Final Report

Stingless bees as effective managed pollinators for Australian horticulture

Project leader:

Professor James Cook

Delivery partner:

Western Sydney University

Project code:

PH16000

Project:

Stingless bees as effective managed pollinators for Australian horticulture (PH16000)

Disclaimer:

Horticulture Innovation Australia Limited (Hort Innovation) makes no representations and expressly disclaims all warranties (to the extent permitted by law) about the accuracy, completeness, or currency of information in this Final Report.

Users of this Final Report should take independent action to confirm any information in this Final Report before relying on that information in any way.

Reliance on any information provided by Hort Innovation is entirely at your own risk. Hort Innovation is not responsible for, and will not be liable for, any loss, damage, claim, expense, cost (including legal costs) or other liability arising in any way (including from Hort Innovation or any other person's negligence or otherwise) from your use or non-use of the Final Report or from reliance on information contained in the Final Report or that Hort Innovation provides to you by any other means.

Funding statement:

Levy funds – R&D projects

This project has been funded by Hort Innovation, using the Hort Innovation Vegetable research and development levy, co- investment from Western Sydney University, Hort Innovation, Syngenta and OLAM and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Hort Frontiers – R&D projects

Project *Stingless bees as effective managed pollinators for Australian horticulture* (PH16000) is funded by the Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Western Sydney University, Syngenta and OLAM and contributions from the Australian Government.

Publishing details:

ISBN 978-0-7341-4892-6

Published and distributed by: Hort Innovation

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Contents

Public summary

This project explored the potential of stingless bees as managed crop pollinators by testing their effectiveness with selected field crops and suitability as glasshouse crop pollinators. The overall objective was to develop the use of stingless bees as managed pollinators for horticultural crops. Honeybees are seen as the industry-standard pollinator, but availability, disease, and the incursion of *Varroa* mite suggest that other avenues need to be explored.

The leading candidates are stingless bees, which live in large colonies (like honeybees), pollinate a wide variety of plants, and can be kept in managed hives. There is a growing number of stingless beekeepers, and stingless bees are already utilised in macadamia farms, where they outperform honeybees. Stingless bees (particularly *Tetragonula* species) are used in crop pollination in several Asian countries, e.g. India and Thailand, but there is underdeveloped potential in Australia. The project was a collaboration involving Western Sydney University, Griffith University, Australian stingless beekeepers ("Sugarbag bees"), Syngenta, and OLAM. There has also been liaison and research exchange with separately funded efforts in India and Thailand - two key countries with established stingless bee use.

The project compiled data and reviewed existing evidence on the potential of stingless bees and other native bees as managed pollinators adding value to Australian horticulture. Experimental studies were carried out on a range of fruit and vegetable crops (both tropical and temperate), testing first if the bees had visited the flowers and transported the crop pollen. Where they had, the effectiveness of stingless bee pollination was then tested, and its impact on crop set/yield/quality as appropriate. For the most promising crop/bee combinations, more detailed studies were conducted to determine the best ways to deploy managed hives within the target crop. In addition, similar tailored studies of the potential of stingless bees to be effective managed pollinators in glasshouse conditions were undertaken. The glasshouse is an unnatural environment for bees, so specific studies were conducted to determine how to manage glasshouses for healthy stingless bee populations.

Our work shows that stingless bees have exciting potential as managed pollinators in mango, lychee and, with some caveats, avocado. In addition, we found that wild stingless bees are the main pollinators of mangoes in Northern Territory orchards. Stingless bees are also excellent pollinators of macadamia and we found that timing of hive deployment relative to crop flowering influenced their fidelity to the crop, and that recently split hives perform poorly because foragers are highly focused on collecting resin after a colony split.

We also carried out experiments with several cucurbits, but found that neither of the two commonly kept *Tetragonula* bees were inclined to visit flowers, precluding further study. In contrast, the same bees were excellent pollinators of strawberries in the NVPCC glasshouse. We also found that colonies deployed in glasshouses initially lost weight rapidly and showed reduced activity, before stabilizing and providing good crop pollination. When returned to the external environment after the crop rotation, they rapidly regained weight and increased activity levels. These results suggest that stingless bees can also be excellent glasshouse pollinators but should only be used for relatively short periods, interspersed by periods in good foraging situations outdoors.

