hesperiidae), four species of Coleoptera (e.g., Cantharidae), and one lacewing (Neuroptera). Some examples are shown in Figure 5.

Overall insect activity on lychee flowers was greatest in the Tully region, with average daily insect abundance in our standardised point-counts being four times higher compared with Mareeba and three times higher than the Sunshine Coast Region. There was large variation between farms within regions. For example, surveys at one farm on the Sunshine Coast yielded an average of 10 insects during the survey period compared with 63 at another farm.

The predominant flower visitors varied across regions; honey bees (*A. mellifera*) were the most abundant visitors of lychee in Tully and Mareeba (38% and 93% of visitors, respectively), while stingless bees (*Tetragonula* sp.) were more abundant on lychee flowers in the Sunshine Coast Region (51%). In Tully, 'nose flies' in the family Rhiniidae (Diptera), were the second most abundant taxonomic group (33%), but these flies only made up 3% visitors in Mareeba and 8% of visitors on the Sunshine Coast (Figure 6). Our 'other taxa' category includes other species of Diptera (all regions) and Lepidoptera, Vespidae (Hymenoptera), and Coleoptera (Tully and Sunshine Coast).

Figure 5. Examples of flower visitors on lychee in Queensland: a) honey bee (*Apis mellifera*), b) nose fly (Rhiniidae), c) pollen foraging stingless bee (*Tetragonula* sp.), d) lepidopteran species, e) nectar foraging stingless bee (*Tetragonula* sp.), f) solitary bee species. Insects in panels (a and e) are on female flowers and panels (b, c, d, f) are on male flowers. Photos: Brian Cutting.

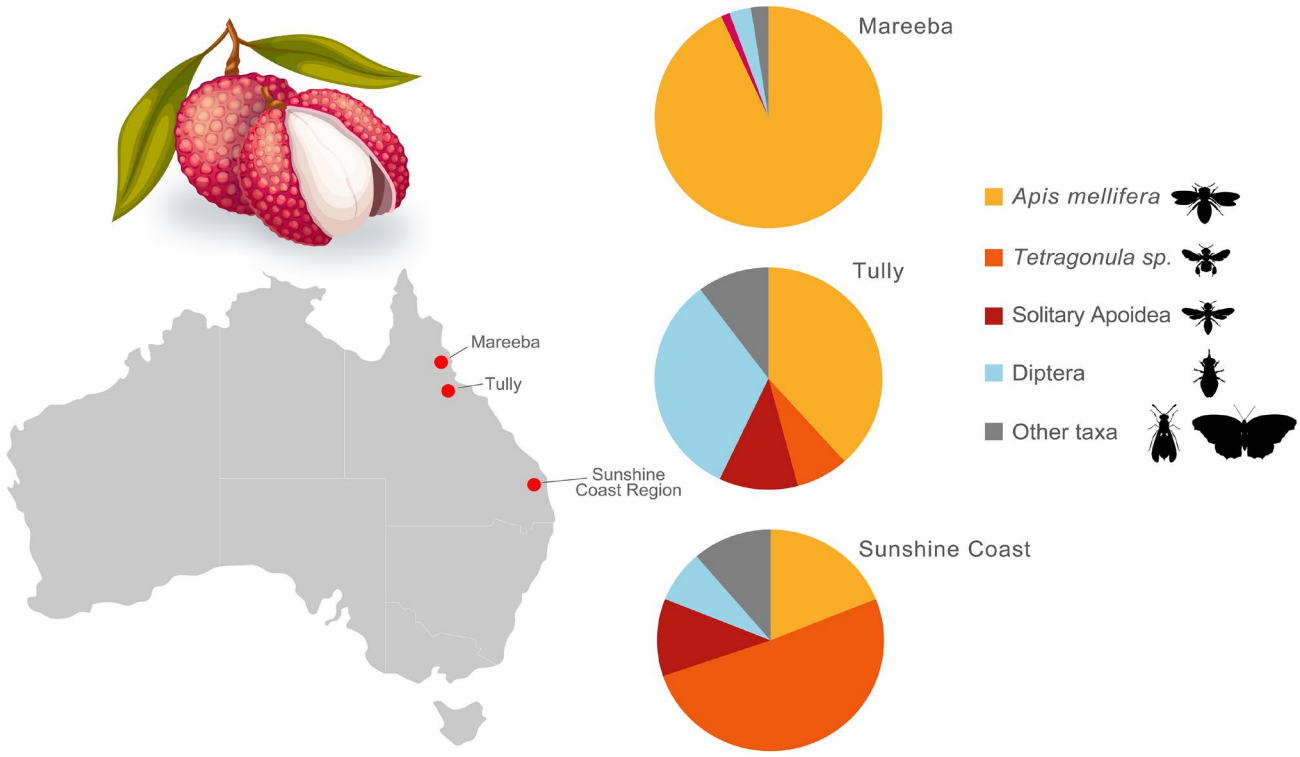


Figure 6. Relative abundance of insect visitors within three Queensland lychee growing regions (Mareeba, Tully, and Sunshine Coast). Insect visitors have been categorised as honey bees (*Apis mellifera*), stingless bees (*Tetragonula* sp.), native bees (solitary Apoidea), flies (Diptera), and other taxa, within each region.

Macadamia

Locations and number of farms: Bundaberg (QLD) 7 farms, Gympie (QLD) 3 farms, Northern Rivers (QLD) 3 farms. All farms were surveyed on 1–2 fine weather days.

Note: some of these data were collected and reported on under MT13060; "Optimising pollination of macadamia & avocado in Australia". They have been reproduced here, with permission, because tech transfer of these data was carried out in the current program and these data were built upon in subsequent trials outlined within this report.

Visiting insects in Queensland macadamia – We observed representatives from the insect orders Hymenoptera, Diptera, and Coleoptera in all three growing regions. In addition, we observed Lepidoptera in Gympie, and species of Neuroptera and Odonata in Northern Rivers. Overall we identified 21 distinct families and 23 genera of insects visiting macadamia flowers. Some examples are shown in Figure 7.

Insect activity on macadamia flowers was greatest in the Gympie region, with average daily insect abundance in our standardised point-counts being 3 times higher compared with Northern Rivers and 2 times higher than in Bundaberg. The predominant flower visitors varied across regions: honey bees (*A. mellifera*) were the most abundant visitors of macadamia in Bundaberg (87%) and Gympie (63%), but in Northern Rivers Coleoptera (*Porrostoma rufipenne* and *Monolepta australis*) were more abundant, contributing 70% of insect visitors. In Gympie, stingless bees (*Tetragonula* sp.) were also in high abundance (28%). Diptera (e.g., Syrphidae and Calliphoridae) were the third most abundant taxonomic group in all three regions (4%–10%), while other taxa such as Ichneumonidae were present in lower numbers in all regions. Solitary Apoidea (e.g., Halictidae and Anthophoridae) were found in low numbers in Gympie and Northern Rivers (Figure 8).

Figure 7. Examples of flower visitors on macadamia in Queensland: a) honey bee (*Apis mellifera*), b) pollen foraging stingless bee (*Tetragonula* sp.), c) Austronomia (Halictidae), d) Masked bees (Hylaeus sp.), e) nose fly (Rhiniidae), f) Lycid beetle (*Porrostoma rufipenne*). Photos: Brian Cutting.

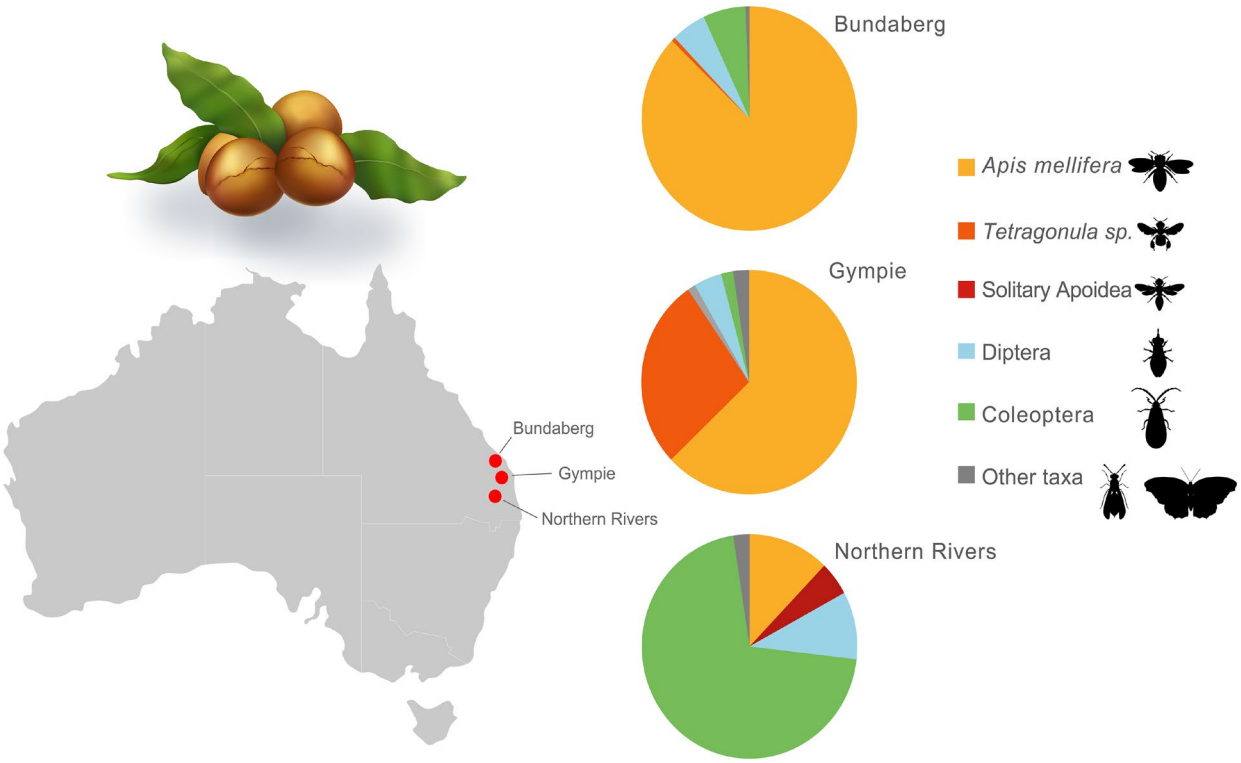


Figure 8. Relative abundance of insect visitors within three Queensland macadamia growing regions (Bundaberg, Gympie, and Northern Rivers). Insect visitors have been categorised as honey bees (*Apis mellifera*), stingless bees (*Tetragonula* sp.), native bees (solitary Apoidea), flies (Diptera), beetles (Coleoptera), and other taxa, within each region.

Blueberry

Locations and number of farms: near Devonport and Cygnet (Tasmania (TAS)) 4 farms (multiple blocks were surveyed per farm). All farms were surveyed on 1–3 fine weather days.

Note: PFR collaborated with UNE and Sydney University to survey a larger number of blueberry farms across regions. The same type of data were collected in additional farms in Tasmania (5 farms – data from Sydney University) and Coffs Harbour, NSW (5 farms – data from UNE). Data from this extended sampling are presented in this report only where specified, but are presented together in the following publication: Kendall LK, Gagic V, Evans LJ, Cutting BT, Scalzo J, Hanusch Y, Jones J, Rocchetti M, Sonter C, Keir M, Rader R 2020. Self-compatible blueberry cultivars require fewer floral visits to maximize fruit production than a partially self-incompatible cultivar. Journal of Applied Ecology 57(12): 2454-2462.

Visiting insects in Tasmania blueberry – We observed representatives from the orders Hymenoptera and Diptera visiting northern highbush blueberry flowers. Nectar-feeding birds (e.g., scarlet honeyeater (*Myzomela sanguinolenta*)), were also observed visiting blueberry flowers but were not recorded in surveys. Some examples of blueberry flower visitors are shown in Figure 9. Honey bees (*A. mellifera*) were the predominant insect visitor to blueberry flowers on these Tasmanian farms, comprising 56% of visitors recorded. Bumble bees (*Bombus terrestris*) were also prominent, comprising 30% of the visitors. The remainder of the insects recorded were Diptera (8%, primarily Syrphidae) and other taxa (3%, including non-social Hymenoptera and Formicidae) (Figure 10).

Figure 9. Examples of flower visitors on blueberry flowers in Tasmania: a) honey bee (*Apis mellifera*), b) honey bee nectar-robbing through a hole chewed by *Bombus terrestris*, c) queen bumble bee (*Bombus terrestris*), d) hoverfly (Syrphidae), e) blow fly (Calliphoridae), f) scarlet honey eater (*Myzomela sanguinolenta*). Photos: Brian Cutting.

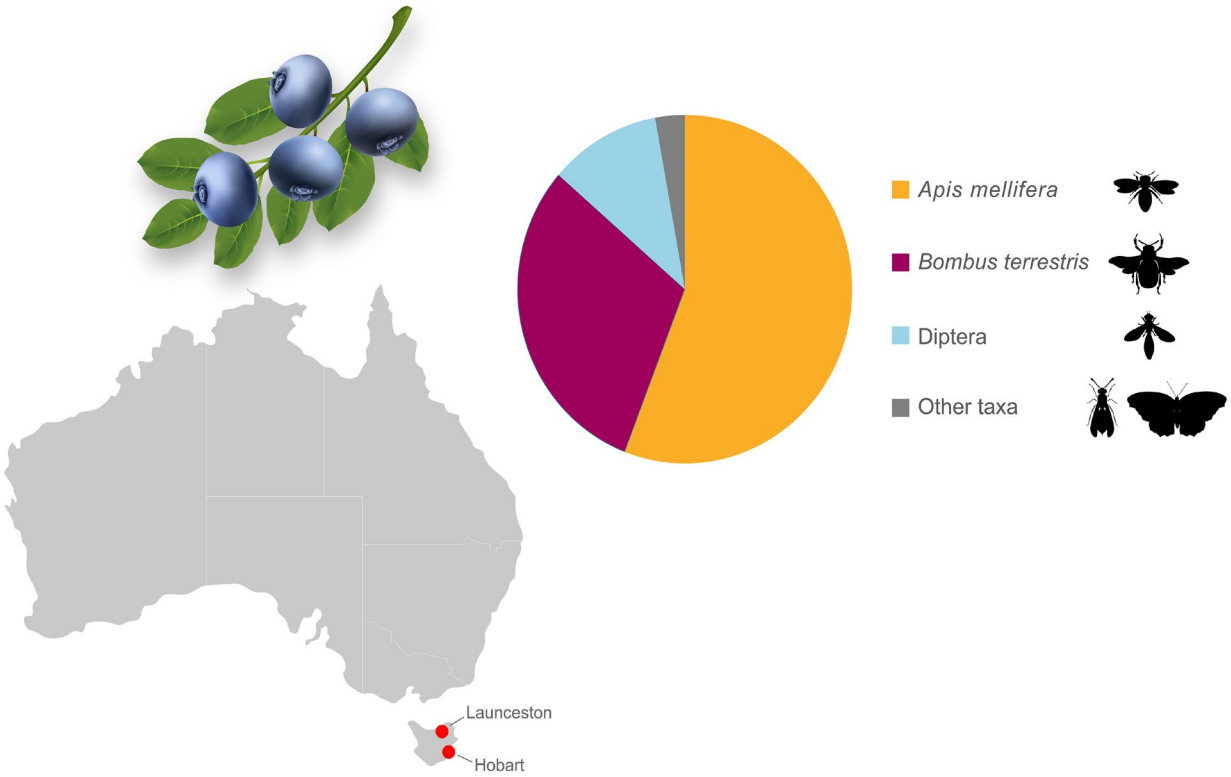


Figure 10. Relative abundance of insect visitors to blueberries in farms located outside of Launceston and Hobart in Tasmania. Insect visitors have been categorised as honey bees (*Apis mellifera*), bumble bees (*Bombus terrestris*), flies (Diptera), beetles and other taxa.

Flower visitation rates to blueberry flowers were also assessed for the most abundant flower visitors across two growing regions (honey bees and bumble bees in TAS and honey bees and stingless bees in NSW), taking into account the number of flowers being observed. Here we looked at three different types of blueberries; northern highbush (Tasmania, Coffs Harbour), southern highbush (Coffs Harbour), and rabbiteye (Coffs Harbour). The most abundant insect visitors included honey bees (at both locations), bumble bees (TAS), and stingless bees (NSW). Flower visitation rates/min varied with insect visitor and blueberry plant type, but for all three plant types honey bees were the most frequent visitors (Figure 11). Overall the rabbiteye blueberries (*V. virgatum*) received the most bee visits (Figure 11).

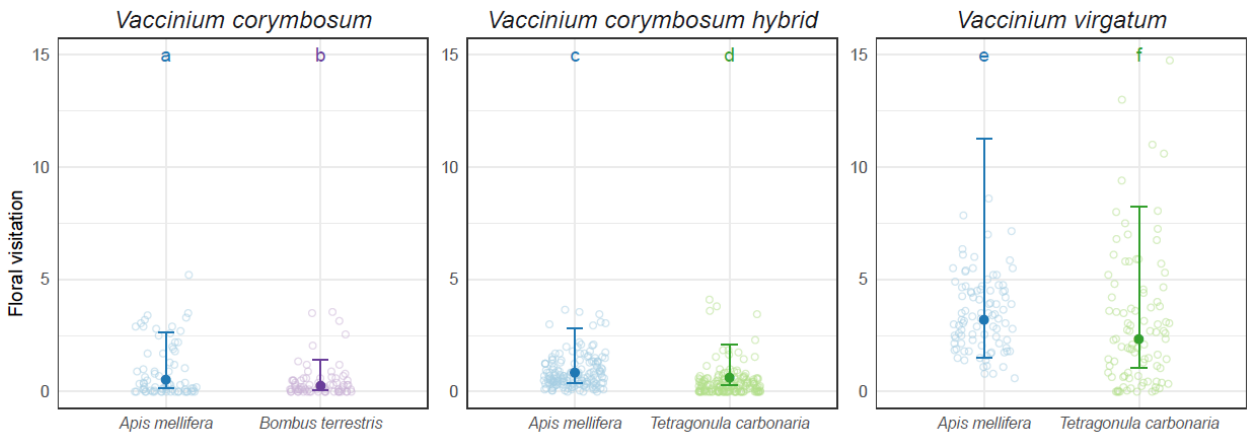


Figure 11. Insect visitors observed/minute: Honey bee (*Apis mellifera*), bumble bee (*Bombus terrestris*) and stingless bees (*Tetragonula carbonaria*) on northern highbush (*Vaccinium corymbosum*), southern highbush (*V. corymbosum* hybrid) and rabbiteye (*V. virgatum*) blueberries. Individual data points represent results from different surveys at each farm. Bold line indicates posterior mean estimate ± 95% CI. Comparisons are made within species between blueberry varieties and between species within a blueberry variety. Different letters denote significant differences.

Avocado

Locations and number of farms: Mildura (Victoria (VIC)) 11 farms, Renmark (VIC) 5 farms, Robinvale (South Australia (SA)) 6 farms, Waikerie (SA) 5 farms. All farms were surveyed on 1–2 fine weather days. Note: these data were collected and reported on under MT13060; "Optimising pollination of macadamia & avocado in Australia". They have been reproduced here, with permission, because further analysis of these data were conducted under the current program and also to maintain reporting format consistency across the crops included in this report.

Visiting insects on avocado in Sunraysia – We observed representatives from the following orders: Hymenoptera, Diptera, Coleoptera, Lepidoptera, and Hemiptera in avocado flowers in all four growing regions. We also found representatives from Blattodea and Dermaptera in Mildura, and representatives from Neuroptera in Mildura and Waikerie. Some examples are shown in Figure 12.

Overall dipteran species were the most abundant avocado flower visitors (all together 52% of visitors). Common dipteran species encountered included species from the following families: Calliphoridae, Rhiniidae, Syrphidae, Anthomyiidae, and Tachinidae. Coleoptera (primarily Coccinellidae, but also Chrysomelidae and Lycidae) were also abundant (all together 35% of visitors). Honey bees (*A. mellifera*) and solitary bees (e.g., Colletidae and Halictidae) made up a small proportion of the recorded flower visitors; 7% and 0.3% respectively. Our 'other taxa' category included Lepidoptera (e.g., Lycaenidae), wasps (e.g., Thynnidae, Ichneumonidae, and Vespidae), Blattodea (cockroaches), Dermaptera (earwigs), and Neuroptera (lacewings), and contributed 5% of the visitors (Figure 13).

Figure 12. Example flower visitors of avocado flowers in the Sunraysia Region: a) common green bottle fly (*Lucilia sericata*), b) black-orange hoverfly (*Melangyna viridiceps*), c) brown blow fly (*Calliphora stygia*), d) honey bee (*Apis mellifera*), e) flesh fly (*Oxysarcodexia* sp.), f) variegated ladybird (*Hippodamia variegate*). Photos Brian Cutting (a,e), Lisa Evans (b,c,f) and Melissa Broussard (d).

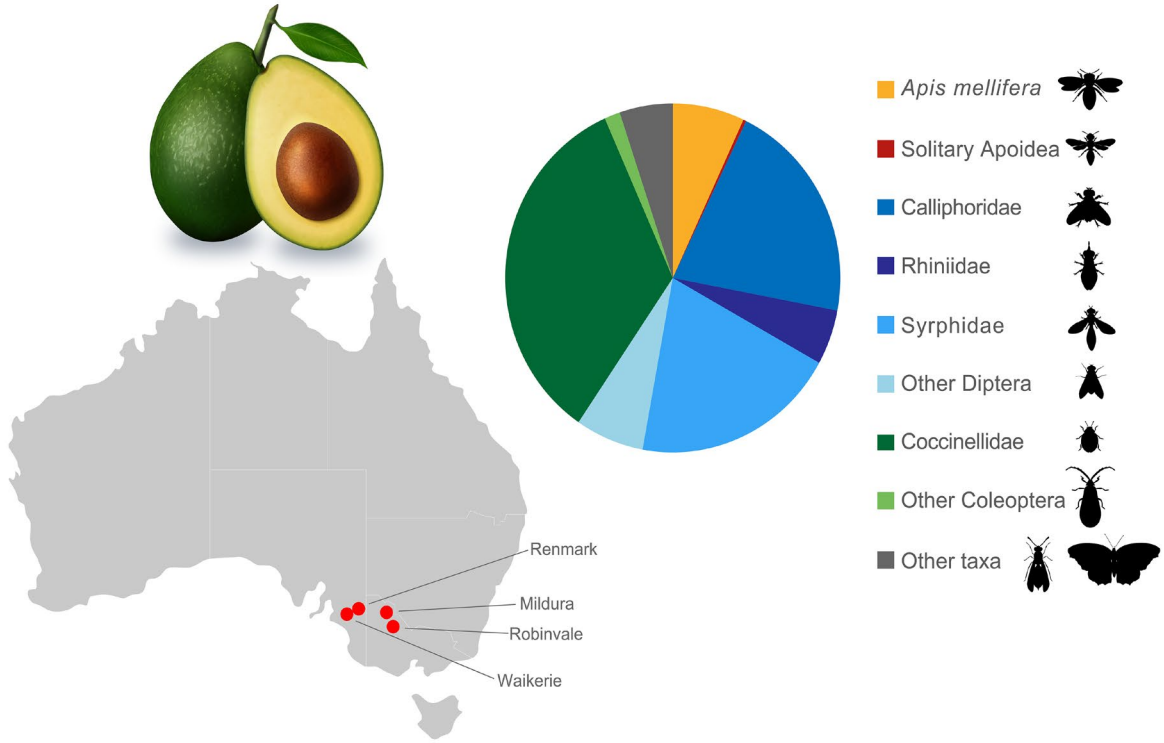


Figure 13. Relative abundance of insect visitors to avocado flowers within the Tri-State growing region. Insect visitors have been categorised as honey bees (*Apis mellifera*), native bees (solitary Apoidea), blow flies (Calliphoridae), nose flies (Rhiniidae), hover flies (Syrphidae), other flies (Diptera), ladybirds (Coccinellidae), other beetles (Coleoptera), and other taxa.

Papaya

Locations and number of farms: Mareeba (QLD) 2 farms, Brisbane (QLD) 1 farm. Video footage of 44 flowers was collected in Mareeba over two days (~83 h including daytime and night time) and 19 flowers in Brisbane over two days (~38 h including daytime and night time). Observations included red fleshed varieties (papaya) and yellow fleshed varieties (paw paw).

Flower visitors on papaya – We observed representatives from the following insect orders: Hymenoptera, Lepidoptera, Diptera. We also found representatives from Araneae and one vertebrate. Some examples are shown in Figure 14.