These outcomes had progressed two of the three strategic investment themes of the Pollination Fund EAC: a) Optimising crop pollination efficiency and b) Identifying alternate crop pollinators.

Keywords

Tetragonula; stingless bees; agriculture; pollination; fruit quality; yield; native bees; alternative pollinators; floral resources; lychee; macadamia; mango

Introduction

Insect pollination of crops depends on varying contributions from managed and wild pollinators. Honeybees are the only pollinators widely available for deployment in managed hives, and are excellent pollinators in many situations, but less so in protected cropping or when buzz pollination is important. In addition, collapse of honeybee populations (especially wild colonies) is expected if Varroa mite (and associated pathogens like Deformed Wing Virus) establishes in Australia.

The future supply of managed honeybees in Australia is also a serious concern due to lack of recruitment into the beekeeping profession, and increasing demand for managed hives, especially from the valuable and expanding almond industry. Therefore, alternative insect pollinators for Australian horticulture need to be considered, their pollination efficiency investigated in different crops, and better ways found to propagate and deploy them. The leading candidates are stingless bees, which pollinate a wide variety of plants, and are already used in some macadamia orchards.

Managed stingless bees may have wide but underdeveloped potential for crop pollination in Australia, and are already used in India and Thailand, from which we may gain useful knowledge. Stingless bees (Meliponini) are the only other tribe of highly social bees after the honeybees (Apini); and number over 500 species globally (Michener 2013). Moreover, they are the only other bees to be managed in persistent hives, and can be easily transported (Heard 2016). They are pollinators of many fruits, including tropical crops, and have also shown promise in preliminary studies for greenhouse pollination of crops such as strawberry (Malagodi-Braga & Kleinert 2004) and capsicum (Greco et al., 2011a,b). Australia has 12 stingless species in two genera – *Tetragonula* and *Austroplebeia* – and a small but rapidly growing stingless bee industry (Halcroft et al. 2013), based largely on three species *T. carbonaria, T. hockingsi* and *A. australis*. Of these *T. carbonaria* is by far the most widely kept. A recent survey (Halcroft et al. 2013) reported a 2.5-fold increase in the number of stingless beekeepers and a 3.5-fold increase in the number of hives kept over a 10-year period.

Few scientific studies have been conducted on the behavior of Australian stingless bees, although valuable anecdotal information may reside with amateur beekeepers (Halcroft et al. 2013). Further, there has been little work on improving colony propagation, the major impediment to colony availability. Nevertheless, the stingless bee industry already provides hives for pollination of macadamia and some other crops where stingless bees can be more effective than honeybees. Crops reported to benefit from managed pollination by stingless bees in Australia include macadamia, lychee, blueberry, mango, avocado, and watermelon (Anderson et al. 1982, Heard 1994, Halcroft et al. 2013), but the data are often limited. Globally, stingless bees have been reported to be important pollinators of 10 and partial pollinators of 60 fruit, vegetable, and medicinal crops (Heard 1999). However, most of these reports are anecdotal and need verification and quantification.

1. What are the key knowledge gaps in stingless bee biology with respect to managed pollination services?
2. Do stingless bees visit the flowers and carry the pollen of a range of major temperate and tropical crops?
3. Are stingless bees good pollinators of these crops with regard to yield and quality?
4. For crops where the answer to questions 1 and 2 is yes, how can stingless bees best be deployed in commercial cropping situations?
5. Can stingless bees be used as effective managed pollinators of key glasshouse crops?

Methodology

WP1. Review of existing knowledge on native bees as alternative pollinators and identification of research gaps

We surveyed the existing literature using a range of online searches to identify: a) current knowledge about the role of Australian native bees in crop pollination, b) native bee species with potential to be further developed as managed pollinators, c) key knowledge and research gap for stingless bees.