The predominant flower visitors differed in the two regions: honey bees (*A. mellifera*) were the most abundant visitors of papaya in Brisbane (56%), whereas Lepidoptera (primarily hawk moths (Sphingidae), including a day- and night-active species, were the most abundant visitors in Mareeba (28%). Of the two regions Mareeba was more diverse, and stingless bees (*Tetragonula* sp.) (31%), Diptera (15%), and honey bees (13%) were relatively abundant. A smaller number of other invertebrates were observed (11% - included wasps and spiders) and one vertebrate (blue faced honeyeater, *Entomyzon cyanotis*). In the Brisbane orchard Lepidoptera (hawk moths) were also relatively abundant, the only other visitors observed were stingless bees (Figure 15). It is noted that the Brisbane observations were conducted during a period of drought which may have impacted abundance of flower-visiting insects.

Figure 14. Example flower visitors of male papaya flowers: a) hawk moth (Sphingidae), b) honey bee (*Apis mellifera*), c) blue faced honeyeater (*Entomyzon cyanotis*).

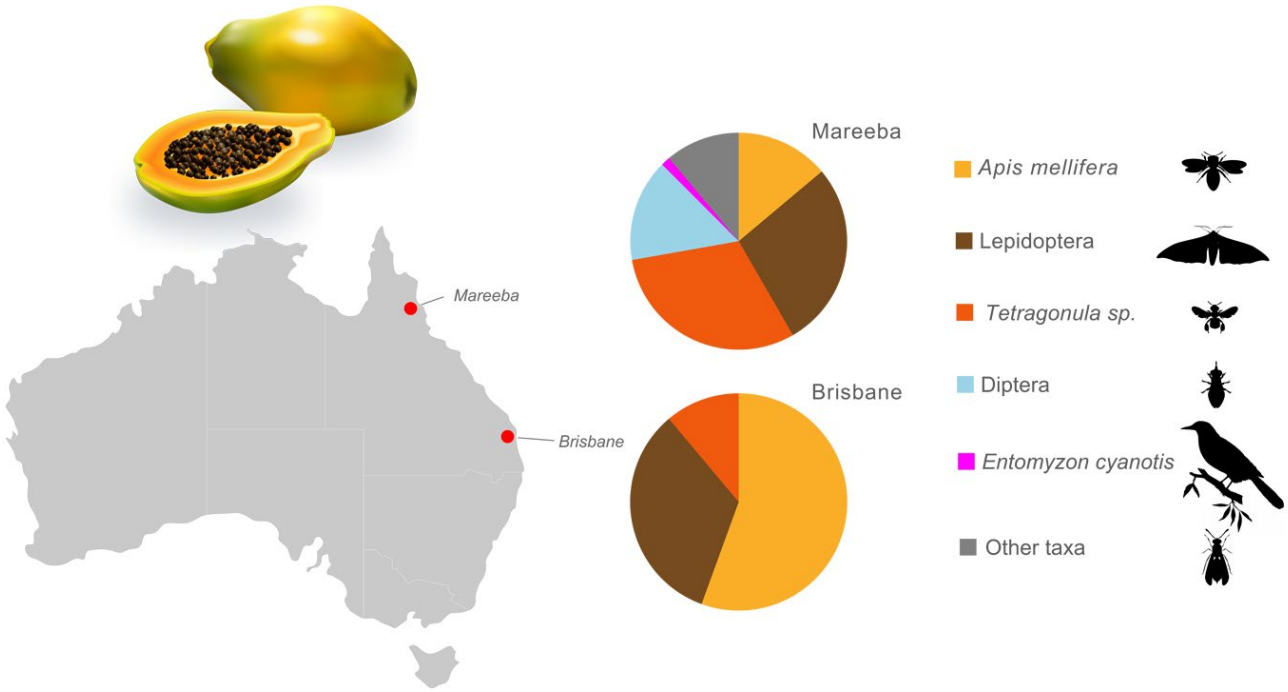


Figure 15. Relative abundance of insect visitors to papaya flowers in the Mareeba and Brisbane regions. Flower visitors have been categorised as honey bees (*Apis mellifera*), moths (Lepidoptera), stingless bees (*Tetragonula* sp.), flies (Diptera), birds – honeyeaters (*Entomyzon cyanotis*), and other taxa.

Almond

Locations and number of farms: Loxton (SA) 2 farms. 70 flowers across 22 recordings were analysed for pollinator visits. This constituted 1,381 hours of video footage of individual flowers.

Visiting insects in South Australia almonds – The diversity of flower visitors captured in our video footage was low, with three different orders/species identified: honey bees (*A. meliffera)*, Diptera, and *Zosterops lateralis* (a passerine bird). Honey bees were the predominant flower visitor, constituting 86% of almond flower visitors recorded. Silvereyes (*Z. lateralis*) were the only other regular visitor recorded – making up 12% of flower visitors. During 41 hours of recording a single species of Diptera was captured (Figure 16).

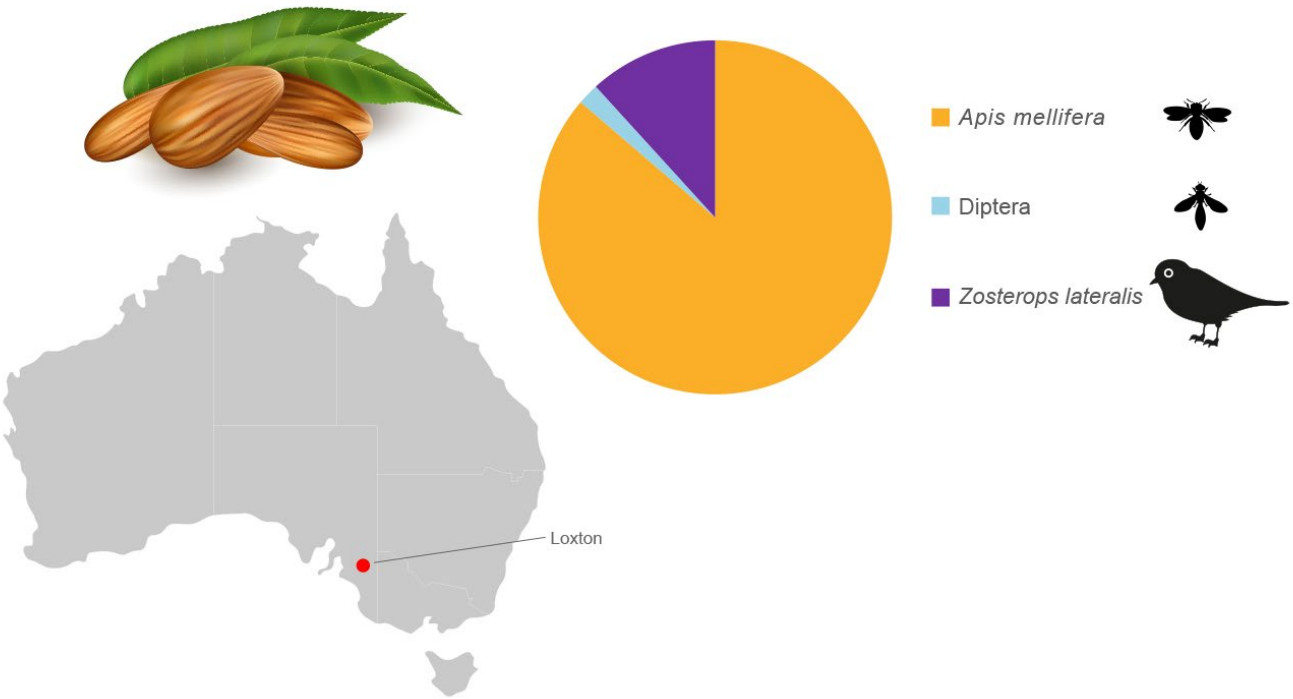


Figure 16. Relative abundance of flower visitors to almond flowers in Loxton (South Australia). Flower visitors have been categorised as honey bees (*Apis mellifera*), flies (Diptera), and silvereyes (*Zosterops lateralis* - a passerine bird).

2.2 Pollination efficacy and behaviour of the most abundant insect groups

Methods

Pollinator effectiveness was measured based on their single-visit pollen deposition on stigmas (watermelon, lychee, and macadamia) or resulting fruit set/quality (blueberry).

To assess single-visit pollen deposition an 'active approach' was used (Howlett et al. 2017) for watermelon, lychee, and macadamia. Previously bagged (unvisited) treatment flowers were presented to insects on flowers (male flowers in watermelon and lychee). Once the targeted insect moved onto the treatment flower, it was allowed to forage uninterrupted (e.g., Figure 17a). 'Method control' flowers were also collected. These flowers were held next to a target insect visitor but the insect was prevented from moving onto the flower. For blueberries, we used a 'static approach'; the flower remained attached to the plant and the researcher waited until an insect visited. Then flowers were re-bagged to prevent further visits.

After receiving an insect visit, each flower was kept moist and stored for at least 24h (precise duration varied among crops) to enable deposited pollen to germinate (e.g., Figure 17b). The number of pollen grains deposited were subsequently counted (e.g., Figure 17d,c). To assess fruit set and fruit quality in blueberry, we counted the number of flowers that developed into fruit and measured fruit weight when ripe (mean 85–90 days after flowering).

Movement patterns of insects between flowers was assessed to determine the likelihood of different insect taxa moving between male and female flowers (watermelon, lychee, and papaya) or different plants (blueberry). Observers followed individual insects, annotating their visits to male/female flowers and stigma/anther contact. Foragers were observed as long as possible, up until a maximum of 10 min. Insects were identified visually without disturbing them. Video cameras were used to record the three different sexual phases of lychee flowers and three types of papaya flowers, to establish whether the most abundant insects visited all flower types.

Figure 17. Steps taken in an 'active approach' to assess single visit pollen deposition. A) Stingless bee foraging uninterrupted on a macadamia flower (typically held amongst other flowers within the raceme), B) insect-visited lychee flowers stored to allow pollen germination/adherence, C) stigmas being prepped for pollen grain counting, D) a lychee stigma under magnification – the small pink-stained pollen grains are evident on and surrounding the stigma.

Results and Interpretation

Watermelon

Pollinator effectiveness – We assessed the number of pollen grains deposited on 387 watermelon flowers after a single insect visit on each flower. Pollen deposition was estimated for honey bees (*A. mellifera*), stingless bees (*Tetragonula* sp.), solitary bees (Apoidea), and flies (Diptera) (Figure 18). All insects/insect groups assessed were capable of pollinating watermelon flowers and deposited more pollen than that observed on unvisited (control) flowers (mean no. pollen grains = 0.65, n = 34). Honey bees and stingless bees deposited similar numbers of pollen grains per flower visit (honey bee mean no. pollen grains ± SE = 39.7 ± 4.3; stingless bee mean ± SE = 26.5 ± 5.5; model Est. = −0.31, SE = 0.22, p = 0.16). Whereas the solitary bees (including *Homalictus* and *Lasioglossum* sp.) and flies (primarily Syrphidae) deposited fewer pollen grains than honey bees (solitary bee mean pollen grains ± SE = 17 ± 5.28; model Est. = −0.77, SE = 0.22, p < 001; flies mean ± SE = 4.75 ± 1.41, model Est. = −2.11, SE = 0.59, p < 001).

Pollen foraging bees (including *Apis*, *Tetragonula*, and solitary bees) deposited more pollen grains on stigmas than nectar collecting bees (model Est. = 0.46, SE = 0.21, t. ratio = 2.21, p = 0.027). Further, more pollen was deposited when a bee moved onto our female test flower after visiting a male flower on a diploid cultivar (polleniser) compared with the bee moving from a male flower on a triploid cultivar (model Est. = 0.52, SE = 0.21, t. ratio = 2.54, p = 0.01; Figure 19).

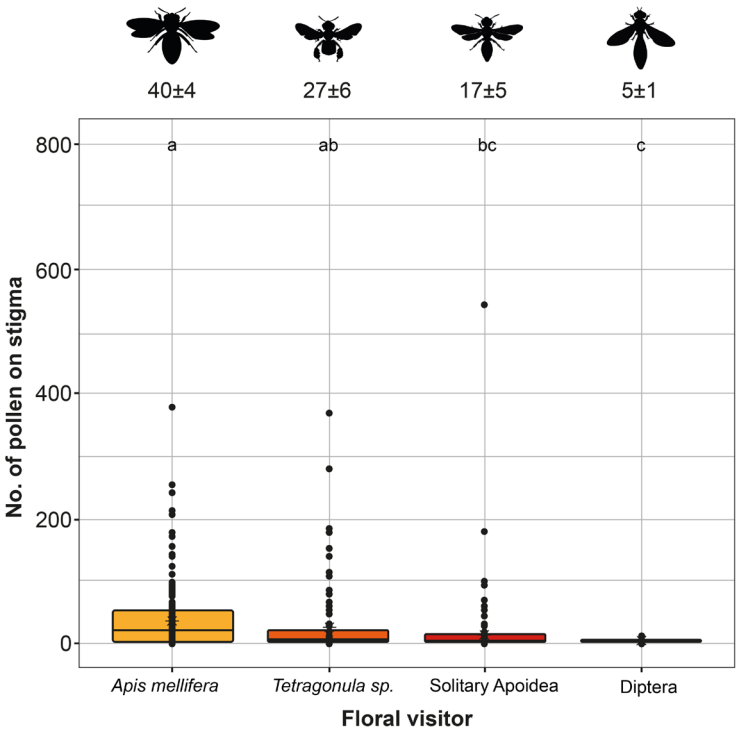


Figure 18. Pollinator effectiveness (single-visit pollen deposition) by honey bees (*Apis mellifera* n = 82), stingless bees (*Tetragonula* sp. n = 103), solitary bees (solitary Apoidea n = 111) and flies (Diptera n = 8) on female watermelon flowers. In each box the bold horizontal line is the median, and means are shown with an asterisk (*). The lower and upper edges of the box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (circular data points). The different letters indicate significant differences among floral visitors (EMMeans pairwise comparisons at α = 0.05 and Confidence Level = 0.95).

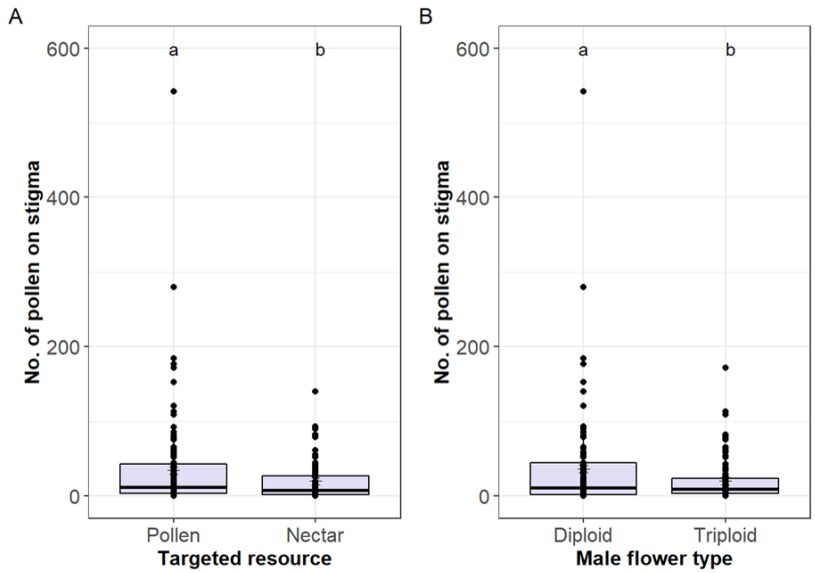


Figure 19. Pollinator effectiveness (single visit pollen deposition) of a) pollen foraging bees (n = 124) versus nectar foraging bees (n = 80), and b) bees whose previous flower visit was to a diploid male (n = 105) versus a triploid male (n = 99). In each box the bold horizontal line is the median, and means are shown with an asterisk (*). The lower and upper edges of the box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (circular data points). Different letters indicate significant differences between variables (EMMeans pairwise comparisons at α = 0.05 and Confidence Level = 0.95).

Movement patterns of insects between watermelon flowers – Movement patterns varied among insect species/taxonomic groups, however all of them visited both male and female flowers while foraging and came into contact with the reproductive parts of the flower (i.e., the anthers and stigma). Honey bees (*A. mellifera*) visited the highest number of flowers per hour (452 flowers/h – prediction based on observed visits per minute), while hoverflies (Syrphidae) visited the lowest number (51 flowers/h). Medium-sized solitary bees visited the highest percentage of female watermelon flowers, with 16% of the flowers the visited being female, compared with a low of 4% female flowers by hoverflies. All bee groups and hoverflies made contact with the anthers in >70% of their visits to male flowers. Honey bees, stingless bees and hoverflies made contact with the stigma in >80% of their visits to female and hermaphroditic flowers (Table 1).

Table 1. Flower movement patterns of insects foraging on watermelon flowers in Queensland.

Species/ taxonomic group	No. of individuals	Mean duration followed (min:s)	Predicted flowers/hr	Predicted % female flowers visited	% anther contact	% stigma contact
Honey bee (*Apis mellifera*)	375	01:59	452.05	9.95	79.40	82.32
Small solitary bee†	10	02:52	61.90	16.42	80.95	40.00
Medium solitary bee†	28	02:22	98.38	9.85	76.67	66.67
Stingless bee (*Tetragonula* sp.)	63	03:24	92.96	5.78	80.79	81.25
Hoverfly (Syrphidae)	11	03:17	51.10	4	83.33	100.00
Other fly	19	01:26	127.82	9.8	56.52	20.00

†Medium-sized solitary bees were between 5 and 10 mm in length, and small-sized solitary bees were those <5 mm in length.

Table 1. Is sourced from the below publication under license: https://creativecommons.org/licenses/by/4.0/. Modifications have been made to the species/taxonomic group labelling and the columns 'Predicted flowers/h' and 'Predicted % female flowers visited' have been added. Subasinghe Arachchige EC, Rader R, Cutting BT, Keir M, van Noort T, Fale G, Howlett BG, Samnegård U, Evans LJ 2022. Honey bees are the most abundant visitors to Australian watermelon but native stingless bees are equally effective as pollinators. Ecological Solutions and Evidence, 3(4): e12189.

In summary, honey bees were the most frequent visitors in all regions and are likely to be very important for Australian watermelon production. Honey bees are excellent pollinators of watermelon; readily moving between male and female watermelon flowers and leaving behind an average of 40 pollen grains with each visit. To put this into perspective, it's estimated that about 1,000 pollen grains are needed for the successful development of watermelon (data for seeded watermelon: McGregor, 1976). Based on this, approximately 25 visits by honey bees are required for fruit production and on a fine weather day this would have been achieved within an hour at the surveyed farms.

Watermelon flowers were also visited by a diverse array of other insects across Australia. While many of these wild insects were present in smaller numbers, native bees, including stingless bees like *Tetragonula* sp., and bees from the families Megachilidae and Halictidae (including *Lasioglossum*, *Homalictus*, and *Lipotriches*) were particularly abundant in the southernmost sampling region – Chinchilla. Several of these insect groups proved to be effective pollinators of watermelon flowers. For example, native stingless deposited similar quantities of pollen as honey bees.

Recommendation – Considering a broader range of managed species (e.g., *Tetragonula*) alongside honey bees, and encouraging wild pollinators, could have positive effects on crop yields and would enhance the resilience of watermelon pollination services. Two species of stingless bees are available commercially in Australia (*Tetragonula hockingsi*, *Tetragonula carbonaria*) and can be managed alongside honey bees. The number of managed stingless bee colonies deployed and the associated cost would be relatively higher than honey bees, so growers may wish to adopt land management practices to encourage natural populations of the bees. Such practices include the preservation of native vegetation, especially large trees that stingless bees use as nest sites (Oliveira et al. 2013). Maintaining areas of bare ground and providing additional floral food sources are practices that can be used to encourage native ground-nesting bees (Venturini et al. 2017; Antoine & Forrest 2021).

Lychee

Pollinator effectiveness – We assessed the number of pollen grains deposited on 159 lychee flowers after a single insect visit to each flower. Pollen deposition was determined for: honey bees (*A. mellifera*), stingless bees (*Tetragonula* sp.), solitary bees (Apoidea), flies (Diptera), and method control flowers (no insect visit). A Kruskal-Wallis test was used to compare the amount of pollen deposited between these five groups of flowers. Note: one stingless bee-visited flower with a reported 160 pollen grains was removed from the data set as this outlier was likely a recording error.

All insects/insect groups assessed appeared capable of pollinating lychee flowers, however large variation among individuals within a group and some pollen recorded on control flowers (38% of flowers) meant we were not able to determine whether certain insects/insect groups were more effective (Figure 20; H = 6.7, df = 4, p = 0.16).

Honey bees transferred lychee pollen onto 49% of flowers they visited, at an average (± SE) of 3 ± 0.7 pollen grains per visit. Similarly stingless bees transferred lychee pollen onto 48% of flowers they visited, at an average of 2 ± 1.1 pollen grains per visit. Solitary bees (63%) and flies (83%) were more likely to transfer at least one pollen grain in a visit and deposited an average of 8 ± 2.6 and 4 ± 3.2 pollen grains per visit respectively (Figure 20).

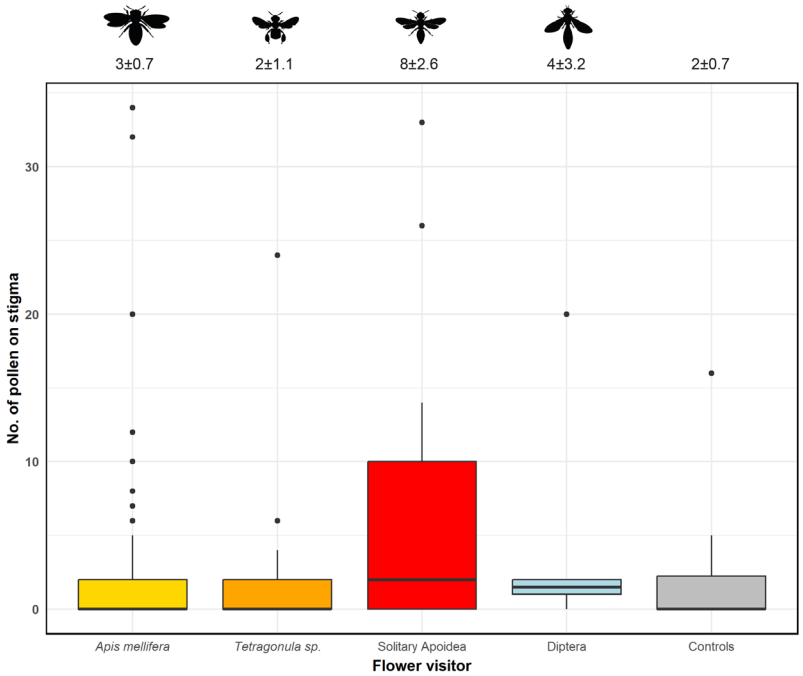


Figure 20. Pollinator effectiveness (single visit pollen deposition) by honey bees (*Apis mellifera* n = 78), stingless bees (*Tetragonula* sp. n = 21), solitary bees (solitary Apoidea n = 19), flies (Diptera n = 6) and method controls (controls n = 24) on female watermelon flowers. In each box, the bold horizontal line is the median. The lower and upper edges of the box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (circular data points).

Movement patterns of insects on lychee flowers – A total of 851 flowers on 10 panicles were filmed on three farms in Tully (QLD). The cultivar used was 'Kwai May Pink'/ 'B3' and filming took place over six days. This included visitation data for 310 M1 flowers across two panicles; 341 F flowers, majority across six panicles; and 200 M2 flowers across two panicles. Only video footage captured between 9:00 and 11:00 h was analysed. All three flower types (e.g., Figure 21) received visits from the most abundant flower visiting insects/insect groups, including: honey bees, stingless bees, solitary bees, and flies (Table 2). Bees were more frequent visitors on M1 and F flowers, while flies were more frequent visitors on the M2 and F flowers.

We annotated the movement patterns of 61 individual insects (honey bees n = 21; stingless bees n = 24; solitary bees n = 9; and flies n = 7) on 'Kwai May Pink' lychee flowers, on two different farms on the Sunshine Coast. Each insect was followed by an observer for an average of 3.23 minutes, while they visited an average of 43 ± 6.6 flowers. The flower visit

duration varied with insect/insect group, with honey bees spending the least amount of time on flowers and flies the longest (mean honey bees visit duration = 2.9 ± 0.2s; stingless bees = 10 ± 0.7s; solitary bees = 9.2 ± 1.3s; and flies 19 ± 2.7s). Overall, 1,300 between-flower movements were recorded. Of these movements, 20% (n = 256) were to a new panicle and 0.7% (n = 9) were to a new plant, but only nine of these movements led to a different flower sex phase being visited (F to M1 = 5, M1 to F = 2, M2 to M1 = 1, and M2 to F = 1), and these movements were made by five individuals insects (3 honey bees, 1 stingless bee, and 1 wasp).

Figure 21. Lychee flower sex phases left to right: male 1 (M1) phase flowers; female (F) phase flower; and male 2 (M2) phase flower. Photos by Brian Cutting.

Table 2. Insect visitation behaviour on the different sex phases of lychee, including: male 1 (M1) phase flowers; female (F) phase flower; and male 2 (M2).

Insect	Flower phase	% of total visits per insect/insect group	Mean visits/flower between 9:00 and 11:00 h	Mean visit duration (s)
Apis mellifera (n = 715)	M1	47%	5.79	2.92
	F	32%	3.54	3.74
	M2	21%	3.96	10.67
Tetragonula sp. (n = 287)	M1	40%	1.02	5.70
	F	41%	0.94	7.94
	M2	19%	0.77	6.52
Solitary Apoidea (n = 141)	M1	69%	1.3	5.25
	F	22%	0.38	8.23
	M2	9%	0.26	4.19
Diptera (n = 87)	M1	14%	0.11	6.86
	F	40%	0.28	12.14
	M2	46%	0.56	13.51

Summary, all focal insect species and groups, including honey bees, stingless bees, solitary bees, and flies, were observed visiting the three different sex phases of lychee flowers, indicating their potential for pollination. However preferences for specific flower types were observed and when following individual insects, very few movements occurred between the different sex phases. When we directly assessed their ability to move pollen from a male flower onto a female flower, per visit, flies were the most likely to deposit some pollen (although note small sample size), followed by solitary bees, honey bees, and stingless bees. However on average, single visits from all insects/insect groups resulted in the transfer of only a small number of pollen grains (range = 0-36, mean = 3.28, median = 1).

Recommendations - to increase instances of insect movement from male flowers, especially M2, onto female flowers, we recommend interplanting cultivars. Ensuring overlap in these flowering phases on different cultivars will provide more opportunity for pollination events to occur. High-density planting may also be a good option for lychee, as we observed minimal insect movement between trees.

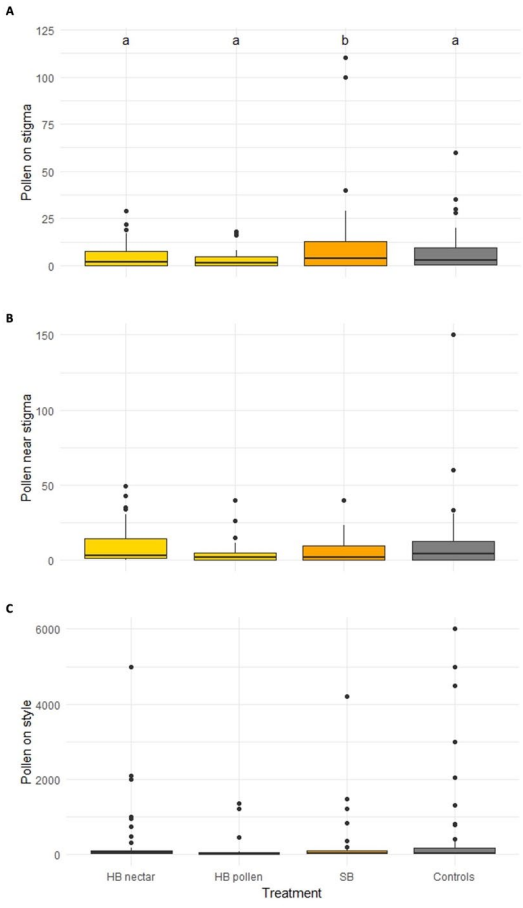
Macadamia

Pollinator effectiveness – The effectiveness of macadamia pollinators has been studied previously in MT13060; "Optimising pollination of macadamia & avocado in Australia", but we evaluated two pollinators – honey bees (*A. mellifera*) and stingless bees (*Tetragonula* sp.) again in this project, for two reasons:

1. The previous data set did not include pollen foraging honey bees, which, when foraging, exhibit a distinctive foraging behaviour where they intentionally 'dab' macadamia stigmas, compared to nectar foragers, which make incidental body contact with stigmas.
2. Previous methods removed test stigmas pollen load before an insect visit. Here we only removed the top half of the self-pollen load to encourage more normal pollen foraging behaviour.

Pollen grains were counted (or estimated) on the stigma, near the stigma (within approximately 2 mm), and on the style. Few of pollen grains developed pollen tubes so linear models (LMs) were used to determine whether there were differences in the placement of pollen on visited macadamia flowers compared with control flowers (which also had the top of their self-pollen load removed but received no bee visit). We observed one instance where a flower visited by a nectar foraging honey bee was identified as a distant outlier during analysis. As this flower did not represent typical behaviour observed in nectar foraging honey bees, we made the decision to exclude it from the analysis.

Macadamia flowers visited by nectar foraging honey bees had a mean ± SE of 5.13 ± 1.04 pollen grains, compared with 3.91 ± 1.24 pollen grains on flowers visited by pollen foraging honey bees, and 11.36 ± 3.17 pollen grains on flowers visited by stingless bees. Only the stingless bee visited flowers had significantly more pollen grains on their stigmas compared with the unvisited controls (mean ± SE = 6.64 ± 1.06); t(3, 191) = 1.995, p = 0.0478; Figure 22). There were no further significant differences in the number pollen grains 'near the stigma' or 'on the style' of the insect visited flowers or the controls (Figure 22). Stingless bees also spent significantly longer on flowers compared with honey bees (mean visit duration ± SE for stingless bees = 9.32 ± 0.81s and honey bees = 3.57 + 0.36s; t(2, 109) = 5.901; p <0.001).



In summary, stingless bees were able to deposit more pollen on the tip of the macadamia stigma (where it needs to be placed for pollination to occur), possibly because of their longer visits and/or direct, full body contact with the stigma. The absence of a statistical difference in pollen on honey bee-visited flowers compared with the controls does not discount the possibility of pollen deposition by honey bees; rather, it could not be distinguished from existing 'self pollen' on the flowers. The lack of difference between 'nectar foraging' and 'pollen foraging' honey bees aligns with our observation that most of our pollen foragers (which had pollen in their corbiculae) were collecting nectar when visiting our test flowers (functionally removing self-pollen), limiting our ability to estimate pollen deposition by bees actively collecting pollen. However, the average visits by both bee species were longer than previously recorded (when the pollen cap was removed from the stigma), suggesting that the earlier estimates of pollen deposition numbers were underestimated.

Figure 22. Pollinator effectiveness (single visit pollen deposition) by nectar foraging honey bees (*Apis mellifera*; HB nectar n = 46), pollen foraging honey bees (HB pollen n = 22) and pollen foraging stingless bees (*Tetragonula* sp.; SB n = 47), as well as testing unvisited controls (Controls n = 83). All flowers retained some of their self-pollen load. In each box, the bold horizontal line is the median. The lower and upper edges of the box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (data points).

Blueberry

Pollinator effectiveness – When comparing fruit set as a function of insect visitor, after a single visit on farms in Tasmania and Coffs Harbour (NSW), there was no difference in fruit set from flowers visited by the different bee species within a blueberry plant type. For instance, on northern highbush (*V. corymbosum*), honey bees and bumble bees were both highly efficient pollinators, achieving >75% fruit set after a single visit. The efficiency of the bees did vary with blueberry plant type; both honey bees and stingless bees single visits resulted in a lower percent fruit set in rabbiteye (*V. virgatum*) compared with southern highbush (*V. corymbosum* hybrid) (Figure 23).

An increasing number of insect visits led to greater fruit set across all three blueberry types (Bayesian R^2: 0.47, Figure 23). While 100% fruit set was achieved in southern and northern highbush varieties after five bee visits, in rabbiteye the positive increase in fruit set in response to the number of visits was significantly lower than the two southern highbush types, increasing from 15% fruit set with zero visits to 62% with 15 visits. This suggests rabbiteye requires >15 visits to achieve 100% fruit set (Figure 24).

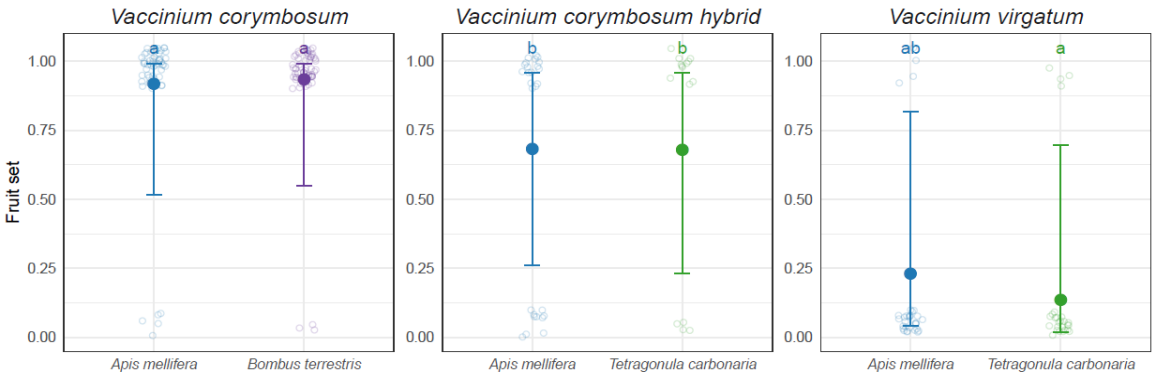


Figure 23. Probability of fruit set as a function of insect visitor: Honey bee (*Apis mellifera*), bumble bee (*Bombus terrestris*) in northern highbush (*Vaccinium corymbosum*), and honey bees and stingless bees (*Tetragonula carbonaria*) in southern highbush (*V. corymbosum* hybrid) and rabbiteye (*V. virgatum*) blueberries. All flowers received one visit from the specified insect visitor. Circles are actual data which have been jittered. Bold line indicates posterior mean estimate ± 95% CI.

Figure 23. Is sourced from the below publication under license: 5656231379827.Modifications have been made to the colour scheme, figure labels and control data has been omitted. Kendall LK, Gagic V, Evans LJ, Cutting BT, Scalzo J, Hanusch Y, Jones J, Rocchetti M, Sonter C, Keir M, Rader R 2020. Self-compatible blueberry cultivars require fewer floral visits to maximize fruit production than a partially self-incompatible cultivar. Journal of Applied Ecology 57(12): 2454-2462.

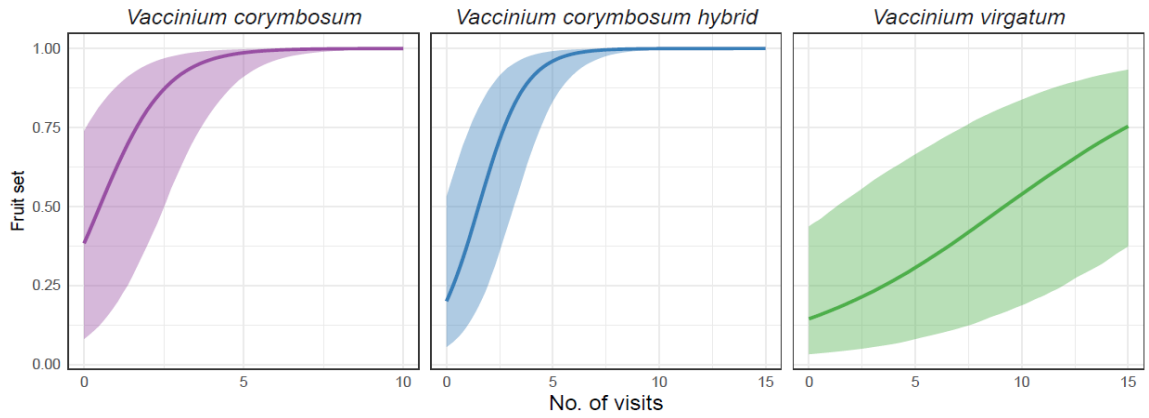


Figure 24. Fruit set as a function of number of insect visits in northern highbush (*V. corymbosum*), southern highbush (*V. corymbosum* hybrid), and rabbiteye (*V. virgatum*) blueberries. Bold line indicates posterior mean estimate ± 95% CI (shading).

Figure 24. Is sourced from the below publication under license: 5656231379827.Modifications have been made to the colour scheme, figure labels and data presentation. Kendall LK, Gagic V, Evans LJ, Cutting BT, Scalzo J, Hanusch Y, Jones J, Rocchetti M, Sonter C, Keir M, Rader R 2020. Self-compatible blueberry cultivars require fewer floral visits to maximize fruit production than a partially self-incompatible cultivar. Journal of Applied Ecology 57(12): 2454-2462.

Movement patterns of insects between blueberry flowers – Different movement patterns were observed among insect species/groups and also within a species between regions (Table 3a and b). Bumble bees were predicted to have visited the highest number of flowers per hour (635 flowers/h), all other flower visitors spent more time on individual flowers and therefore visited fewer flowers overall. Bumble bees also moved between plants and rows more frequently compared with honey bees and flies in Tasmania. Carpenter bees (*Xylocopa* sp.), like bumble bees, readily moved between flowers (445 flowers/h) and plants, although it should be noted that only two individuals were followed. Honey bees visited a similar number of flowers in both Tasmania and Coffs Harbour, but the rate they moved between plants varied, with more movement occurring on Coffs Harbour farms. Stingless bees (*Tetragonula carbonaria*) spent longer on individual flowers and were not recorded moving between plants during the observation period.

Table 3a. Data on visitation frequency of different taxonomic groups to northern highbush blueberry flowers in Tasmania and the percentage of visits that result in a change between plants, row change and robbing behaviour.

Species/ taxonomic group	No. of individuals	Mean duration followed (m:ss)	Predicted flowers/h	% Visits on same cluster	Predicted plant change/h	Predicted row change/h
Honey bees (*Apis mellifera*)	19	2:56	347.13	28.17	12.94	7.55
Bumble bees (*Bombus terrestris*)	66	2:27	635	52.91	35.16	21.84
Flies (Diptera)	2	4:40	128.43	28.57	0*	0*

*A value of zero is an indication that the insects may not have been followed long enough to observe this behaviour, but it likely that this this behaviour occurs infrequently.

Table 3b. Data on visitation frequency of different taxonomic groups to southern highbush and rabbiteye blueberry flowers in Coffs Harbour and the percentage of visits that result in a plant change, row change and robbing behaviour.

Species/ taxonomic group	No. of Individuals	Mean duration followed (m:ss)	Predicted flowers/h	%Visits on same cluster	Predicted plant change/h	Predicted row change/h
Honey bees (*Apis mellifera*)	43	2:12	328.98	14.72	34.86	7.61
Stingless bee (*Tetragonula carbonaria*)	5	3:34	144.89	45.65	0*	0*
Carpenter bees (*Xylocopa* sp.)	2	0:48	444.92	0*	37.08	0

*A value of zero is an indication that the insects may not have been followed long enough to observe this behaviour, but it likely that this this behaviour occurs infrequently.

In summary, single-visit pollination effectiveness was similar across the compared pollinator taxa – honey bees, bumble bees, and stingless bees. The probability of fruit set in all three blueberry types improved with an increasing number of pollinator visits, but this relationship was steeper in the self-compatible highbush cultivars; with >90% probability of fruit set occurring after three to five visits. In the self-incompatible rabbiteye cultivar 58% fruit set was achieved with 15 visits. This suggests >15 pollinator visits are needed for optimal fruit production in partially self-incompatible rabbiteye varieties of blueberry.

Insects that exhibit frequent movement between blueberry plants and/or rows, such as carpenter bees and bumble bees, are likely to be highly effective in facilitating pollen transfer between varieties. This characteristic may make them useful for the pollination of self-incompatible rabbiteye varieties. However, it should be noted that both these bees are known to engage in nectar robbing from blueberry flowers, a behaviour that can diminish their overall pollination effectiveness. Interestingly, robbing behaviour was infrequent among bumble bees (which were mostly queens) in the current trial.

Honey bees demonstrated nearly three times more movement between plants in Coffs Harbour farms compared to the surveyed farms in Tasmania. This increased movement could be attributed to the warmer climate and increased pollinator diversity in the region (possibly leading to increased competition for resource). Previous research suggests that greater pollinator diversity on farms can significantly boost honey bee movement between flowers, thereby potentially elevating their efficacy in cross-pollinating plants (Brittain et al. 2013).

Recommendation – Blueberry growers can potentially improve pollination outcomes for varieties exhibiting self-incompatibility, which require more pollinator visits, by encouraging pollinators that frequently move between plants/trees. By directly observing pollinator activity during flowering, growers may be able to identify insects that move the most frequently. Additionally, promoting pollinator diversity may enhance movement among plants by honey bees.

Papaya

Movement patterns of insects on papaya flowers – Video footage of 63 papaya flowers (13 female, 27 male, and 23 perfect flowers) across 15 trees was analysed to understand the visitation patterns of the most abundant flower visitors on the three types of papaya flowers. Filming occurred over four days and four nights across two farms in Mareeba and Brisbane, resulting in a total of 120 hours of video footage analysis (37 hours for female flowers, 40 hours for male flowers, and 43 hours for perfect flowers).

Female flowers had the highest number of visits per flower per hour (mean = 0.30 visits/flower/h), followed by the perfect flowers (mean = 0.17 visits/flower/h) and male flowers (mean = 0.57 visits/flower/h). However male flowers had the highest diversity of visitors, attracting representatives from all our identified papaya visitors. In contrast, female and perfect flowers were visited by a subset of the identified papaya flower visitors (Figure 25). Moths (Lepidoptera) were observed visiting all three flower types, while honey bees (*A. mellifera*) and wasps (included in the 'Other taxa' category) were exclusively observed on male and perfect flowers. Flies (Diptera) were only observed on female and male flowers. Stingless bees (*Tetragonula* sp.) were exclusively observed on male flowers (Figure 25).

Figure 25. A) Female and B) male flowers of yellow-fleshed pawpaw and C) perfect hermaphroditic flowers of a clonal variety of red fleshed papaya and the relative abundance of their flower visitors.

In summary, the low diversity of visitors to female and perfect flowers is unsurprising – the floral resources provided by these flower types is reduced, and female-only flowers rely on mimicry of insect pheromones (rather than food-rewards) for attracting pollinators (Garrett, 1995). Moths, in particular hawk moths (Sphingidae), visited all flower types so are likely to have a role in pollinating both the dioecious yellow-fleshed pawpaw (male and female plants; as shown by Garrett, 1995) and the clonal varieties of red fleshed papaya (with perfect flowers). Flies were found on both male and female flowers, so also have potential as pollinators of pawpaw. Interestingly, honey bees were the most common visitors to the perfect flowers; while they probably have little to no role in pollination of yellow-fleshed pawpaw, honey bees may play a role in pollinating red fleshed papaya varieties. However, visitation rates generally were very low and we were not able to further explore pollinator efficacy in this crop.

2.3 Pollination deficits

Methods

For blueberry (uncovered and covered plants), lychee, and almond we carried out experimental pollination treatments to determine whether there is the potential for pollination deficits and whether there is value to be gained in cross pollination. The method of applying pollen varied across the different crops but for each crop we set up the following four treatments: (a) closed pollination: organza bags were placed over flowers to prevent insect visitation/pollen transfer, (b) open pollination: flowers were exposed to floral visitors, (c) self- (geitonogamous) pollination: flowers were hand-

pollinated with pollen of the same cultivar, and (d) cross (allogamous) pollination: flowers were hand-pollinated with a different cultivar. For lychee, no 'closed' pollination (a) treatments were undertaken and it was not always possible to complete cross pollination (d) at each farm. For avocado, we correlated insects per 1,000 flowers with mean fruit set per tree across 18 different farms.

Results and interpretation

Lychee

Application of supplementary pollen by hand resulted in increased fruit set in five out of the seven lychee orchards and enhanced mean fruit weight in four orchards. Percent fruit set in open-pollinated panicles ranged from 2.2 to 9.76%, and fruit weights per panicle ranged from 6 to 16g. In comparison, the percent fruit set on panicles that received supplementary pollination ranged from 2.93 to 16.98% (an average increase of 2.7%), and their fruit weights ranged from 7 to 17g (an average increase of 0.71g). The most significant increase was observed in the orchard where pollen from a different variety was used, resulting in a 7.22% increase in fruit set (Table 4).

Summary, the observed improvements in both percent fruit set and fruit weight suggest that many of the visited orchards were experiencing pollen limitations. This limitation could stem from insufficient pollinators or poor synchronization of flowering stages among trees/cultivars.

Table 4. Fruit set and weight of lychee produced by open pollinated and hand pollinated flower panicles across seven different farms. Gains in fruit set/ weight are shown in bold. All hand pollinations are 'B3 X B3' except for those in Mareeba, which were cross pollinated with 'Tai So' (green box).

		Open pollination treatment					Supplementary pollination treatment				
Region	Farm	Panicles	Flowers followed	Fruit set	Mean fruit/panicle (% fruit set)	Mean fruit weight (g)/ panicle (±SE)	Panicles	Flowers followed	Fruit set	Mean fruit/panicle (% fruit set)	Mean fruit weight (g)/panicle (±SE)
Mareeba	1	25	625	61	2.4 (9.76)	16 (±1.5)	25	630	107	**4.3 (16.98)**	**17** (±1.1)
Sunshine	1	20	2093	46	2.3 (2.20)	8 (±1.3)	11	1057	31	**2.8 (2.93)**	8 (±1.4)
	2	52	2950	267	5.1 (9.05)	10 (±0.8)	20	720	90	4.5 **(12.50)**	**12** (±1.2)
	3	44	1644	50	1.1 (3.04)	6 (±1)	44	1753	82	**1.9 (4.68)**	**7** (±1)
	4	44	2419	103	2.3(4.09)	9 (±1)	44	2267	107	**2.4 (4.72)**	9 (±1.1)
Tully	1	10	-	46	4.6	16 (±1.9)	10	-	33	3.3	13 (±2.9)
	2	10	-	5	0.5	7 (±4.9)	10	-	5	0.5	**11** (±5.8)

Blueberry

We compared fruit set in northern highbush cultivar 'Blue Rose', after four different pollination treatments: no pollination, open-pollination, self-pollination, and cross-pollination (hand-pollinated with pollen from 'Brigitta'). Fruit set was compared with a chi-squared test and fruit weight with an ANOVA (the Bonferroni correction was applied to account for multiple comparisons) and a Tukey's post hoc comparison.

Fruit set varied with pollination treatment (χ^2_1, $p < 0.05$), with open pollination (n flowers = 125, 96% fruit set, p <0.001), self-pollination (n = 100, 92% fruit set, p <.001) and cross pollination treatments (n = 20, 95% fruit set, $p < 0.001$) producing significantly more fruit than unpollinated flowers (n = 178, 26% fruit set).

Fresh fruit weight also varied with pollination treatment ANOVA F(3, 139) = 21.76 g, $p < 0.001$. Following the same pattern as fruit set, all pollination treatments; open-pollination, self-pollination, and cross-pollination, produced significantly larger fruit than un-pollinated flowers; Tukey's HSD ($p < 0.001$). There was no significant difference in fruit weight between the open-pollination and cross-pollination treatments (Tukey's HSD; $p = 0.608$) or the cross-pollination and self-pollination treatments (Tukey's HSD $p = 0.052$; Figure 26).

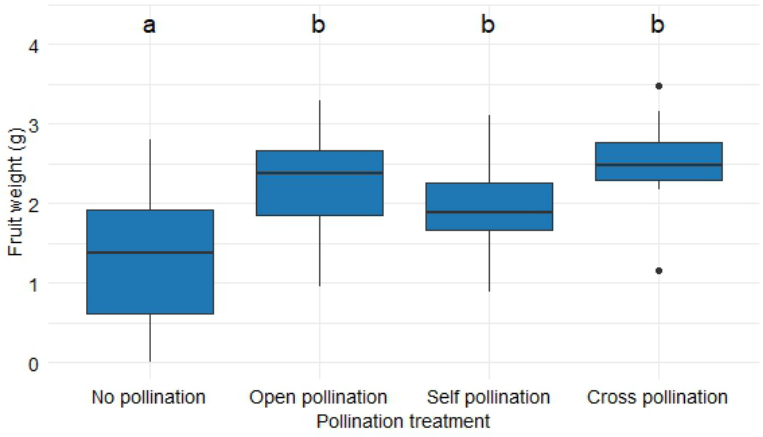


Figure 26. The fresh fruit weight of blueberries produced from four different pollination treatments: no pollination (bagged), open-pollination (flowers marked but otherwise unmanipulated), self-pollination (hand-pollinated with pollen from the same variety), and cross-pollinated (hand-pollinated with pollen from a different variety). In each box, the bold horizontal line is the median. The lower and upper edges of the box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (circular data points). Different letters indicate significant differences between variables (Tukey's HSD at α = 0.05).

As blueberries are increasingly being grown under protective covers, we also compared pollination in a covered and uncovered block of highbush blueberry, cultivar: 'Legacy'. We again compared the same four different pollination treatments as above. Fruit set results were compared with a logistic mixed-effects model (GLMM, binomial family) and fruit weight with a linear mixed model (LMM). Both models included pollination treatment and an interaction with block type (covered versus open) as fixed effects and plant ID as a nested random effect to account for treatments taking place on multiple flowers on the same plant.

One day of open-pollination (n flowers = 60, 65% fruit set, p <0.001), open-pollination (n = 70, 96% fruit set, p <0.001), self-pollination (n = 70, 76% fruit set, p <.001), and cross-pollination treatments (n = 55, 81% fruit set, $p = 0.003$) all produced significantly more fruit than unpollinated flowers (n = 70, 34% fruit set). There was no significant difference in fruit set between cover types for any of the pollination treatments.

Similarly, fruit weights resulting from the open-pollination, self-pollination, and cross-pollination treatments were significantly heavier than the fruit produced by unpollinated (bagged control) flowers (Table 5; Figure 27). Interestingly, there was also a difference in fruit weight between block types (covered versus open), but only among flowers exposed for one day – with flowers producing significantly smaller fruit in the covered block (Table 5; Figure 27).

Table 5. Results from a linear mixed model (LMM) which included pollination treatment (no pollination, one day of exposure, open-pollination, self-pollination, and cross-pollination) and an interaction with block type (covered versus open) as fixed effects and plant ID as a nested random effect to account for treatments taking place on multiple flowers on the same plant. Significant p-values shown in bold.

Factor	Estimate	Std. Error	t-value	p-value
Intercept (no pollination)	0.37	0.08	4.48	**<0.001**
One day	0.20	0.1	1.98	0.05
Open	0.43	0.1	4.5	**<0.001**
Self	0.27	0.1	2.7	**<0.05**
Cross	0.43	0.11	4.48	**<0.001**
Block type	0.13	0.11	1.12	0.26
Treatment One day: Block type	0.31	0.15	2.1	**0.04**
Treatment Open: Block type	0.02	0.13	0.15	0.90
Treatment Self: Block type	-0.08	0.14	-0.61	0.54
Treatment Cross: Block type	<0.001	0.14	-0.004	1.0

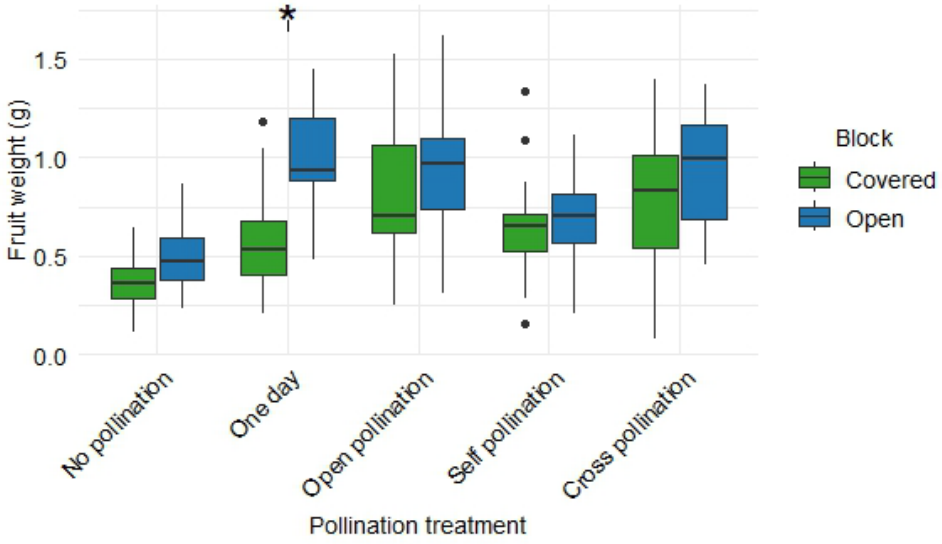


Figure 27. The fresh fruit weight of blueberries produced from four different pollination treatments: no pollination (bagged), open-pollination (flowers marked but otherwise unmanipulated), self-pollination (hand-pollinated with pollen from the same variety), and cross-pollination (hand-pollinated with pollen from a different variety) in covered and uncovered (open) blocks. In each box, the bold horizontal line is the median. The lower and upper edges of the box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (circular data points). Asterisks show significant differences within a treatment between block types (GLMM at $\alpha = 0.05$).

In summary, in uncovered blocks, background pollination rates were sufficient for fruit set and fruit quality in the assessed highbush cultivars. However, under a protective crop cover, it may take longer for pollination to occur, even in self-compatible cultivars. Smaller fruit were produced under cover when flowers were exposed for just one day compared with being exposed for the full duration that the flowers were open (i.e., open pollination). In contrast, fruit size was similar under these treatments in the open block. This difference may be attributed to lower pollinator activity or variations in flower-visiting behavior under covers (e.g., Kendall et al. 2021).

Recommendation – Growers should be mindful of differences in pollinator activity between growing environments, particularly during periods of wet/ very cold weather when there may be a reduced opportunity for fruit to reach their optimum quality under covers. In such instances, larger numbers of managed pollinators may need to be introduced into covered blocks.

Avocado

To investigate the relationship between insect visits to avocado flowers and fruit set, a survey of insect activity on flowers was conducted on nine 'Hass' avocado trees in each of 18 orchards. The survey was carried out at three different time points under fine weather conditions (13°C –36°C). These data were collected and described in MT13060: "Optimising pollination of macadamia & avocado in Australia". We then counted mature fruit produced by these trees; recording fruit set within the same tree area (1.5 m around the tree circumference) as the insect surveys were performed.

Across the 18 farms, the average fruit set per tree ranged from 2 to 135 (mean ± SE = 56.03 ± 8.58). Fruit set exhibited a positive correlation with the number of insects per 100 flowers (Pearson's r = 0.008, R-squared = 0.36; Figure 28), indicating that trees with higher insect counts tended to produce more fruit.

In summary, this result suggests a link between insect pollination and fruit set in avocado orchards. It is also an indication that some orchards are pollination limited and are not reaching their full potential for fruit set.

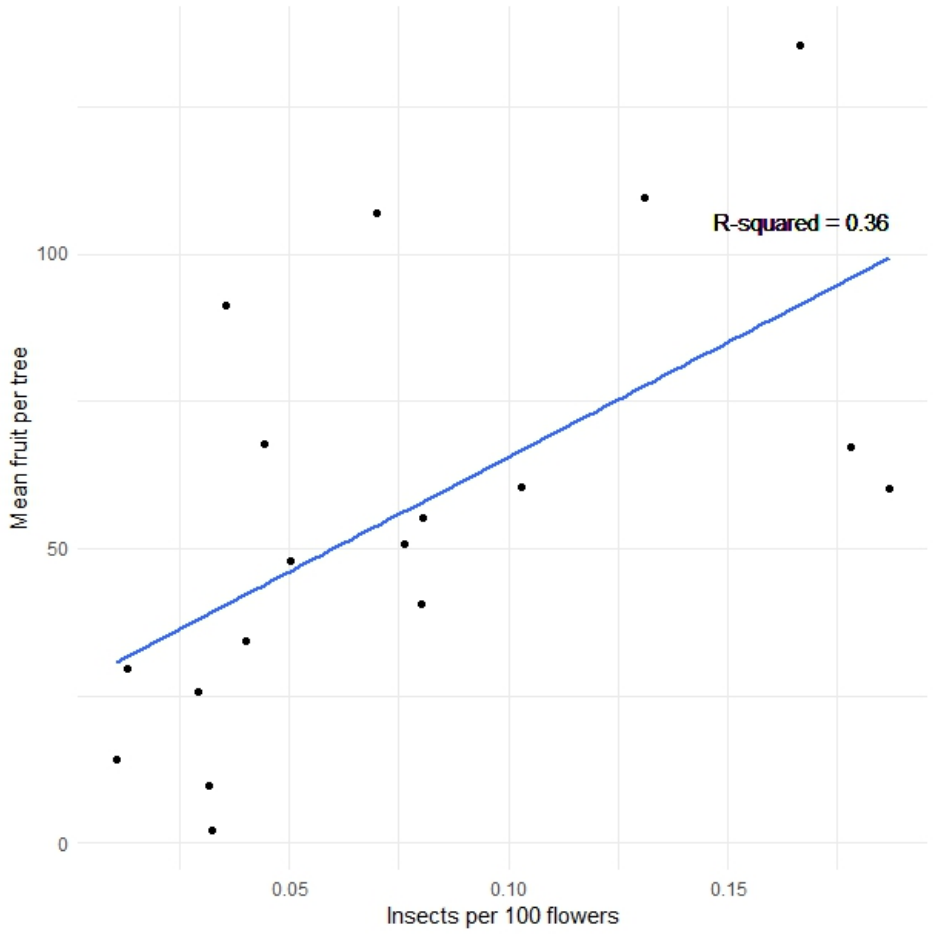


Figure 28. Relationship between the mean number of avocado produced per tree for each farm and insects observed per 100 flowers.

Recommendation - Insect activity on flowers should be regularly monitored throughout avocado orchards. Based on our data, growers should aim for an average of at least two insects per panicle (which has approximately 1000 flowers open daily) during fine weather. Beneficial insects for avocado pollination include both bees and fly species.

Almond

Nut set was compared on one almond cultivar ('Carina') selected for self-compatibility, and the standard commercial cultivar ('Nonpareil'), after four different pollination treatments: no pollination, open-pollination, self-pollination, and cross-pollination (hand-pollinated with pollen from alternate cultivar). Initial fruit set results were compared with a Kruskal-Wallis rank sum test and post hoc Dunn's test. The Bonferroni correction was applied to account for multiple comparisons.

Nut set in 'Carina' did not differ across open (n flower clusters = 28, 49% set), self- (n = 28, 49% set), and cross-pollination treatments (n = 30, 50% set), suggesting that in our trial orchard background pollination rates were sufficient for nut production (Figure 29). Even when 'Carina' flowers were bagged to prevent pollinators from visiting, on average 30% of flowers in a given cluster produced nuts.

'Nonpareil' flowers that were cross-pollinated produced nearly three times more nuts compared with open-pollinated flowers (open-pollination fruit set = 13%; cross-pollination fruit set = 38%; Figure 29). As to be expected of a self-incompatible cultivar, the no-pollination (bagged) and self-pollination treatments resulted in negligible nut set.

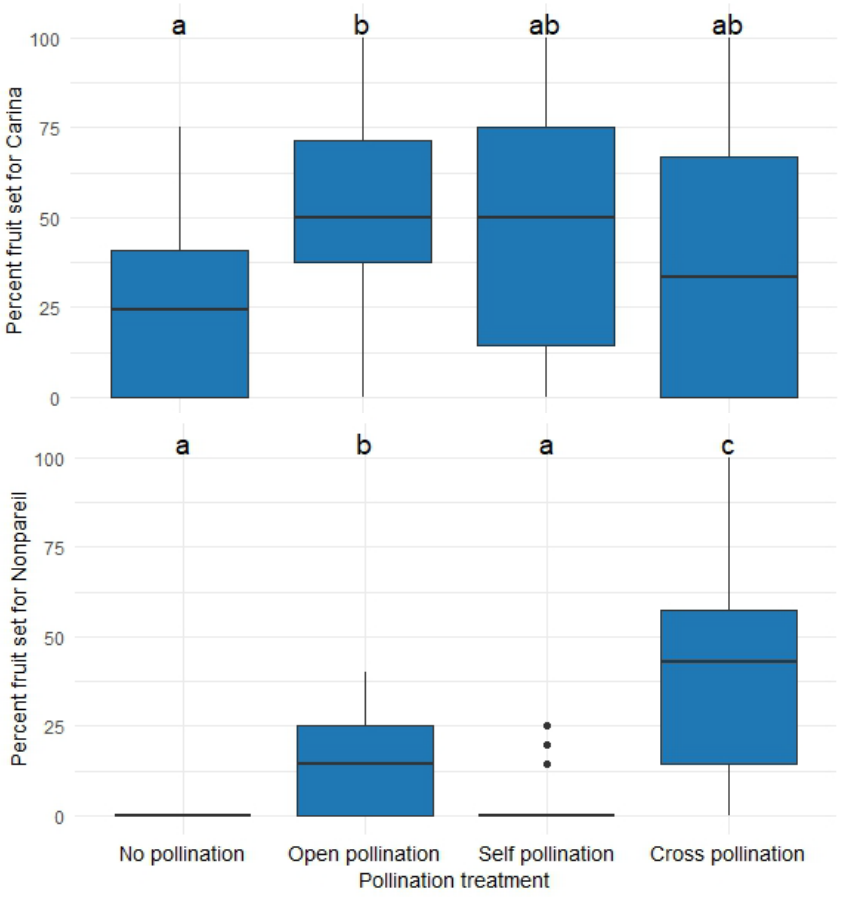


Figure 29. Percent fruit set for almond varieties 'Carina' and 'Nonpareil' after four different pollination treatments: no pollination (bagged), open-pollination (flowers marked but otherwise unmanipulated), self-pollination (hand-pollinated with pollen from the same cultivar), and cross-pollinated (hand-pollinated with pollen from a different cultivar). In each box, the bold horizontal line is the median, and means are shown with an asterisk (*). The lower and upper edges of the

box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (circular data points). Different letters indicate significant differences between variables (Kruskal-Wallis test and Dunns post hoc comparison).

In summary, our results suggest that animal pollinators are only responsible for an average 16% of nut set in 'Carina' compared with 100% in 'Nonpareil'. Furthermore, 'Nonpareil' produced close to three times as many nuts when cross pollinated (with 'Carina'). This is an indication that pollination of 'Nonpareil' was not optimised at this orchard, resulting in a loss of potential yield of 25% or more; however, it is noted that the weather conditions during this trial were sub-optimal for pollination by bees.

Recommendations – Unlike 'Nonpareil', 'Carina' and other self-compatible cultivars are not reliant on pollen being moved from another cultivar to set nuts. Hence these varieties are likely to be more resilient against pollination failure, and present a good option to reduce dependence on honey bees as the impacts of *Varroa* in Australia are realised.

However growers 'Carina' and other self-compatible cultivars will likely still benefit from insect visits and should undertake an economic analysis of the cost of hive rental against the potential yield increase with increased cross-pollination.

Almond is a good candidate for pollination by artificial means – with a substantial portion of producers in the USA applying mechanically collected pollen to flowers to ensure adequate yields. These should be explored further in Australia to provide additional tools for resilience against *Varroa* impacts.

2.4 Management practices for key pollinators

2.4.1 Watermelon – assessing different honey bee stocking rates and honey bee movement across blocks

Methods

This trial was conducted in two blocks; a 21.6 ha bock in Gumlu, QLD (block A; 17 October – 6 November) and a 4.6 ha block in Chinchilla, QLD (block B; 8 – 20 December). To achieve the desired stocking rates, existing hives were blocked – and/or additional hives were introduced. In Block A stocking rates were assessed in an ascending order, in Block B the low and high stocking rates were alternated (thus controlling for crop age/flowering progression at different stocking rates).

All hives were fitted with powder dispensers above their normal entrance (Figure 30a), that marked thorax bees with fluorescent powder as they exited their colony (Figure 30b). A different colour powder was used for hives located in each location around the block (Figure 31). Fluorescent powder was placed into the dispenser between 05:30 and 07:00 hours. To estimate the marking efficiency of the dispensers, the number of marked versus unmarked bees leaving and returning to the hive in 10 minutes was recorded. These observations were conducted for 40% of the colonies, after the powder was added to the dispenser and again, for the same colonies, after the point counts were completed. The marking efficiency was accounted for in our analysis.

Surveys of marked foragers on flowers were conducted between 08:00 and 10:00 h along four crop rows. Four-minute counts of marked bees were conducted every 100 m in block A (15 counts total) and every 50 m in block B (9 counts total). During each point count, a ca. 1 m^2 area of the crop was observed. The approximate number of flowers within the observation area was recorded. Point counts were conducted on two separate days for each of the different stocking rates trialled. A GLMM was performed to assess whether the number of marked bees on flowers was predicted by hive stocking rate, distance from hives, and an interaction between stocking rate and distance.

Figure 30. Honey bees being marked with fluorescent powder as they pass through a powder dispenser attached to the front of a hive (A), and marked bees foraging on watermelon flowers (B). Photos by Lisa Evans.

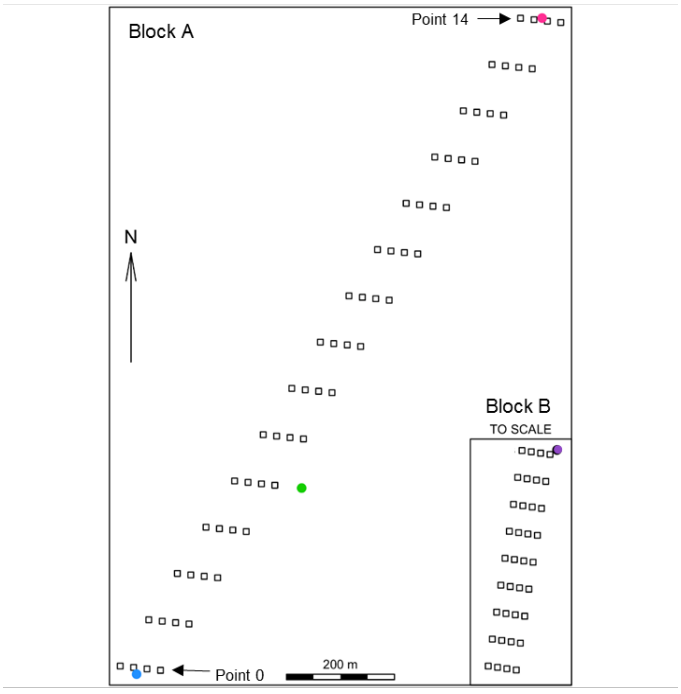


Figure 31. Trial setup to scale, block B shown in insert. Hive position within each block is provided with coloured dots. The colours correspond to the colour of the powder being used to mark bees in hives at that location. The small squares give the approximate location of each survey location.

Results and Interpretation

The number of marked bees visiting flowers was influenced by both colony stocking rate (Est. = 0.62, SE = 0.16, $p < 0.001$) and distance from colonies (Est. = -0.001, SE = 0.0004, $p = 0.002$). Marked bee numbers were directly proportional to stocking rate and inversely proportional to distance from colonies (Figure 32). Importantly, there was no combined (interactive) effect of stocking rate and distance on bee visits to flowers, indicating that bees did not forage further into the block when more hives were placed at a given location within the block.

In summary, increasing hive stocking rates from <1 hive/ha to 3.5 hives/ha led to a corresponding increase in the number of marked bee visits to flowers. However, as expected, the number of bees visiting flowers declined with distance from hives. This occurred irrespective of the localised hive number.

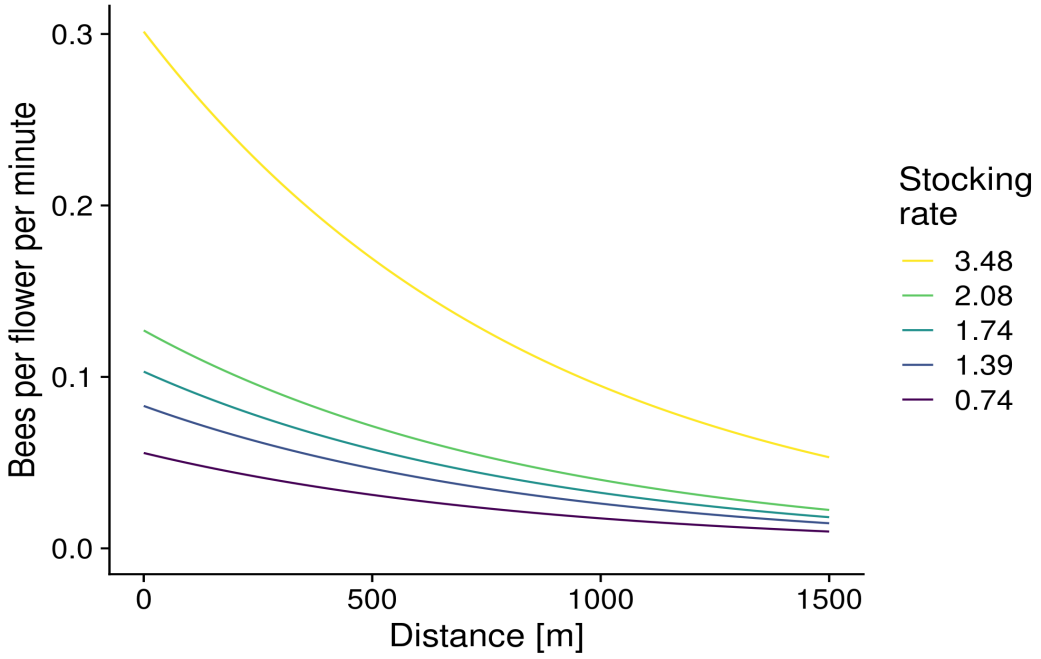


Figure 32. Number of marked honey bees (*Apis mellifera*) visiting watermelon flowers per minute compared with distance from their hives. Different coloured lines represent different honey bee hive stocking rates.

Recommendation

Current recommended stocking rates for watermelon vary (between 1 and 13 hives/ha), but most commonly between 2 to 4 hives/ha. Our data suggest that high visitation rates can be achieved by using 4 hives/ha.

Given the decline in bee numbers with distance from hives, we recommend spreading hives across the block, as opposed to introducing a large number at one location. Based on our pollinator efficacy data, watermelon flowers require approximately 25 honey bee visits to set fruit. Considering that watermelon pollen availability is typically limited to the first three hours of the day (during fine weather), flowers need at least eight visits per hour during this three-hour window. To achieve this level of visits across the block, at a stocking rate of 4 hives/ha, hives should be placed approximately every 750 m.

Pollinator activity on the crop is influenced by weather conditions and the presence of competing floral resources in the surrounding environment, growers should monitor pollinator activity across their block to ensure they have adequate numbers of pollinators. Introducing more honey bee hives may be necessary to optimise pollination in varying conditions.

2.4.2 Watermelon – Sucrose feeding in field

Some growers provide sucrose for honey bees within their blocks with the intention of increasing bee activity on the crop. There is also evidence from other crops that providing colonies with sucrose (within their hives) will satisfy the colony's need for carbohydrates and, as a consequence, the colony will focus its efforts on pollen collection. As we show in this report (Section 2.2) pollen foragers are more effective pollinators of watermelon flowers. However, providing sucrose is labour intensive and, contrary to the intent, it may remove bees from the crop by encouraging them to visit feeders instead of flowers to obtain their carbohydrates. We tested whether providing sucrose within a watermelon block i) affects the number of honey bees visiting flowers, ii) affects the type of foragers present (pollen or nectar), and/or iii) affects pollination.

Methods

This trial was conducted in an 11.3 ha block in Gumlu, QLD, between 21 October and 5 November. Sucrose was supplied in 13 feeders across the block (e.g., Figure 33), with three to four feeders placed in every other row. Over the course of the trial, sucrose was supplied for three consecutive days (2.5 L per feeder, installed between 06:00 h and 07:30 h), followed by three consecutive days of no sucrose feeding. Each three-day feeding treatment was repeated three times. The empty sucrose feeders were removed from the field at the end of each three-day feeding period to avoid bees being attracted to the empty feeders on the non-sucrose days.

Bee numbers and flower visiting behaviour were surveyed on the third day of each feeding treatment. Point counts of bees on flowers were conducted along a crop row at: 07:30, 08:30, 09:30, 10:30, 11:30, and 12:30 h, every 50 m for 500 m. At each point we recorded the number of male and female flowers observed and the number of pollen and nectar (no pollen in their corbiculae) foragers visiting these flowers in a four-minute period. The flower-visiting behaviour of honey bees was also video recorded over the course of the day. To assess pollination with feeding treatment, on each survey day, flowers were collected from each point in the surveyed crop row (n = 20) and the pollen grains per stigma were subsequently counted.

The effect of sucrose treatment on the number of honey bee visits to watermelon flowers and the number of pollen grains per stigma was modelled with a negative binomial generalised mixed-effects model. The visitation model included a random effect for time bin nested within replicate nested within date and the pollen grains model included an offset term for the number of stigmas in sample and a random effect for date. Significance of treatment was established with a likelihood ratio test (LRT).

Figure 33. Sucrose feeder within a flowering watermelon block.

Results and Interpretation

Providing honey bees access to sucrose within the field had no significant effect on the number of honey bee visits watermelon flowers received LRT: $\chi^2 = 0.433$ at 1 df; $p = 0.51$ (Figure 34a), the type of forager (nectar vs pollen) visiting the flowers $p = 0.78$, or the number of pollen grains transferred onto flowers LRT: $\chi^2 = 0.67$ at 1 df; $p = 0.41$ (Figure 34b).

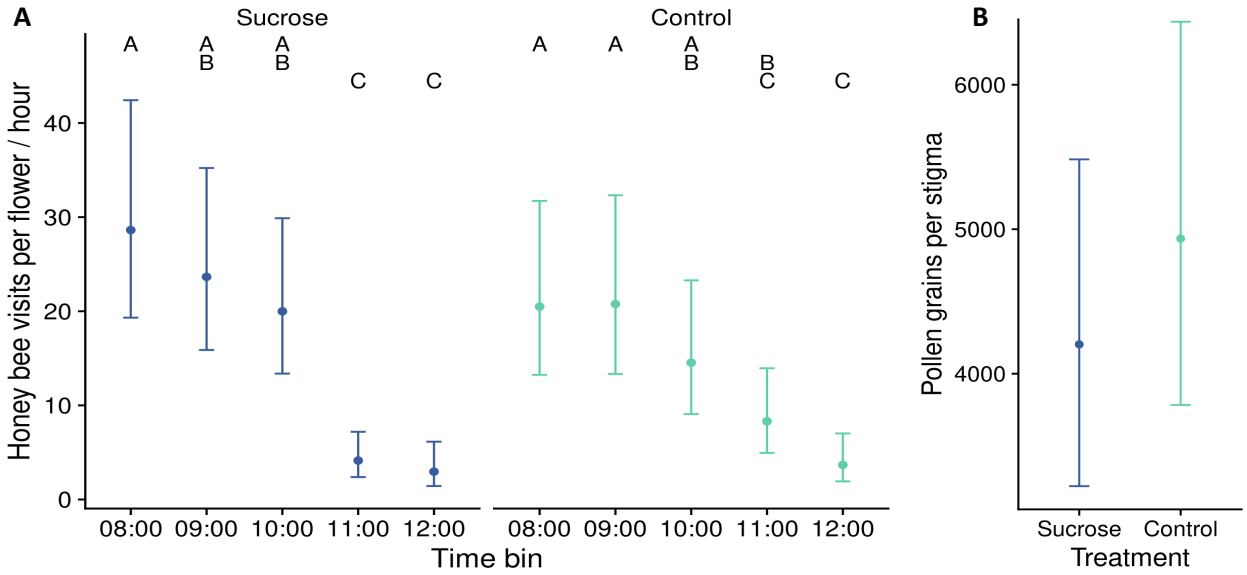


Figure 34. A) Honey bee visits to melon flowers per hour when sucrose was provided in the field (blue) and when sucrose was absent (control – green). Visit numbers are shown over the course of the day, for the entire period in which individual watermelon flowers are open. B) The number of pollen grains per stigma on flowers collected on sucrose feeding and non-sucrose feeding days. Data shown are modelled means ± 95% confidence intervals.

Recommendations

We found no evidence that feeding honey bees sucrose within watermelon blocks increases the number of bee visits to flowers or increases the amount of pollen transferred onto flowers. Given that providing sucrose is labour intensive and therefore costly for the grower, we do not recommend it as a method for increasing pollination of watermelon. We note however that within this trial we have not assessed the potential for supplied sucrose to benefit managed honey bee colonies. The volume of nectar secreted per watermelon flower is low, and the sugar composition and concentration vary between cultivars (Wolf et al. 1999). Therefore, there is potential for sucrose provisions to enable managed honey bee colonies on large watermelon farms to meet their caloric requirements.

2.4.3 Macadamia – Distribution of foraging honey bees and stingless bees in macadamia

Established guidelines for macadamia cultivation suggest strategic placement of managed bee colonies, considering "appropriate spacing" throughout the orchard (Manning 1995; Rhodes 1986). However, there exists a deficiency in precise recommendations and a consensus on how to effectively manage bee colonies for this crop (Grass et al. 2018). We determined the factors influencing the foraging patterns of honey and stingless bees in cultivated macadamia.

Methods

In a 4-hectare macadamia block near Bundaberg, twenty colonies of stingless bees (*T. carbonaria*) were placed at one end of the block, near the end of the rows. Fifty-two honey bee (*A. mellifera*) colonies were placed in a group at the opposite end of the block, 325 m away (Figure 35). The next-nearest honey bee colonies were at least 500 m away. We then conducted standardized surveys of bees on flowers on each of 48 trees, at 12 different distances across the block. Counts were conducted at three times of day (09:00–10:00 h, 12:00–13:00 h and 15:00–16:00 h) for eight days. The number of racemes with open flowers on each survey tree was recorded to allow analysis of how flower density affected visitation rates.

To ensure that these results were relevant across Australian conditions, we conducted lower-intensity surveys of bee distribution in 17 additional macadamia blocks in QLD, including in Bundaberg, Gympie, Glass House Mountains, and Northern Rivers. In these blocks we conducted similar surveys over one or two days each, surveying 12 trees per block. Managed stingless bees were not introduced to these sites, and the distance to the nearest managed honey bee colonies was unknown, but both were present on flowers. Models were used to assess which factors best explained the observed distribution patterns of bees in all surveyed blocks.

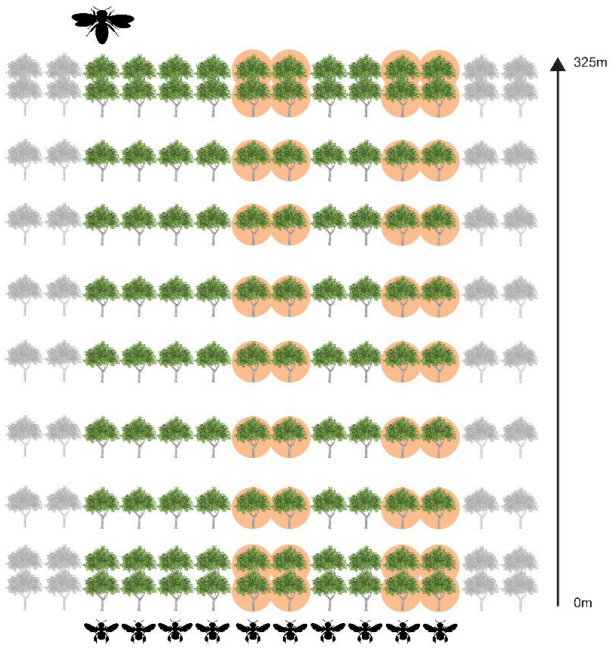


Figure 35. Relative position of our 120 marked macadamia trees; 10 rows (within the middle of a 4-hectare block) with 12 trees marked per row. Twenty stingless bee (*Tetragonula carbonaria*) colonies (small bee pictograms) were placed at one end of the block, two per row under marked trees at 0 m and 3 m. Fifty-two honey bee (*Apis mellifera*) hives (large bee pictogram) were placed in a group at the opposite end of the block, 325 m away from the stingless bees. Raceme point counts of bees were conducted on all 120 marked trees (dark green trees), and whole-tree surveys were conducted on 48/120 marked trees (trees in orange circles).

Results and Interpretation

Stingless bees and honey bees had different patterns of distribution in macadamia blocks. Generally, honey bees were encountered foraging much further away from their colonies, whereas stingless bees stayed very close to their colonies (Figure 36). Honey bee distribution was more strongly driven by flower density than it was by distance-from-hive. Trees with high numbers of flowers (either end of the block) received a greater-than-expected number of honey bee visits, while parts of the block where flower density was low received very few visits.

More than half (57.4%) of the stingless bees surveyed were within the two rows nearest to the colonies, and more than 96% of stingless bees were encountered within 100 m of the colonies (Figure 36). While *Tetragonula* is known to travel as far as 700 m to forage, they are likely to forage close to home when resources are plentiful. As a result of foraging close to the colony, stingless bees were less likely to be encountered in the middle of the block (Figure 36).

The same patterns were found in the additional 17 blocks surveyed; floral display was a good predictor of honey bee numbers (LRT: $x^2 = 31.61$ at 1 df, $p < 0.001$) and location predicted stingless bee numbers (LRT: $x^2 = 4.98$ at 1 df, $p = 0.03$). A larger number of stingless bees were observed on trees near the block edge (42 bees; 77.8% stingless bees) compared with trees in the middle of the block (12 bees; 12.2% of stingless bee; Figure 37). While the locations of colonies at these 17 sites were not known, most of the stingless bees observed were around the edge of the block (presumably closer to their colonies in surrounding vegetation).

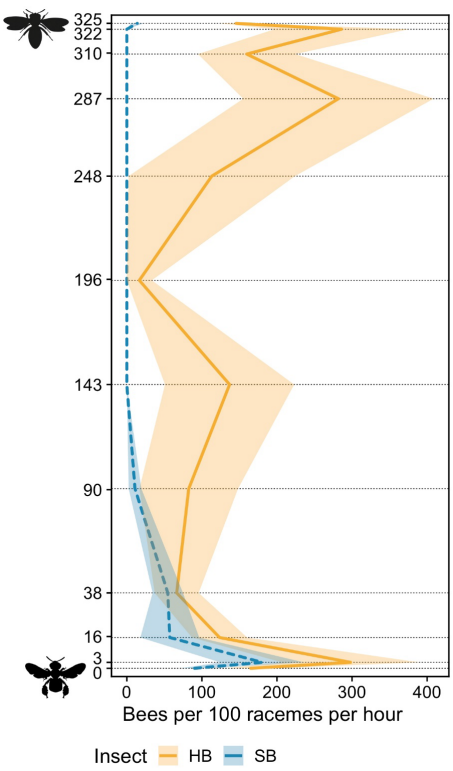


Figure 36. Spatial distribution of honey bees (HB) (*Apis mellifera*) and stingless (*Tetragonula carbonaria*) bees (SB) observed on macadamia trees at different distances from hives, in the manipulated survey block. Stingless bee hives were located at 0 m and the honey bee hives were located at 325 m. Mean number of bees per ~100 flowers per hour, across 48 trees (4 trees per distance). Means (solid line) ± SE (band around mean) for each distance were estimated from per tree counts of bees (i.e., 3 × 3 min surveys over 2 days = 18 min of survey time x 3.33 to achieve bees per hour) that were multiplied by the percentage of open racemes (obtained in daily quadrant counts). Insect pictograms indicate location of hives. Horizontal dashed lines correspond to distances from stingless bee hives at which observations were recorded.

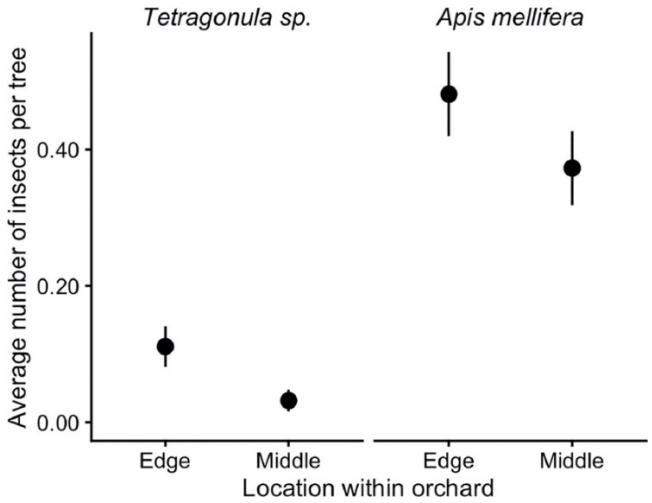


Figure 37. Mean number (± SE) of stingless bees (*Tetragonula carbonaria*) and honey bees (*Apis mellifera*) on trees on the edge versus in the middle of 17 macadamia blocks.

Figure 37. Is sourced from the below publication under license: **https://creativecommons.org/licenses/by-nc-nd/4.0/.** Evans, L.J., Jesson, L., Read, S.F.J., Jochym, M., Cutting, B.T., Gayrard, T., Jammes, M.A.S., Roumier, R,. and Howlett, B.G., 2021. Key factors influencing forager distribution across macadamia orchards differ among species of managed bees. Basic and Applied Ecology, 53:, pp.74-85.

Recommendations

We suggest a mixed approach to macadamia pollination management, employing honey bees, stingless bees, and other insect pollinators that are not directly managed (i.e., flies, beetles, and solitary bees).

Typically, hives are placed on the periphery of blocks in spaces that are easy to reach for beekeepers, however if some hives can be located in the center of the block, this may help with even pollination in harder-to-reach spots. As honey bee activity is influenced by flower density, undertaking management to promote evenness of flowering may help to achieve consistent pollination – this includes selective pruning to promote flowering, nutrition management and/or application of floral stimulants, and tree girdling (cincturing) to promote flowering.

The localised distribution of stingless bees is a clear challenge for their use for macadamia pollination – to get adequate coverage, colonies must be placed at regular intervals through a block (one colony approximately every 100 m) requiring large numbers of colonies per hectare for full pollination. Colonies placed within the block may be an obstacle for other orchard management, and these colonies are at increased risk of damage from pesticides. These challenges can be overcome by removing colonies during sprays, or blocking bees into their colonies for hours or days until the risk is diminished. Foraging close to home can also be a strength for pollination management. Stingless bee colonies can be used for targeted pollination of areas where bee activity is low (such as areas with low flower density, and therefore low honey bee numbers).

Unmanaged pollinators can also provide pollination and so their activity should be monitored for changes.

2.4.4 Sucrose feeding to increase pollen foraging in macadamia

There is evidence from other crops that providing colonies with sucrose will satisfy the colony's need for carbohydrates and, as a consequence, the colony will focus its efforts on pollen collection (Gemeda et al. 2018). This has the potential to be beneficial for pollination, if pollen foragers are more effective pollinators than nectar foragers, which is possibly the case for macadamia (Heard 1994).

Methods

Sixteen honey bee colonies were introduced into a macadamia orchard in West Brisbane at peak flowering. All colonies were fitted with modified Mann-lake pollen traps (the baffle plate within the traps was removed). Eight of these colonies were fed 1 L of 50% sucrose solution around 09:00 h every day for five days (treatment colonies), while the remaining eight colonies were not provided with any supplementary food (control colonies). As the strength of these hives were variable, we ensured that an even number of strong to weaker hives were included in the 'treatment' versus 'control' groups.

Pollen trap content was collected every 24h for six days, including on day 0 before the colonies were fed, but not on day 1 – the first day of feeding. The percentage of macadamia pollen in trapped pollen was determined on trial days 0, 3, and 6. A linear mixed model (LMM) was used to determine whether percentage change in pollen collected was predicted by sucrose feeding, trial day number, or an interaction between these two fixed effects. Colony number was included in the analysis as a random effect. A betareg model was used to compare the percentage of macadamia pollen collected.

Results and Interpretation

We found no significant effect of the treatment (sucrose feed) or trial day on the change in the percentage of pollen collected by colonies (treatment: χ^2 (1, 95) = 0.0156, p = 0.9; trial day: χ^2 (5, 95) = 10.074, p = 0.073, Figure 38). However, there was a significant interaction between treatment and trial day (χ^2 (5, 95) = 14.5422, p = 0.012), indicating that the change in pollen collection varied between treatment groups over time. Pairwise comparisons revealed that the percentage change in pollen collected by sucrose-fed colonies increased significantly between day 0 (baseline) and day 5 (p < 0.001) and between day 0 and day 6 (p < 0.001). In contrast, there were no significant differences in the percentage change in pollen collected by control colonies (no sucrose) between day 0 and day 5 (p = 0.517) or between day 0 and day 6 (p = 0.857).

The percentage of macadamia pollen (compared with all other pollen types) ranged from 0.7% – 43% per colony, with a mean of 10%. The amount of macadamia pollen collected by colonies decreased over time (Est. = -1.147; z =-4.057; p <0.001). The only effect of treatment on the percentage of macadamia pollen collected was a significant difference on day 0 (prior to feeding sucrose), where control colonies collected significantly more macadamia pollen (Est. = -0.734; z =-2.816; p =0.005).

In summary, by day five of our six-day trial, the percentage of pollen collected by sucrose-fed colonies had significantly increased compared to the baseline (trial day 0). This effect was not observed in non-sucrose control colonies, indicating the potential of sucrose feeding to enhance pollen foraging in macadamia orchards over time. The decline in macadamia pollen collection observed during the trial is likely attributed to a slowdown in macadamia flowering. Overall macadamia pollen collection was relatively low in our orchard, possibly influenced by the presence of more attractive pollen resources in the surrounding native bush. The impact of sucrose feeding on macadamia pollen has the potential to be more pronounced in isolated farms, such as those in the Bundaberg growing region.

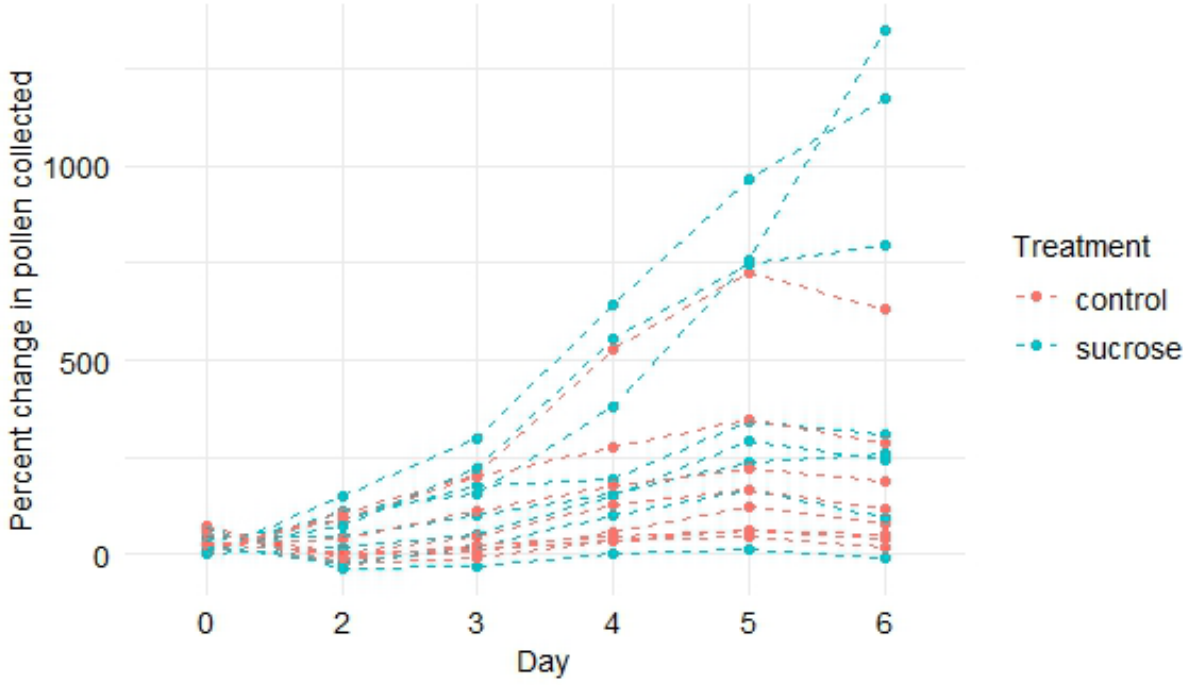


Figure 38. Percent change in pollen collected by honey bee colonies adjacent to a macadamia orchard. Colonies were either fed sucrose daily (sucrose, n = 8 colonies) or not fed (controls, n = 8). Day 0 serves as the baseline, measured prior to sucrose feeding. Subsequent trial days represent the percentage change compared to day 0. Note – no pollen was collect on day 1 – when the colonies were first fed.

3. Innovations to improve the health of honey bees in Australia

3.1 American Foulbrood (AFB)

Polymerase Chain Reaction (PCR) assays have the potential to dramatically decrease surveillance costs for AFB, thereby increasing the scope and thus effectiveness of control and eradication efforts. We aimed to authenticate these PCR assays as a diagnostic tool for AFB. To do this it was necessary to optimise the PCR assay against established culture methods used in AFB surveillance and test it against strains present in Australia and New Zealand. We calibrated sample source (honey bee, hive debris) and PCR assay results with clinical, and sub-clinical symptoms, in infected hives.

Methods

We worked with two commercial beekeeping operations in New Zealand that had an AFB prevalence of ~10% in autumn and spring 2017. Honey bee colonies were initially recruited into the trial if they were AFB negative based on field examination, but were situated in an apiary site where one or more colonies had recently been diagnosed with AFB. We considered these hives to be at high risk of AFB through movement of honey bees between infected and uninfected colonies. There was a delay of up to one month between recruitment to the trial and experimental sampling. During sampling all colonies received a complete brood disease inspection and a sample of bees was collected from the brood comb of colonies and stored at -20°C. Samples of 30 bees per colony were pooled in a plastic bag with 10 mL of ddH_2O and crushed. The resulting extract was used for culture plate testing following the protocol of Goodwin et al. (1996) and used in the DNA extraction protocol as below.

Bee extract (400uL) was loaded with 2 steel beads (3.2mm) and homogenised in 2 mg/ml Lysozyme solution in TNES

buffer (10mM Tris, 400mM NaCl, 100mM EDTA pH 8.0, 0.6% SDS). Following overnight incubation at 37°C, Proteinase K (10mg/ml) was added. Samples were vortexed for 10 sec and spun briefly, followed by a 30 min. incubation at 65°C. 5M NaCl and Chloroform:Octanol (24:1) was added to each well followed by a quick vortex. Samples were centrifuged at maximum speed for 20 min at room temperature. The supernatant was transferred into cold 100% Ethanol and incubated at -20°C for 30 min. After washing with 70% Ethanol and drying of the pellets the samples were rehydrated in TE buffer. A literature and empirical review was completed on published *P. larvae* specific primers. The primer set 16SN-233 (Han et al. 2008) gave the best amplification efficiency. Briefly, 10µl reactions included 2.5mM $MgCl_2$, 1x Master Mix (Roche), 200 nM of each primer and 2.5 µL DNA. Primers 16SNF (5' - GTGTTTCCTTCGGGAGACG - 3') and 16SNR (5' - CTCTAGGTCGGCTACGCATC - 3') amplify a 233bp fragment of the *P. larvae* 16S rRNA following thermal cycling: 5 min at 95°C, 45 cycles at 95°C for 10 sec, t 58°C for 10 sec, 72°C for 25 sec.

Results and Interpretation

One-hundred and thirty-three honey bee colonies were identified as high risk of developing AFB based on their proximity to confirmed AFB positive hives. These colonies were spread over 11 apiary sites in two different beekeeping operations. While the colonies were initially considered AFB negative based on field inspection, 12 colonies developed unambiguous symptoms of AFB between recruitment and the first data collection point and were classified as AFB positive. There were also two colonies that were classed as indeterminate based on field inspections, meaning they had abnormal brood but could not be unambiguously defined as AFB positive. We collected nurse bee samples for all colonies and completed both a culture plate test for *P. larvae* spores and qPCR detection for *P. larvae* for all colonies.

The culture plate test following the protocol of Goodwin et al. (1996). This includes a pasteurisation step that devitalised *P. larvae* vegetative rods while leaving the *P. larvae* spores unaffected to germinate on the culture plates. We measured the number of *P. larvae* colonies. Culture plate testing can also produce an inconclusive result where growth of non-*P. larvae* microbes can overgrow a plate, making the detection of *P. larvae* spores impossible. Finally, we ran a Qpcr assay on a DNA extract from the bee sample. We measured the cycle threshold (Ct) for each sample. A Ct of 0 indicates that no *P. larvae* DNA was detected in the sample, a Ct value between 1 and 45 indicates *P. larvae* DNA was detected in the bee sample in an inversely proportional relationship (i.e., the lower the Ct value, the more *P. larvae* detected).

Of the 133 colonies assessed, 78 were negative for AFB symptoms and for *P. larvae*, as detected by culture plate testing and qPCR. Of the remaining 55 colonies, 12 of them showed AFB symptoms, 16 were positive for *P. larvae* by culture plate testing and 27 were positive for *P. larvae* detection by qPCR. For qPCR to be a useful diagnostic tool it is important to understand the false negative rate and the relationship between *P. larvae* detection (Ct value) and likelihood of AFB symptoms. The false negative rate, where confirmed AFB positive colonies are not detected by qPCR, is very high in our study with eight out of the 12 clinical AFB colonies coming back negative for *P. larvae* in our qPCR assay. False negatives have also been reported from culture plate testing (Goodwin et al. 1996) and we have also found some false negatives in our culture plate testing presented here. In culture plate testing studies, possible explanations for false negatives include low germination rates of *P. larvae* spores *in vitro* and inconclusive results from non-specific overgrowth on the culture plates. Explanations for such a high rate of false negatives for qPCR could include the difficulty of extracting DNA from *P. larvae* spores (D'Alessandro et al. 2007) and the potential for honey bee tissue to affect PCR efficiency (Boncristiani et al. 2011).

We also looked for a relationship between the numbers of *P. larvae* spores from the culture plate test and the Ct value from qPCR (which is inversely proportional to the quantity of *P. larvae* DNA in the reaction). Bees from AFB positive colonies more likely to carry *P. larvae* spores than those without AFB symptoms and so the number of spores detected on a sample of bees by culture plating can be used to predict the likelihood of AFB symptoms (Goodwin et al. 1996). By plotting the cycle threshold values against *P. larvae* colony counts on culture plate for each the colonies (excluding the honey bee colonies with inconclusive plate reads from non-specific overgrowth) and fitting a linear trend line (Figure 39), we observed the expected relationship whereby the honey bee colonies with high *P. larvae* colony counts trended towards a lower Ct value (indicating a higher level of *P. larvae* template). The r-squared value of 0.239 reflects the high

proportion of hives that were positive for the *P. larvae* on the culture plate test yet negative by qPCR, or vice versa. This could reflect variable spore germination and variable *P. larvae* DNA extraction and amplification as discussed above.

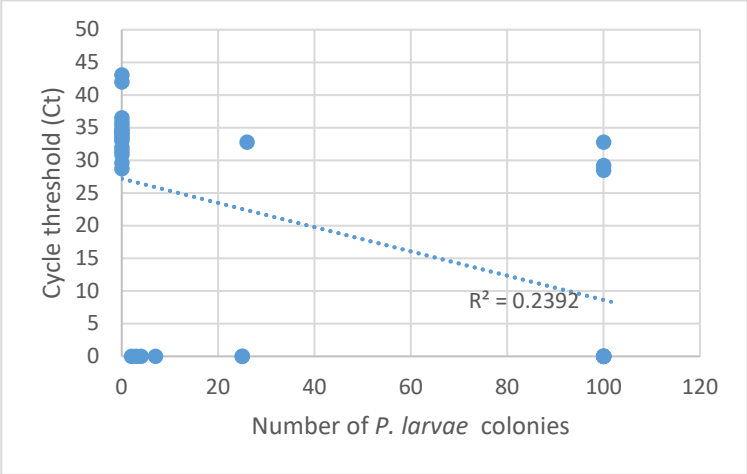


Figure 39. Number of *P. larvae* colonies detected on culture plate plotted against qPCR Ct values for individual honey bee colonies (n = 35; each blue dot represents a different honey bee colony). Trend line fitted and r-squared generated using standard settings in Excel.

Recommendations

Based on the results presented here, the use of qPCR to estimate the likelihood of a colony exhibiting clinical AFB disease in the same way that the culture plate test is used will require further technical refinement. A possible explanation for the high false negative rate using qPCR screening, and the poor correlation between Ct values and *P. larvae* colony counts on plates, could be the difficulty in reliably extracting DNA from *P. larvae* spores which are notoriously resilient (Goodwin et al. 1998). One possible approach could be to stimulate spore germination as a preliminary step of any DNA extraction protocol. As with all epidemiological models, the predictive power of such a test would increase as more cases are tested and we are aware of similar trials conducted after this research programme was completed.

3.2 Varroa Sensitive Hygiene (VSH)

If the identified genetic markers correlate with VSH in local populations, there is the potential to genetically select for VSH traits in queen breeding programmes as well as the queen management of individual beekeepers.

Methods (Trial I)

The SNP 9-9224292 is an adenine/guanine polymorphism observed to associate with VSH behaviour in North American honey bees. The purpose of this study was to assess if selection of SNP 9-9224292 variation associates with *Varroa* numbers in New Zealand honey bees. Note: this work was conducted in New Zealand as Australia was *Varroa* free at the time of experimentation.

Genotyping SNP 9-9224292 – Mated queens (<1 year of age) were genetically sampled at the commercial beekeeping operation Coast to Coast Bees Ltd (CTOC) by taking a wing clipping and storing in ethanol at -20°C. The HotSHOT protocol was used to extract genomic DNA from the wing fragment (Truett et al. 2000). The queens were genotyped for SNP 9-9224292 using a PCR amplification/restriction digest assay as described by Kirrane et al. (2014). Queens that carried two copies of the protective SNP variant (homozygous for guanine) were designated as 'treatment queens' while queens identified as carrying two copies of the wildtype SNP variant (homozygous for adenine) were designated as 'control queens'. The queens at CTOC were obtained from a range of different sources which was recorded to include as a

covariate in the analysis. Queens heterozygous at this SNP were excluded from the analysis. All queens were openly mated in the same environment, which is expected to reduce variation between the treatment and control colonies rather than create arbitrary differences.
Colony set up – In early summer of 2017, forty colonies headed by genotyped honey bee queens (treatment queens, n = 18; and control queens, n = 22) were set up in a single CTOC registered apiary site in Te Kowhai, New Zealand. Hives comprised of two three-quarter depth boxes placed in two rows in a north-facing semicircle arrangement. Colonies were inspected fortnightly and fed with sugar syrup when it was necessary. No efforts were made to manage inter-colony drift in the research apiary, a phenomena expected to reduce variation between colonies rather than create arbitrary differences.

Determination of *Varroa* levels – At the beginning of the field trial (day 7), both sides of each frame of every hive were photographed and the colony size (number of bees) was estimated by two observers based on the percentage of the comb surface covered by bees (Delaplane et al. 2013). An alcohol wash at day 15 provided an estimate of phoretic mites per 100 bees. The total *Varroa* load at the start of the trial was then estimated by multiplying the total colony size by the phoretic mite rate to provide a covariate (*initial Varroa load*) for subsequent analysis. Bayvarol (flumethrin) and Apivar (amitraz) strips were applied to each hive at day 83 following the manufacturer's instructions and *Varroa* fall determined using sticky boards smeared with canola oil. Sticky boards were replaced weekly and mites were counted manually. The total number of mites were calculated as the sum of the four weekly counts. To assess if the genotype of the queen had an impact on final *Varroa* levels we employed a Generalized Linear Models with a negative binomial distribution and *log* link function (GLM). Significance of terms was established with a likelihood ratio test (LRT).

Results and Interpretation

Varroa levels – We first confirmed that the differences in the initial *Varroa* estimates (*initial Varroa load*) in experimental colonies assigned to *genotype* and *source of queen* groups were not statistically significant. For that purpose, we fitted a series of GLMs to the *initial Varroa load*. We found no evidence for the differences between the *genotype* and *source of queen* groups, fitted either as simple terms (LRT against null model; *genotype*: $\chi^2 = 0.049$ at 1 df, p = 0.82; *source of queen:* $\chi^2 = 0.049$ at 1 df, p = 0.39), or in an interaction (LRT against simplified model: $\chi^2 = 0.98$ at 1 df, p = 0.32). Subsequently we fitted a GLM to the estimate of the total *Varroa* counts (at the end of the experiment); the saturated model included a three-way interaction between *genotype, source of queen*, and *initial varroa load*. In the course of manual model simplification we established that neither the three-way interaction (LRT: $\chi^2 = 0.081$ at 1 df, p = 0.77) nor any of the two-way interactions (LRT; *genotype:source of queen*: $\chi^2 = 0.19$ at 1 df, p = 0.65; *genotype:initial varroa load*: $\chi^2 = 0.82$ at 1 df, p = 0.36; *initial varroa load:source of queen:* $\chi^2 = 1.05$ at 1 df, p = 0.30) were significant. Simple term for *source of queen* was non-significant (LRT: $\chi^2 = 0.26$ at 1 df, p = 0.61), and was thus dropped from the model. When reanalysed, we found a significant simple effect of genotype (LRT: $\chi^2 = 4.92$ at 1 df, p = 0.027) and a significant simple effect of *initial varroa load* (LRT: $\chi^2 = 10.0$ at 1 df, p = 0.0016). Figure 40 shows the intercepts and the slope over initial *Varroa* load, as predicted by the minimal model; overall mean total *Varroa* levels in treatment (*genotype:* GG) was 948 (SE: 134.4) and 1301 (SE: 127.7) in control (*genotype:* AA).

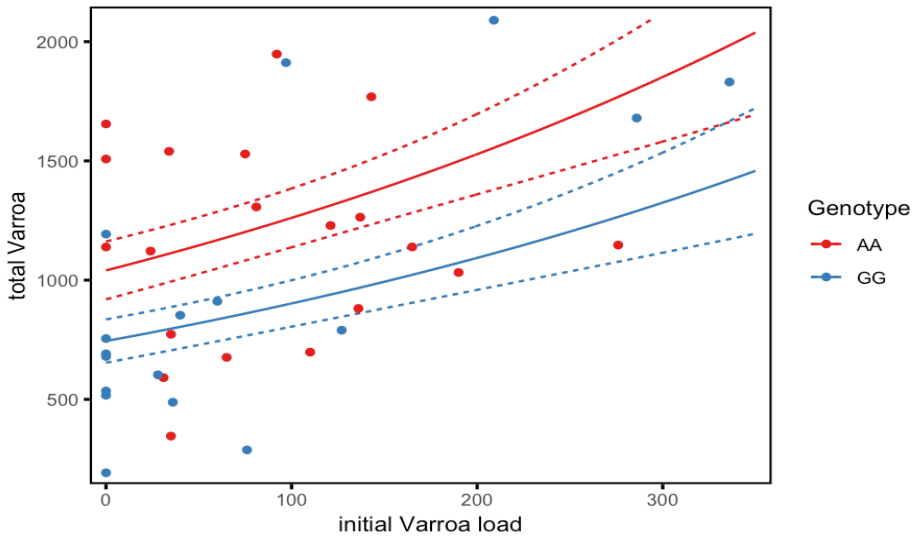


Figure 40. Total *Varroa* levels predicted by the Generalized Linear Model (GLM) for AA (control) and GG (treatment) genotypes with slope over *initial Varroa load* (solid lines) and corresponding standard errors (dashed lines). Three data points fall outside of displayed range.

We did not measure VSH behaviour directly in this trial, which requires extensive colony manipulation and direct observation of bee activity. To assess *Varroa* control, the total autumn *Varroa* population was estimated using mite drop counts following treatment with Bayvarol and Apivar, which are expected to kill all mites. When testing for a difference between the treatment and control colonies we needed to include both the source of the queen and the *Varroa* load at the start of the trial. CTOC sources their queens from a range of sources and our modelling concluded that specific sources do not have an impact on the final *Varroa* populations. The results of the modelling are presented in Figure 40 the mean *Varroa* population level is 28.5% lower in the treatment colonies compared with the control colonies. A possible explanation for this difference would be that the treatment colonies express higher levels of VSH (or other *Varroa* resistance) traits significantly suppressing the *Varroa* population growth over the 2017/18 season. This is consistent with other observations of the guanine allele of SNP 9-9224292 associating with VSH behaviour. In addition, *Varroa* levels were highly variable across colonies (Figure 40) regardless of any genotype effect which is not surprising as other factors are known to contribute to *Varroa* population growth, including the initial *Varroa* load in the colony.

Methods (Trial II)

Trial I (above) relied on genotyping each queen, which was invasive and expensive (and therefore less practical in a commercial operation), so we completed a second trial, testing if genotyping the breeder queen rather than the individual daughter queens is sufficient to predict beneficial traits of *Varroa* resistance. Briefly, virgin queens were produced at a commercial queen breeding operation. When the virgin queens were grafted from breeder queens that carried two copies of the protective SNP variant (homozygous for guanine) the virgin daughter queens were designated as 'treatment queens' while queens grafted from breeder queens that carried two copies of the wildtype SNP variant (homozygous for adenine) were designated as 'control queens.

In early summer 2018, twenty colonies had the existing queen removed and the resources (brood, honey, pollen and bees) of the colony split equally between two daughter colonies. By equally splitting colonies at the beginning of the field trial the *Varroa* levels (and other disease) levels were equalised between the treatment and control colonies. Each doublet of the daughter colony pair was re-queened with either a treatment or a control virgin queen (assigned at random) and managed in parallel at the same PFR registered apiary sites at Ruakura, New Zealand for the duration of the trial.

Ninety days after re-queening, approximately 300 bees were sampled from each colony and an alcohol wash used to

dislodge phoretic *Varroa* and provide a measure of *Varroa* levels (mites/100 bees). A cluster size assessment was completed on each colony following the protocol of Nasr et al. (1990). The Varroa levels (mites/100 bees) were multiplied by the cluster size estimate for each colony to generate a mite load index for each colony at the end of the trial. We used a linear mixed-effects model to assess differences in *Varroa* levels between the treatment and control colonies at day 90; the Mite load index (the response variable) was log-transformed to approximate a normal distribution. During the trial 13 colonies failed for reasons unrelated to *Varroa* infestation (primarily queen problems and robbing) meaning that only nine colony pairs (total 27 colonies) were included in the analysis.

Result and Interpretation

In Figure 41 we see there was no significant difference in *Varroa* levels between the treatment and control colonies (likelihood ratio test: $\chi^2 = 0.64$ at 1 df, $p = 0.43$). In our original study (Part I; above), we genotyped the queen of the colony and were able to correlate with *Varroa* levels of the colony, despite the open mating of the queen. In this trial, we established that the genotype of the mother of the colony queen does not correlate with *Varroa* level in a colony. A logical explanation is that two generations of open mating returned the frequencies of the SNP 9-9224292 polymorphism to open mating ratios, in the process neutralising any benefits associated with a colony having a higher frequency of the guanine allele.

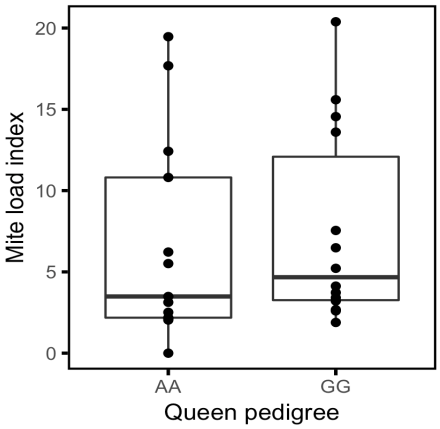


Figure 41. Mean *Varroa* mite (*Varroa destructor*) load index ± SE.

Recommendations – The honey bee SNP 9-9224292 shows promise as a marker for selection when aiming for colony-level *Varroa* control traits as part of an integrated pest management plan. The recent incursion of *Varroa* into Australia highlights the potential of this research as the Beekeeping Industry adapts to the reality of managing this pest. Another avenue of investigation would be the use of this research, combined with artificial insemination of queens from genotyped drones, to further increase the frequency of the protective genetic variant in colonies and thus assist with *Varroa* control.

Outputs

Table 6. Output summary for PH15000

Output	Description	Detail
Overall	86 outputs	Tangible products or services that were produced from the project activities.
Grower engagement	36 examples of grower engagement	This includes workshops, talks on farms, grower updates, industry meetings, and webinars. Estimated number of attendees are reported – reaching an estimated total of 1,720 growers and industry people across Australia receiving information from the project. June 5-6, 2018. Howlett: Understanding and managing macadamia pollination, Macadamia consultants meeting, Caloundra, QLD, Australia. (est. number of ppl: 60) July 3, 2018. Howlett, Cutting, Evans: Managing pollination in your orchard, Macgroup meeting, Nambucca, NSW, Australia. (est. number of ppl: 45) July 4, 2018. Howlett, Cutting, Evans: Managing pollination in your orchard, Macgroup meeting, Alstonville NSW. (est. number of ppl: 50) July 5, 2018. Howlett, Cutting, Evans: Managing pollination in your orchard, Macgroup meeting, Alstonville NSW. (est. number of ppl: 55) July 10, 2018. Cutting: Macgroup presentation. Glasshouse Mountains, Australia. (est. number of ppl: 30) July 11, 2018. Cutting: Macgroup presentation Gympie, Australia. (est. number of ppl: 30) July 12, 2018. Cutting: Macgroup presentation Bundaberg, Australia. (est. number of ppl:40) July 2018. Individualised grower updates provided to 11 different blueberry and watermelon growers. August 2018. Sainsbury: DNA-informed Queen Selection: Varroa Control. New Zealand Beekeeping South Island Seminar Day, Christchurch, New Zealand. October 8, 2018. Cutting: Lychee Pollination, ALGA meeting and AGM, Bundaberg, Australia. (est. number of ppl:40) October 2018. Sainsbury: DNA-informed Queen Selection: Varroa Control. Rotorua Honey Bee Club Bee Educated Day, Rotorua, New Zealand. November 13, 2018. Cutting: presentation during pollination workshop. Aus Mac conference, Gold Coast, QLD. Approx. 100 in workshop, 300+ at conference May 2019. Sainsbury: DNA based detection of *Paenibacillus larvae*: the infectious agent of American Foulbrood. New Zealand Beekeeping Inc Auckland Branch Seminar Day, Auckland, New Zealand. June 7, 2019. Evans: Honey bee foraging under netting covered

		crops. Fruit Growers Tasmania (FGT) Conference. Orchard presentation, Tasmania, Australia. (est. number of ppl: 80) September 5, 2019. Evans (Invited): Honey bee health and foraging under netting. Berry Growers workshop, Tasmania, Australia. (est. number of ppl: 60) September 11, 2019. Cutting (Invited): Lychee Pollination. Annual meeting of the Australian Lychee Growers Association, Sarina Beach QLD. (est. number of ppl: 30) August 3, 2020. Evans: 20-minute webinar/podcast delivered on melon pollination, recorded by Firetelle as part of the Talking Melons Series: https://www.stitcher.com/podcast/talking-melons/e/76656872 (number of views: 60) September 9, 2020. Cutting: Webinar to lychee industry. Approx. 20 representatives in attendance. June 6, 2021. Cutting: Macadamia pollination best practice and future directions. New Zealand Macadamia Society AGM. Approx. 85 representatives in attendance. September 15, 2021. Cutting: Webinar to lychee industry Approx. 40 representatives in attendance. September 23, 2022. Cutting: live webinar: Blueberry pollination best practice. Miro Berries technical group, NZ. January 17, 2023. Broussard: Pollination research – berryfruit and beyond. Berryfruit Days. Motueka, New Zealand. February 16, 2023. Cutting: pre-recorded presentation (with technical staff present in person for questions). Watermelon pollination research update. Melons Australia industry roadshow, Chinchilla QLD. Approx. 30 representatives in attendance. March 23, 2023. Cutting: Watermelon pollination research update. Melons Australia industry roadshow, Bundaberg QLD. Approx. 15 representatives in attendance. July 7, 2023. Cutting: pre-recorded presentation, Blueberry pollination, best practice and future directions. Miro Berries grower day, Gisborne, NZ. Approx. 20 representatives in attendance.
Best practice manuals	6 electronic 4-page pollination manuals for 6 different cropping industries: avocado, macadamia, watermelon, blueberry, lychee, and papaya.	Crop pollination manuals were published online between 2018 and 2020 (two manuals per year during this period). They are available online on the Hort Innovation, Plant Health Australia, and BeeAware websites. Paper copies of the manuals were also printed by PFR and given out to growers/industries at workshops and conferences. 1. Howlett, Evans, Cutting, Pattemore – Maximise your macadamia crop with better pollination: https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/avocado-pollination-brochure/ https://www.planthealthaustralia.com.au/industries/avocados/ https://beeaware.org.au/wp-

		content/uploads/2018/03/Macadamia-pollination-brochure.pdf 2. Howlett, Evans, Cutting, Pattemore – Maximise your avocado crop with better pollination: https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/avocado-pollination-brochure/ https://www.planthealthaustralia.com.au/industries/avocado/ https://beeaware.org.au/pollination/pollinator-reliant-crops/avocado/ 3. Evans, Cutting, Howlett – Maximise your melon crop with better pollination: https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/melon-pollination-brochure/ https://www.planthealthaustralia.com.au/industries/melon/ https://beeaware.org.au/pollination/pollinator-reliant-crops/melon/ 4. Evans, Cutting, Howlett – Maximise your blueberry crop with better pollination: https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/blueberry-pollination-brochure/ https://www.planthealthaustralia.com.au/industries/blueberry/ https://beeaware.org.au/pollination/pollinator-reliant-crops/blueberry/ 5. Cutting, Evans, Howlett – Maximise your papaya crop with better pollination: https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/lychee-pollination-brochure/ https://www.planthealthaustralia.com.au/industries/lychee/ https://beeaware.org.au/pollination/pollinator-reliant-crops/lychee/ 6. Cutting, Evans, Howlett – Maximise your lychee crop with better pollination: https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ph15000-assets/papaya-pollination-brochure/ https://www.planthealthaustralia.com.au/industries/papaya/ https://beeaware.org.au/pollination/pollinator-reliant-crops/papaya/ When completed, all the manuals were promoted by Hort Innovation: https://www.horticulture.com.au/hort-innovation/news-events/media-releases/20152/getting-the-

		facts-on-pollination/ Evidence of engagement – page views in the last 12 months (4 years after the first manuals were published online). Note: no information on webpage views is available for the BeeAware website. Plant Health Australia: 1. Melon = 3 downloads 2. Blueberry = 17 downloads 3. Papaya = 5 downloads 4. Lychee = 35 downloads 5. Macadamia = 1 download 6. Avocado = 27 downloads Hort Innovation: 1. The general report page = 109 visits 2. Melon = 9 visits 3. Blueberry = 5 visits 4. Papaya = 5 visits 5. Lychee = 6 visits 6. Macadamia = 8 visits 7. Avocado = 13 visits
Published grower articles	16 published grower articles. This includes write-ups in established industry and beekeeping association newsletters and industry journals.	July 2017. Willcox, B., Robson, A., Rader, R and Howlett, B. Can we increase avocado production via pollination? Talking Avocados Magazine. October 2018. Evans LJ, Cutting BT, Keir M, Van Noort T, Howlett B. Beeing proactive about pollination. Melon Industry newsletter. Spring 2018. Howlett B**,** Cutting B, Evans L, and Pattemore D. Pollination of macadamia: what to do? AMS News Bulletin:40-41 (See Appendix 4). October 2018. Cutting B, Evans L, Keir M, Nathan T, Fale G, and Jochym M. Sweet Potential for Lychee yields. Living Lychee newsletter. 2018. Cutting BT & Evans LJ. Scientists aim towards perfecting pollination. Papaya Press. October 2018. Cutting B, Evans L, Keir M, Nathan T, Fale G, and Jochym M. Improve yield with better pollination. Living Lychee newsletter, 77 (Appendix 3). September 2019. Sainsbury J, Cross, S. Beekeeper Science: DNA-Informed Queen Selection. The New Zealand Beekeeper 27(8): 26-27. July 2020. New research project brings Australian lychee pollination into focus. Living Lychee newsletter (See Appendix 3). August 2020. Cross S, Fale F, Sainsbury J. Project on selecting queens for varroa control shows value of citizen science. The New Zealand Beekeeper: 28(7):27 December 2020 Cutting BT, Evans L, Keir M, Nathan T, Fale G, Jochym M Increased lychee yields through managed pollination. Tree Crops Magazine. February 2021.Wijesinghe SAEC, Evans LJ, Cutting BT, Keir M,

		and Rader R. Insects that visit watermelon flowers in Australia. Melon News, p21. August 2021. Cutting BT, Evans LJ, Read S, Jochym M, Jesson L, Howlett B. Research Update: Stingless Bees for Macadamia Pollination. Cross-pollinator, Australian Native Bee Association. July 2021. Cutting BT, Read S, Jochym M, Jesson L, Howlett B, Evans L. Managing Pollination: Will bees go the distance? AMS bulletin. March 2023. Cutting BT, Wijesinghe SAEC, Rader R, Evans L. Stingless bees are effective pollinators of seedless watermelon. Cross-pollinator, Australian Native Bee Association (See Appendix 1). January 2023. Cutting B, research update on melon pollination. Melons Australia e-news. January 2023 Cutting BT, Wijesinghe SAEC, Rader R, Evans L. Research Update: Identifying key melon pollinators and getting the most out of honey bees. Melons Australia grower resources. (See Appendix 2)
Grower conferences	11 presentations at industry led conferences.	February 13–15, 2018, Evans (Invited): Covering all the bases: what do we know about pollination in protected cropping environments. BerryQuest International, Launceston, Tasmania, Australia. (est. number of ppl: 160) June 18–20, 2018, Evans: Covering all the bases: what do we know about honey bee behaviour and pollination in protected cropping environments? Hort Connections, Brisbane, Australia. (est. number of ppl: 80) June 18–20, 2018, Evans & Cutting: Pollination in Protected Cropping & Future Environments, Hort Connections, Brisbane, Australia. (est. number of ppl: 70) June 28, 2018, Pattemore: Providing a pollination service of value to growers. 3rd Australian Bee Congress, Gold Coast, Australia. (est. number of ppl: 180) September 17–19 2018, Evans: Pollination of hybrid watermelon in Australia. Melon Conference, Townsville, Australia. (est. number of ppl: 220) November 13, 2018, Howlett: Macadamia pollinator efficiency. Australian Macadamia Industry Conference, Gold Coast, Australia. (est. number of ppl: 160) June 2019, Sainsbury: DNA-informed Queen Selection: Varroa Control. Apiculture New Zealand 2019 Conference, Rotorua, New Zealand. August 2019, Sainsbury: DNA-informed Queen Selection: Varroa Control. NZ Beekeeping Inc. Mini-conference, Hamilton, New Zealand. June 8, 2019, Evans (Invited): Honey bee health and foraging under netting. Fruit Growers Tasmania (FGT) Conference, Tasmania, Australia. (est. number of ppl: 65) June 26, 2019, Howlett: Pollination for improved yields. 2019 New Zealand Macadamia Annual Conference, Patetonga, New

		Zealand. September 15, 2022, Cutting (invited). Managing Blueberry pollination: best practice and future directions. Blueberries NZ AGM and conference, Hamilton NZ. Approx. 100 representatives in attendance.
Scientific conferences	8 presentations at scientific conferences	September 13–14, 2017. Howlett: Cross pollination mostly increased final raceme nut counts in macadamia compared with self or open pollination. International Macadamia Research Symposium, International Macadamia Research Symposium, Hilo, Hawaii. September 13–14, 2017. Evans: Quantifying the effect of deploying honey and stingless bee hives on macadamia pollination. International Macadamia Research Symposium, Hilo, Hawaii. June 18–20, 2018. Howlett (Invited): Macadamia pollination, 3rd Yunnan Conference on International Exchange of Professionals (YCIEP), Kunming, China. July 1, 2018. Evans: Distribution of managed stingless bees in a macadamia orchard and their effect on nut set. Australian Native Bee Conference, Gold Coast, Australia. July 1, 2018. Cutting: Efficiency of Australian native bees for pollination of watermelons. Australian Native Bee Conference, Gold Coast, Australia. October 17–19 2018. Howlett (Invited): Macadamia pollination, 8th International Macadamia Symposium, Lincang, Yunnan Province China. July 3, 2019, Van Noort: Watermelon pollination in Queensland, Australia. Plant Science Central Conference 2019, Palmerston North, New Zealand. December 6–9, 2021. Subasinghe Arachchige E: Honey bees are the dominant and effective pollinators in watermelon in Australia. Australian Entomological Society 52nd AGM and Scientific Conference, Adelaide.
Published scientific papers	8 papers published in international journals and 1 PhD thesis. Note – 6 further publications on lychee, watermelon, avocados (x2) and macadamia are in preparation.	Willcox BK, Howlett BG, Robson AJ, Cutting B, Evans L, Jesson L, Kirkland L, Jean-Meyzonnier M, Potdevin V, Saunders ME, Rader R 2019. Evaluating the taxa that provide shared pollination services across multiple crops and regions. Scientific Reports 9: 1-10. (as of December 2023: 709 reads online via Research gate) Wijesinghe SAEC, Evans LJ, Kirkland L, Rader R 2020. A global review of watermelon pollination biology and ecology: The increasing importance of seedless cultivars. Scientia Horticulturae 271: 109493. (as of December 2023: 206 reads online via Research gate) Kendall LK, Gagic V, Evans LJ, Cutting BT, Scalzo J, Hanusch Y, Jones J, Rocchetti M, Sonter C, Keir M, Rader R 2020. Self-compatible blueberry cultivars require fewer floral visits to maximize fruit production than a partially self-incompatible cultivar. Journal of Applied Ecology 57: 2454-2462.

		(as of December 2023: 123 reads online via Research gate) Evans LJ, Jesson L, Read SFJ, Jochym M, Cutting BT, Gayrard T, Jammes MAS, Roumier R, Howlett BG 2021. Key factors influencing forager distribution across macadamia orchards differ among species of managed bees. Basic and Applied Ecology 53: 74-85. (as of December 2023: 266 reads online via Research gate) Sainsbury J, Nemeth T, Baldo M, Jochym M, Felman C, Goodwin M, Lumsden M, Pattemore D, Jeanplong F 2022. Marker assisted selection for *Varroa destructor* resistance in New Zealand honey bees. PloSOne: https://doi.org/10.1371/journal.pone.0273289 (as of December 2023: 141 reads online via Research gate) Subasinghe Arachchige ECW, Rader R, Cutting BT, Keir M, van Noort T, Fale G, Howlett BG, Samnegård U, Evans LJ 2022. Honey bees are the most abundant visitors to Australian watermelon but native stingless bees are equally effective as pollinators. Ecological Solutions and Evidence, 3(4): e12189. (as of December 2023: 285 reads online via Research gate) Arachchige ECW, Evans L, Campbell J, Delaplane K, Rice E, Cutting B, Kendall L, Samnegård U, Rader R 2022. A global assessment of the species composition and effectiveness of watermelon pollinators and the management strategies to inform effective pollination service delivery. Basic and Applied Ecology 66. DOI: 10.1016/j.baae.2022.11.006 (as of December 2023: 205 reads online via Research gate) Arachchige ECW, Evans L, Samnegård U, Rader R 2022. Morphological characteristics of pollen from triploid watermelon and its fate on stigmas in a hybrid crop production system. Scientific Reports 12: 3222. DOI: 10.1038/s41598-022-06297-2 (as of December 2023: 142 reads online via Research gate) PhD thesis: Subasinghe Arachchige ECW 2022. Pollination Ecology of Watermelon and Other Global Food Crops. Thesis submitted to University of New England. Principal supervisor: A/Prof. Romina Rader, co-supervisors: Dr. Lisa Evans and Dr. Ulrika Samnegård

Outcomes

Table 7. Outcome summary for PH15000

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Intermediate project outcome: New knowledge on crop	Our project outcomes have progressed all three of the strategic	The project has made good progress on all intended	Collected data and its interpretation have been

specific pollinators, pollination, pollinator management and honey bee disease management.	investment themes of the Hort Innovation Pollination Fund EAC: 1. Management of European honey bee. 2. Optimisation of crop pollination efficiency. 3. Identifying alternative crop pollinators.	objectives: 1. Establishing and disseminating current best practice information – this was completed for the following crops: avocado, macadamia, melon, blueberry, lychee and papaya. 2. Identify crop specific pollinators and developing diverse pollination strategies. We identified crop visiting insects and measured their abundances for watermelon, lychee, macadamia, blueberry, papaya, avocado, and almond. The efficacy of the most abundant pollinators/taxonomic groups was determined for melon, blueberry, lychee and macadamia. We further determined whether there is a pollination deficit and the value of cross pollination (for blueberry, lychee, avocado, and almond). Finally, 3. Assessing innovations to improve honey bee health, for example, we show that the honey bee SNP 9-9224292 shows promise as a marker for selection when aiming for colony-level *Varroa* control traits as part of an integrated pest management plan.	provided in annual milestone reports to Hort Innovation, public summaries, and subsequent publications (for list of publications see Table 6).

Intermediate project outcome: Development of techniques and methods to improve pollination of focal crops, as well as honey bee colony health.	Performance expectations (KPIs): New methods described for improving pollination success. New methods described for improving/monitoring honey bee colony health in terms of disease management.	As described above, we determined the role different taxa/groups of taxa have in the pollination of focal crops and provided suggestions on how these pollinators could be encouraged on farms. We also assessed management techniques for honey bees and native stingless bees in macadamia and watermelon and provided recommendations for improved pollination service delivery. As described above, we showed that the honey bee SNP 9-9224292 shows promise as a marker for selection when aiming for colony-level *Varroa* control traits as part of an integrated pest management plan. Artificial insemination from genotyped drones could further increase the frequency of the protective genetic variant in colonies with a further increase in *Varroa* control.	As described above, collected data and novel method recommendations have been provided in annual milestone reports to Hort Innovation, public summaries, and subsequent publications (for list of publications see Table 6).
End of project outcome: Research findings communicated to growers/industry leading to an improved awareness of crop pollination requirements and honey bee management.	Performance expectations (KPIs): Communication of scientific results to growers/industry and uptake of recommendations to improve pollination.	Our research findings have been regularly communicated to project stakeholders (growers/cropping industries/beekeepers) in a wide range of different formats including: best practice manuals, workshops, grower up-dates, meeting/conference presentations, webinars, popular articles and scientific articles. Howlett surveyed 44 avocado growers to help determine the understanding of avocado grower with regard to the diversity and value of pollinators within their	A complete list of grower engagement activities that have been delivered over the life of the project is provided in Table 6. Some examples are given in Appendices 1- 5. We also report grower/industry participation numbers (evidence of improved awareness) in these activities and website views for online content in Table 6. Survey results

		crops, and to determine whether scientific knowledge is being transferred adequately to growers.	were published as part of a global, multi-crop paper: Osterman J, Landaverde-González P, Garratt MP, Gee M, Mandelik Y, Langowska A, ... & Howlett BG 2021. On-farm experiences shape farmer knowledge, perceptions of pollinators, and management practices. Global Ecology and Conservation, 32, e01949.

Monitoring and evaluation

Table 8. Key Evaluation Questions for PH15000

Key Evaluation Question	Project performance	Continuous improvement opportunities
Has new knowledge been generated on i) crop specific pollinators, pollination, ii) pollinator management, and iii) honey bee disease management?	Yes - extensive new knowledge has been generated for each of these three objectives. We provide evidence of this in presentations, publications, and milestone reports. These outputs are summarised in Table 6.	- Developing methods for managing a wider diversity of pollinators (we are currently helping to address as part of PH19001). - Further information on the pollination requirements for different cultivars/plant types within crops (we are currently addressing this as part of PH20001).
Have techniques and methods been developed to improve pollinator management/pollination of focal crops, as well as honey bee colony health?	Yes - new techniques and methods have been developed as part of this program and are described in presentations, publications, and milestone reports. These outputs are summarised in Table 6.	- Ways of managing honey bees in a wider variety of cropping systems (we are helping to address this as part of ST19006).
Have the research findings been communicated to growers/industry?	Yes – the key results have regularly been communicated with stakeholders in a wide range of formats detailed in Table 6.	- There is potential to update best the practice manuals that were produced earlier in the research program. - Continued dissemination of research findings through scientific and popular articles.
Have the research findings lead to an improved awareness of crop pollination requirements and honey	Yes – we are aware of growers and industries (e.g. macadamia) that now more likely to plan for pollination	-Continued dissemination of research findings through scientific and popular articles.

bee management and/or changes in industry practices?	and pay for honey bee hives during crop flowering. Growers we have worked directly with have modified the positioning of their honey bee hives within their crop and/or modified their cropping environment based on the recommendations coming from this program. We have also had some growers tell us that they frequently refer to our pollination manual.	

Recommendations

Watermelon growers

- Consider a broader range of managed species (e.g., *Tetragonula*) alongside honey bees, and encouraging wild pollinators could have positive effects on crop yields and would enhance the resilience of watermelon pollination services.
- To achieve necessary visits across the block, at a stocking rate of four honey bee hives/ha, hives should be placed approximately every 750 m.
- Pollinator activity on the crop is influenced by weather conditions and the presence of competing floral resources in the surrounding environment, growers should monitor pollinator activity across their block to ensure they have adequate numbers of pollinators. Introducing more honey bee hives may be necessary to optimise pollination in varying conditions.
- We do not recommend sucrose feeding within the field as a method for increasing pollination of watermelon.

→ Future RD&E: assess the pollination requirements and compatibility of different watermelon cultivars and their pairings.

Lychee growers

- To increase instances of insect movement from male flowers, especially M2, onto female flowers, we recommend interplanting cultivars, ensuring that there is good overlap between flower stages in your local environment. Ensuring overlap in these flowering phases on different cultivars will provide more opportunity for pollination events to occur.
- High-density planting may also be a good option for lychee to enhance insect movement between trees.
- Unmanaged insects (native bees and flies) are likely to be important for pollination and so their activity should be monitored for changes. Add managed pollinators if a decrease in activity is noted.

→ Future RD&E: assess the pollination requirements, compatibility, and flowering overlap of different lychee cultivars and potential their pairings.

→ Future RD&E: the effect of protective crop covers on lychee pollination.

Macadamia growers

- As honey bee activity is influenced by flower density, undertaking management to promote evenness of flowering may help to achieve consistent pollination – this includes selective pruning to promote flowering, nutrition management and/or application of floral stimulants, and tree girdling (cincturing) at appropriate times of year to promote flowering.
- Positioning some honey bee hives in the center of blocks may help with even pollination in local positions that are more difficult to pollinate due to low attractiveness to pollinators.
- Stingless bee colonies can also be used for targeted pollination of areas where bee activity is low (such as areas with low flower density). They are likely to forage locally and pollinate all flowers near their colony.
- Stingless bee colonies placed within the block should be removed during spraying, or blocked into their colonies for hours or days until the risk is diminished.
- Unmanaged pollinators can also provide pollination and so their activity should be monitored for changes. Add managed pollinators to compensate for any noticed reduction in other flower visitor activity.

Blueberry growers

- To improve pollination outcomes for varieties exhibiting self-incompatibility, which require more pollinator visits, encourage pollinators that frequently move between plants/trees. Additionally, promoting pollinator diversity

may enhance this type of movement among honey bees.

- Be mindful of differences in pollinator activity between growing environments, particularly during periods of wet/ very cold weather when there may be a reduced opportunity for fruit to reach their optimum quality under covers. In such instances, larger numbers of managed pollinators may need to be introduced into covered blocks.

→ Future RD&E: assess the pollination requirements and compatibility of different blueberry cultivars and their potential pairings.

→ Future RD&E: the effect of protective crop covers on blueberry pollination.

Papaya growers

- At least seven species of hawk moths (Sphingidae) have been identified as key pawpaw pollinators in Queensland, and they are also noted visitors to red-fleshed papaya. These moths are often most active just after dusk. Their larvae depend on particular host plants, including native and introduced species. Conserving or promoting natural or semi-natural environments on and around farms will help to provide breeding habitat for these species.
- Honey bees do not visit female flowers of dioicous plants, but do visit perfect (hermaphroditic) flowers of papaya varieties. Their total contribution to pollination is not entirely understood, but ensuring some honey bees are present in the environment may help to prevent pollination shortfalls, particularly in the cooler months when hawk moths aren't foraging.

→ Future RD&E: Assess the pollinator efficacy of honey bees on red-fleshed varieties, and confirm the potential for these varieties to set fruit in the absence of insect visits.

Avocado growers

- Insect activity on flowers should be regularly monitored throughout avocado orchards. Based on our data, growers should aim for an average of at least two insects per panicle (which has approximately 1000 flowers open daily) during fine weather. Beneficial insects for avocado pollination include both bees and various fly species.

→ Future RD&E: A better understanding of the factors influencing the presence/absence of pollinators (e.g. flies) and the lifecycles of specific species is required to enable rowers to more reliably 'manage' these wild insects for pollination.

Almond growers

- 'Carina' and other self-compatible cultivars are not reliant on pollen being moved from another cultivar to set nuts. Hence these varieties are likely to be more resilient against pollination failure, and present a good option to reduce dependence on honey bees as the impacts of *Varroa* in Australia are realised.

→ Future RD&E: self-compatible cultivars will likely still see some benefit from insect visits. An economic analysis should be undertaken to compare the cost of hive rental against the potential yield increase with increased cross-pollination.

→ Almond is a good candidate for pollination by artificial means. Existing tools should be explored further in Australia to provide additional tools for resilience against *Varroa* impacts.

Beekeepers - honey bee health

- The honey bee SNP 9-9224292 shows promise as a marker for selection when aiming for colony-level *Varroa* control traits as part of an integrated pest management plan. Further, when combined with artificial insemination of queens from genotyped drones, the frequency of the protective genetic variant in colonies could be increased, assisting with *Varroa* control.

→ The use of qPCR to estimate the likelihood of a colony exhibiting clinical AFB disease in the same way that the culture plate test is used will require further technical refinement. Spore germination could be stimulated as a preliminary step of any DNA extraction protocol. As with all epidemiological models, the predictive power of such a test would increase as more cases are tested.

Refereed scientific publications

Published journal articles produced as part of PH15000

Willcox BK, Howlett BG, Robson AJ, Cutting B, Evans L, Jesson L, Kirkland L, Jean-Meyzonnier M, Potdevin V, Saunders ME, Rader R 2019. Evaluating the taxa that provide shared pollination services across multiple crops and regions. Scientific Reports 9: 1-10.

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Intellectual property

No project IP or commercialisation to report.

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3. Innovations to improve the health of honey bees in Australia
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Appendices

Example popular articles produced for Australian horticultural and bee sectors:

Appendix 1: March 2023. Cutting BT, Wijesinghe SAEC, Rader R, Evans L. Stingless bees are effective pollinators of seedless watermelon. Cross-pollinator, Australian Native Bee Association.

FEATURE ARTICLE

This month's feature article is brought to you by a team of researchers funded by the Australian government who are doing terrific work investigating the pollination of crops. In this article they show that unmanaged stingless bees are important and efficient pollinators of watermelon in Northern Australia. We offer an extra special congratulations to two of the authors, Lisa Evans and Brian Cutting on the birth of their son Lewin.

Stingless bees are effective pollinators of seedless watermelon

Brian T. Cutting, Erandi C.W. Subasinghe Arachchige, Romina Rader, Lisa J. Evans

Few foods signal that summer has arrived quite like a sweet and cool slice of watermelon. *Some* of the authors reminisce about lazy afternoons with sticky hands, a pink shirt collar, and the singular goal of spitting a seed further than a sibling. Though our collectively heightened sensitivity to hygiene may have put an end to this tradition (if the delightfully simple triploid seedless varieties hadn't already) we suspect we're not alone in this daydream; about half of all Australian households purchase fresh watermelon each year, and most of these are Australian grown, with more than 182,000 tonnes produced in 2021 (Australian Horticulture Statistics Handbook 2020/21, 2022).

While watermelon seeds may occasionally sail through the air – watermelon pollen does not. At about 70 µm across, it's a bit larger than most wind-blown pollen, and it's covered with a sticky 'pollenkitt' that tends to keep it where it is. However, move it must as most watermelon plants have separate male and female flowers which open for a single day – and pollen must be moved between the two for fruit set to occur. In seedless watermelons, the pollen must be moved from another variety (Arachchige et al. 2022a).

The pollen, of course, is moved by insects – but *which* insects is an important question. Historically, most of what is known about watermelon pollinators comes from North America and little is known about watermelon pollinators here in Australia. What we do know is that some of the potential candidates are quite a bit different. Some of the most important watermelon pollinators in North America include some species of bumble bees (*Bombus sp.*) and Longhorn bees (Eucerini), especially in the genus *Peponapis* (Figure 1) (Spicer 2007). This group has a close association with cucurbits and are commonly known as 'squash bees'. Squash bees and bumble bees are both completely absent from the Australian mainland – Australian growers rely nearly ubiquitously on managed honey bees to ensure adequate watermelon pollination (Arachchige et al. 2022b)

Figure 1. In North America, Squash Bees (*Peponapis*, top) and Bumble Bees (Bombus, bottom)) are both important pollinators of cucurbit crops. Both are absent from Australia. (Photo: B. Cutting)

An over-reliance on a single pollinator poses a risk to pollination dependent producers – it is a well-established principle of pollination ecology that pollinator diversity leads to better and more consistent yields through pollinator complementarity and pollinator redundancy (Rader et al. al; Garibaldi et al. 2013). Additionally, in recent years bushfires and the incursion of *Varroa destructor* in New South Wales have underscored the vulnerabilities if pollination hives are lost or their movement becomes restricted. However, growers cannot anticipate and rely on pollination services from unmanaged insects until there is a better understanding of when, where, and how they are moving pollen in the crop. As a starting point, we set out to:

- Identify which insects were present on watermelon flowers around Australia,
- Quantify insect abundance and behaviour that might reasonably affect watermelon pollination, and
- Directly demonstrate the ability of certain insects to move pollen between flowers.

Methods

We visited 15 seedless watermelon farms in Queensland, Northern Territory, and New South Wales, and at each site we assessed the diversity and abundance of flower visiting insects, as well as their visitation rates, their patterns of movement between flowers, and the amount of pollen that they deposited per flower visit.

At all farms visited, we collected insects (netted after confirmed flower visit (Figure 2), and identified them to the finest taxonomic resolution practicable (bees were identified by Toby Smith).

In the field, we conducted 45-second point-counts of insects on flowers at 50 points along a 50 m transect extending from the edge of each watermelon field towards the centre. These observations were recorded at three times of day (0700 – 0900 h, 0900 – 1100 h, and 1100 – 1300 h) on fine days. Because of the challenges of multiple observers providing consistent and accurate identification of moving insects, we used course groupings in these surveys; bees were identified as honey bees, stingless bees, or other native bees (mostly Megachilidae and Halictidae).

Per-visit effectiveness was tested by presenting unvisited female flowers (protected with mesh bags) to insects foraging on male flowers. Once an insect visited, the female flower was processed so that the number of pollen grains transferred onto the stigma could be counted. Movement patterns were assessed by following individual insects for as long as possible (up to 10 minutes) and recording whether they visited male or female flowers, the distances that they moved, and whether they contacted flower anthers or stigmas during their visits.

Figure 2. Confirmed flower visitors were collected for later identification. Study sites were typically large-scale farms, with rows up to 3km long, often with bush/scrub remnants in the immediately surrounding landscape.

Results

We identified a diverse group of flower visiting insects, but honey bees were the most dominant visitors to watermelon flowers in all regions studied – between 73 % and 94% of all observed flower visits were attributable to honey bees (Figure 3). This is unsurprising, as all surveyed farms had introduced managed colonies of honey bees for pollination (between 2 and 7.5 hives per hectare).

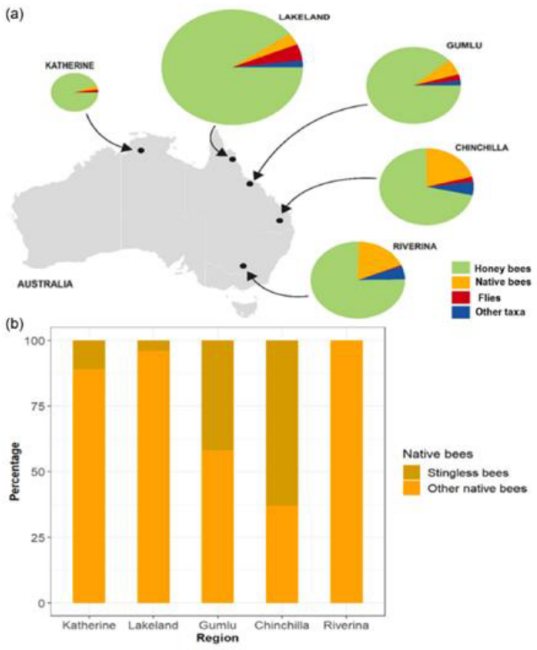

Figure 3 Abundance of insect visitors within five watermelon-growing regions in Australia (Katherine, Lakeland, Chinchilla, Gumlu and Riverina), shown as (a) the relative abundance of honey bees, native bees, flies and other taxa within each region and (b) the proportion of stingless bees (*Tetragonula* sp.) compared to other native bees (from Arachchige et al. 2022c)

The remainder of flower visits observed were from stingless bees and other native bees, flies, butterflies and moths, and beetles, with native bees being the second-most frequent visitors at most sites after honey bees. We found that stingless bees were more abundant on flowers than other native bees, accounting for up to 63% of non-Apis bee visits (Figure 4).

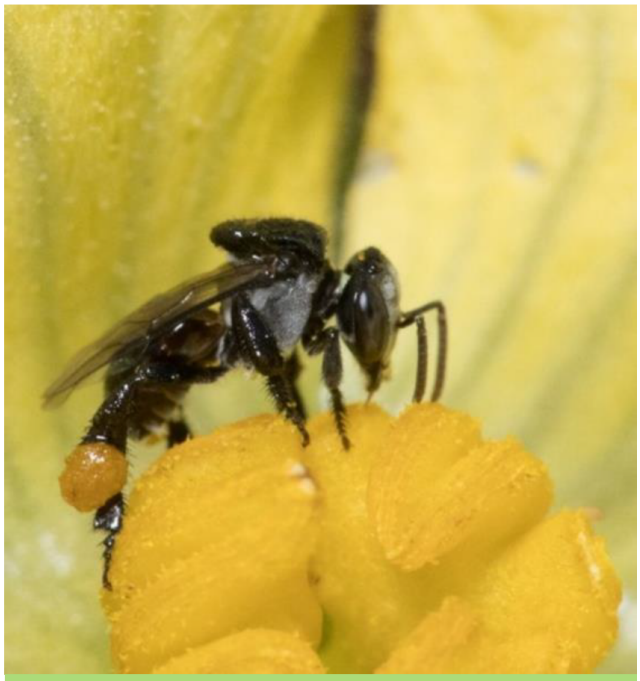

Figure 4. Stingless bees (*Tetragonula* sp. or *Austroplebeia* sp.) were one of the more frequently observed flower visitors at some of the sites within their native range – here pictured collecting pollen on a male flower. While they visit fewer flowers per hour than honey bees, each visit deposits a similar amount of pollen as a honey bee visit. (Photo: B. Cutting)

Honey bees, stingless bees, native solitary bees, and some flies were all confirmed to be capable of moving pollen onto watermelon stigmas. Each honey bee visit deposited an average of 40 pollen grains – this was not significantly different from stingless bees, with an average of 27 pollen grains deposited per visit. Solitary bees and flies both deposited pollen, but were not as effective per-visit (17 and 4.7 grains per visit respectively) (Figure 5). Honey bees were observed moving between male and female flowers more frequently than other taxa, and visited more flowers per hour than other insects.

Discussion/Implications:

Watermelon producers in Australia, as in many places elsewhere in the world, are heavily dependent on honey bees for pollination, however it was confirmed that stingless bees, solitary native bees, and some flies all visit watermelon flowers and are capable of depositing pollen on stigmas. Recently, Nacko et al. (2022) also found a pattern of honey bee dominance in field-grown watermelon, however unlike in our study, they recorded only one stingless bee visit in their study plots. Meanwhile, in commercial-scale sites surveyed in our study, stingless bees were consistently found on flowers within their range, and together with other native bees they were responsible for up to 22% of flower visits observed. Although they visit flowers more slowly than honey bees (and may visit female flowers a lower percentage of the time), each stingless bee visit deposits similar quantities of pollen as a honey bee visit.

The differences between these studies underscores the benefit in sampling across multiple sites and regions. We are consistently finding that pollinator communities can vary within and between regions. Practically for growers and beekeepers, this highlights the need for continued development of both 'permanent' pollination management strategies (e.g., prescribed base rates for hive stocking per hectare and conservation of probable pollinator habitat on farms) and real-time reactive strategies (monitoring activity of known pollinators on flowers and deploying managed pollinator when and where required).

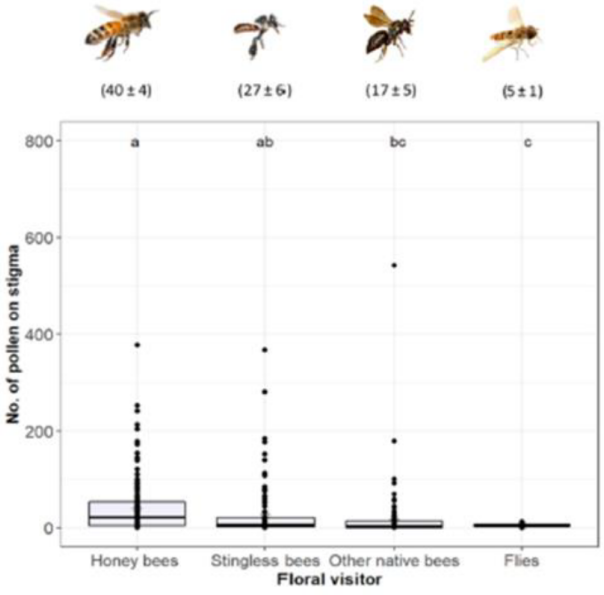

Figure 5. Pollinator effectiveness (single visit pollen deposition) including honey bees, stingless bees , other native bees and flies on watermelon. In each box, the bold horizontal line is the median, and means are shown with an asterisk (*). The lower and upper edges of the box represent the 25% and 75% quartiles, respectively. Whiskers indicate the maximum and minimum values that are not outliers (circular data points). The different letters indicate significant differences among floral visitors (from Arachchige et al. 2022c).

Whether managed stingless bees can be deployed in such a way to affect watermelon pollination in an open field-grown setting remains an active question but based on the abundance and per-visit pollination efficacy of *un-managed* stingless bees on Australian watermelon farms, we conclude that they are likely providing an important pollination service to this industry.

Our paper 'Honey bees are the most abundant visitors to Australian watermelon but native stingless bees are equally effective as pollinators' in *Ecological Solutions and Evidence* is open access. You can find it in full, without charge here: https://besjournals.onlinelibrary.wiley.com/doi/full/10.1002/2688-8319.12189

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Many people who were involved in the substantial field and lab work underpinning this research, and they may as well have been authors on this article, but we didn't want them to be accused of spitting seeds in public. Thanks to Matthew Keir, Theo van Noort, Grant Fale, Brad G. Howlett, Ulrika Samnegård, and Toby Smith, and especially to the watermelon growers around Australia who welcomed us onto their farms.

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Figure 6. The collaborative project team from University of New England and Plant & Food Research in a typical (above) and less typical (below) pollination study site. From top left: Dr. Lisa Evans, Dr. Erandi Subasinghe Arachchige, Dr. Romina Rader, Brian Cutting, Lisa again, Matthew Keir.

Appendix 2: January 2023 Cutting BT, Wijesinghe SAEC, Rader R, Evans L. Research Update: Identifying key melon pollinators and getting the most out of honey bees. Melons Australia grower resources.

Melon News, Jan/Feb 2023

Research Update: Identifying key melon pollinators and getting the most out of honey bees

Brian T. Cutting[1], Erandi C.W. Subasinghe Arachchige[2], Romina Rader[2], Lisa J. Evans[1]
[1]Plant & Food Research Australia & [2]The University of New England

The 2022 *Varroa destructor* incursion in New South Wales underscores the risks and challenges to pollination-dependent industries, especially those with a heavy reliance on honey bees. Pests and disease outbreaks can rapidly alter the availability of hives for pollination and impose uncertainty for growers and beekeepers alike. The good news is that Australia is the last major land mass in the world to have a serious *Varroa* incursion – this has given researchers time to study the ecology of our production systems to help growers make informed decisions about pollination management.

How dependent are Australian watermelons on honey bees?

Watermelon pollen must be moved from staminate (male) to pistillate (female) flowers for pollination to occur. In seedless varieties, this pollen needs to originate from another, diploid variety. As watermelon pollen is relatively large and sticky, it does not blow on the wind, but depends on animal (mostly insect) flower visitors to move pollen. To better understand the contributions of various insects to watermelon pollination in Australia, our team conducted surveys of flower visitors at 15 commercial farms in New South Wales, Queensland, and Northern Territory. We also quantified the behaviour of these insects and measured how much pollen they moved with each visit.

Figure 1 Honey bees (A) were the dominant flower visitor to watermelon flowers in all regions studied. The crop is also visited by flies (B) native stingless bees (C), butterflies and moths (D), native solitary bees (E), and true bugs (F). Honey bees and stingless bees are the best at moving pollen.

We found that honey bees (Figure 1) were by far the most abundant visitors to watermelon flowers in Australia – between 73% and 94% of the visits that we observed were made by honey bees. This is unsurprising as all of the visited farms introduced managed honey bees. As well as being abundant, honey bees also moved between flowers quickly, visiting over 300 flowers per hour (predicted) and about 10% of those visits were to female flowers. Each honey bee visit to a female flower deposits 40 pollen grains on the stigma on average, which is a considerable contribution towards full

pollination (seeded varieties generally require 1000 pollen grains). These numbers suggest that Australian watermelon growers are highly dependent on honey bees for adequate pollination.

Other insects can contribute to pollination

While honey bees were the dominant flower visitor in all regions, other insects including native bees were also observed on the crop, and are thought to be important contributors to pollination. Within their natural range, native stingless (sugarbag) bees are considered to be as effective per visit as honey bees, depositing an average of 27 pollen grains. Some of these species are kept by beekeepers, and colonies could be manipulated to provide managed pollination, however these bees move between flowers less often and are less likely to forage far from their colonies. Solitary bees and flies were also found to deposit some pollen on female flowers. It is suggested that growers familiarise themselves with the native insects visiting flowers as these may play an increasingly important role if *Varroa* continues to spread in Australia.

Pollination by unmanaged honey bees

The recommended honey bee stocking rates for watermelons typically fall between two and 7.5 managed hives per hectare, but currently most farms also benefit from pollination by unmanaged (feral) European honey bees. At one farm each in Gumlu and Chinchilla, we fitted introduced hives with devices which marked bees with a fluorescent powder as they left the hive (Figure 2). We then surveyed the crop and counted the numbers of marked and unmarked bees at various points across the block. We found that at a stocking rate of 2.5 hives per hectare about half of the bees observed in the block were unmarked, and probably from feral colonies.

Figure 2 Honey bees leaving managed hives were marked with fluorescent powder to assess their movement across the crop, and to estimate the contribution by unmanaged (feral) honey bees.

If *Varroa* spreads, the contribution by feral colonies will decline substantially. With *varroa,* survival of colonies becomes dependent on human intervention to keep numbers of mites low, and untreated colonies will perish requiring greater contribution by managed bees. We estimate that at the farms studied, a rate of 5.6 hives per hectare would mean the vast majority (90%) of bees would be from managed colonies.

The good news is that our study showed that in the presence of feral colonies, pollination was not especially sensitive to bee stocking rate – though this was at only two farms and the

density of feral colonies will vary strongly across a landscape. We observed that female flowers received adequate numbers of pollen grains, even at the lowest stocking rate tested of 0.74 hives per hectare. This suggests that if *Varroa* caused a temporary reduction in the ability of migratory beekeepers to provide hives, growers outside of the range of *Varroa* could still benefit from substantial pollination from unmanaged European honey bees.

Getting the most of managed and unmanaged pollinators

Our bee marking trials also provided information about how managed honey bees move across a block, and showed that a more even distribution was achieved when hives were placed at multiple drop-points around the field. This was especially true at the lower hive stocking rates. Some growers have placed liquid sugar feeders among the crop to encourage bees into the field, however our tests have showed that this does not have a substantial effect on bee distribution or resultant pollination.

Figure 3 Download the watermelon pollination guide and checklist for more information and guidelines.

To get the most from managed hives, speak with your beekeeper to help ensure that introduced colonies are healthy and strong, with lots of foraging bees, and when possible, spread the introduction of these colonies around the growing area. Periodically walk through the crop, paying attention to bee activity on flowers. If activity is low in certain areas, target those spots for hive drops and/or increase the density of introduced hives.

You'll also want to pay attention to native bees and other pollinators. Many of these bees nest in the ground or in hollow stems of plants – and they may be living among the crop or travelling in from surrounding areas. Therefore, on-farm management or changes in surrounding land use may affect their abundance. By taking some basic notes about their numbers, you can get a feel for what is driving their abundance and adopt practices that promote their persistence on farm.

The full results of the pollinator monitoring study are published in the peer-reviewed journal *Ecological Solutions and Evidence*. It can be accessed in full, for free here: https://besjournals.onlinelibrary.wiley.com/doi/full/10.1002/2688-8319.12189

Additional information can be accessed in the melon pollination manual, available for free here: https://www.planthealthaustralia.com.au/wp-content/uploads/2021/07/Melon-pollination-brochure.pdf

This research was conducted for PH15000 'Strengthening and enabling effective pollination for Australia', funded by Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation and Plant & Food Research.

Appendix 3: October 2018. Cutting B, Evans L, Keir M, Nathan T, Fale G, and Jochym M. Improve yield with better pollination. Living Lychee newsletter, 77.

Improve yield with

The information presented below comes from the *Lychee pollination** brochure. The brochure provides information for growers on how to maximise their lychee crop with better pollination.

Lychee produces large numbers of flowers grouped in dense clusters, but fruit set per flower is generally low.

Even so, by increasing pollinator visits to flowers it's possible to enhance fruit set, and small increases in fruit set can lead to significant increases in yield.

Lychee flowering is a complex process and generally occurs in three distinct stages.

Each cluster of flowers, or panicle, on a tree may contain hundreds of individual flowers.

The first flowers to open are male (Figure 1, M1). They are followed by the female flowers, which open after the male flowers have finished.

Female flowers are easy to recognise by their forked style (Figure 1, F) and stunted anthers without any pollen.

Finally, a second type of male flower (Figure 1, M2) open, with anthers that produce a large amount of pollen.

Even though they have a rudimentary style (like female flowers), these M2 flowers do not produce fruit.

This sequence of flowers means that most of the fruit on a single tree are pollinated by M2 male flowers that overlap with the female flowers.

So that you have both female and M2 pollen–producing flowers present at the same time, multiple cultivars with offset flowering times need to be planted.

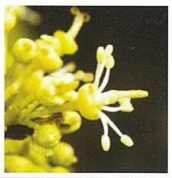
	M1	F	M2
			
Nectar	**yes**	**yes**	**some**
Pollen	**some**	**no**	**yes**
Fruit set	**no**	**yes**	**no**

Figure 1. Stages of lychee flowering. The sterile male phase (M1) opens first and produces nectar and pollen, but does not contribute to fruit set.
The second phase is the female phase (F) which can be fertilised and produce fruit, but does not produce pollen.
Finally, the second male phase (M2) that cannot produce fruit, but is the source of most of the pollen, causes fruit set in female flowers.

Growing the trees where the microclimate affects flowering time (e.g. on a slope) may also help to ensure that plenty of M2 pollen is available for female flowers.


Native stingless bee

Insect pollination

In most regions, lychee flowers are visited by many types of insects. Most insects are likely to move pollen between flowers both within and between trees.

The efficiency of these pollinators will differ: insect body size, shape, and behaviour influence the number of pollen grains they pick up and transport to pollinate other flowers.

Even small insects can pollinate lychee flowers. For example Australian native stingless bees (*Tetragonula* sp.) contact the parts of male and female flowers and occasionally deposit pollen.

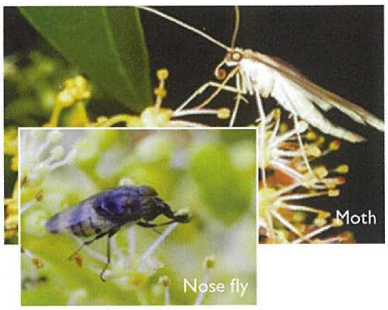
Honey bees

The European honey bee (*Apis mellifera*) is a frequent visitor to lychee flowers, and numerous studies show that its presence results in good fruit set and production.

The generally recommended honey bee hive stocking rate for lychee is 2.5 hives per hectare. The actual number

* The brochure was produced as part of the Hort Innovation Pollination Fund project *Strengthening and enabling effective pollination for Australia* (PH15000). The program identified key pollinators across a range of Australian crops and provides pollination management recommendations to maximise yields and reduce risk of pollination failure. The project involved almond, avocado, blueberry, lychee, macadamia, melon and papaya. **Download the brochure:** horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/lychee-pollination-brochure/

better pollination



needed will depend on how many other pollinators are in the area, and other flowering plants in the surrounding landscape.

It's best to spread honey bee hives throughout the growing area to cover all parts of the crop.

It is important that pollination hives contain strong colonies with a good number of foraging bees, as well as space within the hive for collected resources.

Developing a relationship with your beekeeper, and establishing a pollination contract and a plan for auditing colony strength, can help to ensure that the hives on your farm are suitable for pollination.

The Asian honey bee (*Apis cerana*), which is a little smaller in size than the European honey bee, is also a frequent visitor to lychee flowers around Cairns. However, growers are reminded that they are required to report Asian honey bee to Qld Dept of Agriculture and Fisheries, and that it is a biosecurity offence to keep or move them.

Other pollinators

The contribution of insects like beetles, flies and moths to lychee pollination is not fully understood, but studies show that in some regions they may have a substantial effect on pollination and boost yields.

Having a diverse range of pollinators in the orchard ensures that pollination can occur throughout the day and in varying weather conditions, including when it's cold or wet when honey bees may be absent.

Wild pollinators may come from habitat on the farm or in surrounding areas. It is important to keep in mind that land management and land-use changes can influence the numbers and activity of these important insects.

Many of these insects shelter within the crop (rather than in a remote hive) so they can be vulnerable to pest control measures, even during the night and early morning when the risk to honey bees is low.

Protected cropping

It is common for farms to have protective netting covers to prevent damage from storms and pest animals.

These covers can disrupt normal pollinator behaviour and beekeepers may be reluctant to move hives into covered blocks.

Where possible, it is recommended to leave the sides of protected areas open during flowering to allow natural foraging of honey bees and wild pollinators.

Some unmanaged pollinators and Australian stingless bees appear to be less affected by covers and may be particularly useful as pollinators in these situations.

Hort Innovation

Appendix 4: Spring 2018. Howlett B, Cutting B, Evans L, and Pattemore D. Pollination of macadamia: what to do? AMS News Bulletin:40-41.

IMPROVING POLLINATION IN THE ORCHARD

Brad Howlett, Brian Cutting, Lisa Evans and David Pattemore

Do you know how much pollination is happening in your orchard? It is an important question because research has shown that good pollination can increase both nut set and dry kernel weight. This article summarises how you can better understand how much pollination is currently occurring in your orchard and what you can do to improve it.

Keeping track of nut set from year to year will help you understand the rates of pollination in your orchard and ensure you notice if something changes.

Macadamias usually respond positively to pollen being moved from one variety onto the flowers of a different, compatible variety. This cross-pollination generally improves the number of nuts set and increases dry kernel weight.

A recent study in Bundaberg also hinted at the potential for increased crop yields. Researchers cross-pollinated 100 racemes each in six 741 trees using pollen from 816. Nut counts were 70 per cent higher in the cross-pollinated trees than in six non-crossed control trees. In addition, the researchers found evidence that many farms in Australia had inadequate levels of cross-pollination.

Five steps to improved cross-pollination

Here are five easy steps you can take to learn more about pollination in your orchard and improve cross-pollination rates.

Step 1. Record fruit set in your orchard from year to year. Loss of production due to pests or disease is usually immediately apparent, but unrealised production due to poor pollination is not obvious; you don't miss nuts that were never on the tree. Tracking nut set from year to year will help you identify what your orchard's pollination potential is.

To do this, simply mark about 10 trees diagonally across your block. On each tree, mark 10 fully flowering racemes using tape. Count the nuts on these racemes when fully developed and keep track of your results from different areas of the farm and from year to year.

Step 2. Assess whether you have a pollination deficit by pollinating some flowers by hand. This will allow you to detect whether your block can benefit from better cross-pollination. This can be tested on the same 10 trees by simply cross-pollinating one raceme per tree and comparing it with a control raceme in the same tree.

An easy way to cross-pollinate a raceme is to use an A4 transparent plastic sheet sold as a document cover from stationary stores. Form a 'pollination tube' by rolling the plastic into a cylinder of about the same diameter as a macadamia raceme.

Mark two fully flowering racemes per tree. Toss a coin and mark one raceme with an 'x' (this will be cross-

pollinated). Collect pollen from a different variety to pollinate your racemes; slide a fully flowering raceme inside the pollination tube and rotate the tube a few times. Light-coloured pollen should be visible dusting the inside of the tube. Return to the marked tree, place the tube over the raceme marked 'x' and rotate it to complete the cross pollination. For pollination to occur, the pollen must reach a small spot on the very tip of the flower called the stigma. Repeat the process until all your marked racemes are cross-pollinated.

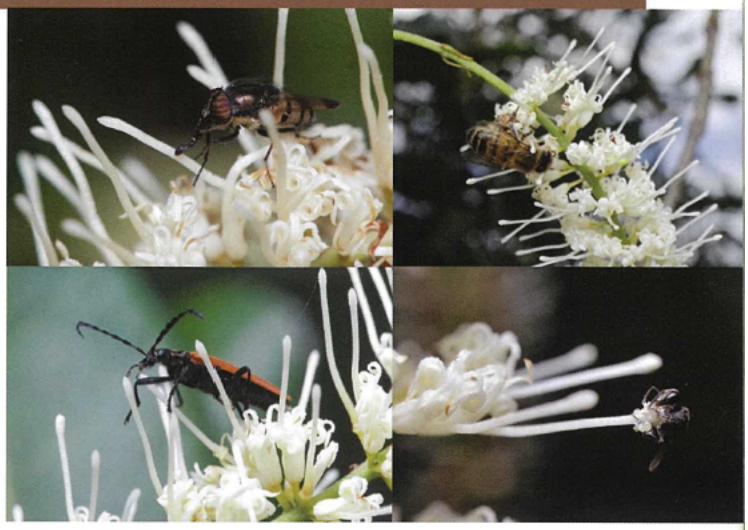
Before harvest, count the nuts on the marked racemes. If there are a lot more nuts on the crossed racemes than on the controls, it indicates a pollination deficit. If the counts are similar it suggests good cross-pollination in your orchard.

Step 3. Monitor and know your pollinators. Recent work has shown that beetles, bees and flies can pollinate macadamia. Net-winged beetles (lycids) and soldier beetles (cantharids) are slower at moving between flowers than bees but move much more randomly, making them very useful cross pollinators. Stingless bees can be very efficient at moving pollen to the most important parts of the flower, and honey bees also do a pretty good job. Becoming familiar with the pollinators that are present on your farm is an important step to understanding what may be moving pollen, and how cross-pollination could be improved.

Step 4. Orchard design. Incorporating multiple varieties in the one orchard block greatly increases the chance of cross-pollination. When planting a new block or selectively replacing trees, consider planting cross-compatible varieties, ensuring that timing of flowering and nut drop are well matched.

Step 5. Work with your beekeeper. Not all beehives are the same; those best for pollination are managed differently from those used for collecting honey. As well, both honey bees and stingless bees have limited movement ranges and patterns of dispersal in orchard blocks. Work with your beekeeper to make sure that you are getting hives that are adequate for pollination and spread these hives throughout your farm to promote pollination in all areas.

Working with your beekeeper can also help to avoid problems with accidental pesticide exposure and ensure your apiarist is familiar with the biosecurity code of practice – preventing spread of honey bee and plant diseases.



A variety of insects can pollinate macadamia, including (clockwise from top left) rhiniid flies, honey bees, lycid beetles, and stingless bees. Because of the way stingless bees interact with flowers, they are very good at moving cross pollen to the stigma, the pollination 'target' at the very tip of the flower.

Tailoring your pollination management

Macadamia pollination can be subject to many factors and varies from farm to farm, but by building your knowledge of what is happening at your site using the above steps, you will be able to better understand which factors are the most important for you. You can then tailor your management strategy. This will help to safeguard your production against environmental changes, and in most cases will help you to develop an active pollination management strategy to ensure your farm is getting the highest possible yields.

Information
The steps in this article are from a guide produced by researchers at Plant & Food Research and funded by Hort Innovation and the AMS. You can download the free guide from the BeeAware website, http://beeaware.org.au/pollination/pollnator-reliant-crops/macadamias/

About the authors
Brad Howlett and David Pattemore are with the New Zealand Institute for Plant and Food Research Limited, and Brian Cutting and Lisa Evans with Plant & Food Research, Australia.

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Report prepared by:

Lisa Evans
Scientist, Beneficial Biodiversity
December 2023

Report approved by:

Jill Stanley
Science Group Leader, Productive Biodiversity and Pollination
December 2023

For further information please contact:

Lisa Evans
Plant and Food Research, Ruakura, New Zealand
Email: Lisa.Evans@plantandfood.co.nz