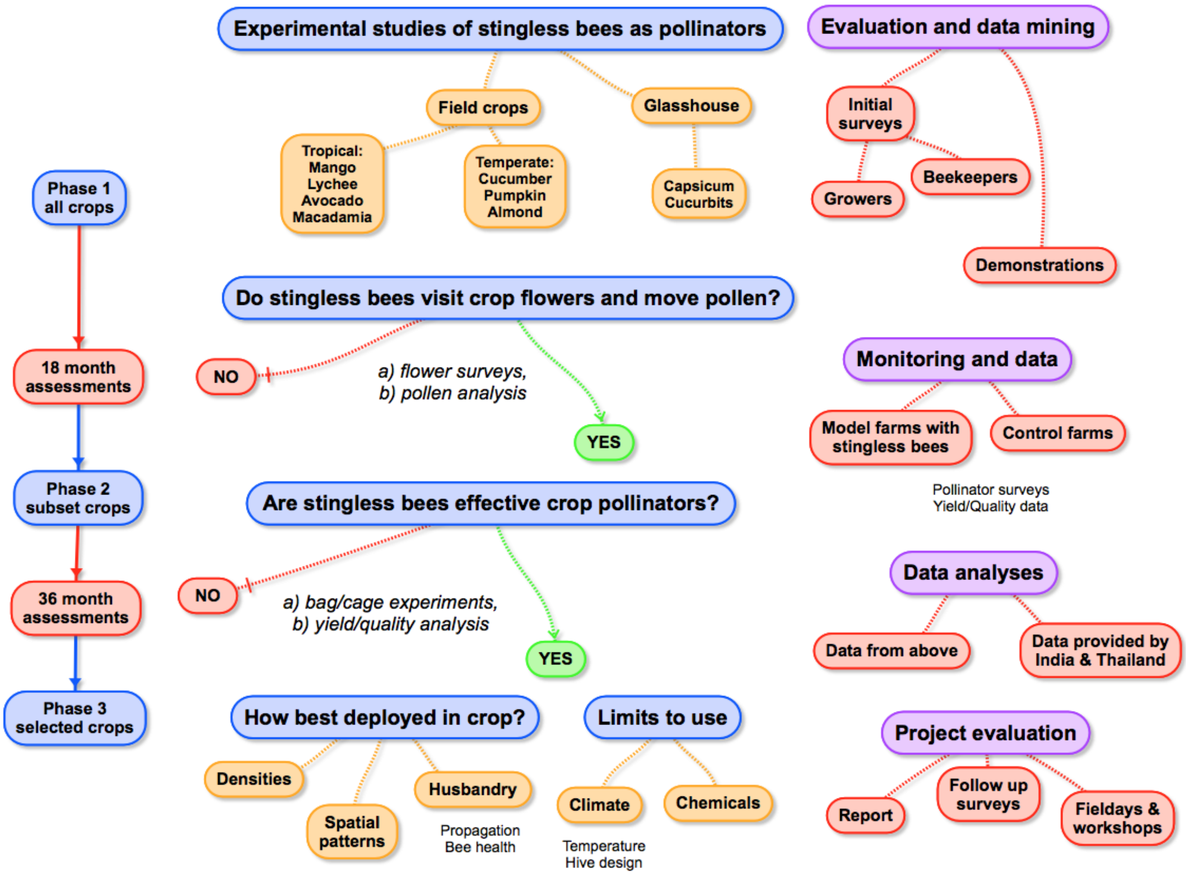


Figure 1. Schematic diagram of the high level "triage" design of the project

Work Packages 2-4. Testing the potential of stingless bees for a range of crops

These activities formed the main body of PH16001 and involved testing the potential of stingless bees as managed pollinators under different cropping conditions – temperate field crops (WP2), tropical orchard fruits and nuts (WP3) and glasshouse cucurbits and berries (WP4). In each case, we adopted a three-phase "triage" approach, with studies only moving to the next stage in the previous one was answered with a clear "yes":

1 – do the bees visit the crop flowers and carry pollen?
2 – are stingless bees effective pollinators of the crop?
3 – how can we optimise deployment of stingless bees in the crop?

In Phase 1, we used field or glasshouse surveys of crops during the flowering period to assess whether stingless bees were visiting the flowers. In most cases, this involved introducing hives at appropriate times and positions, but in NT mangoes we assessed the activity of large numbers of wild stingless bees that were present. Bees were also caught in order to remove pollen from their bodies and test whether this was (at least partly) the target crop pollen. This was done either under the microscope and/or with pollen DNA metabarcoding. Both methods involve matching the pollen (anatomy or DNA sequence) to known reference samples.

In Phase 2, we made more detailed assessment of the bees' ability to be effective pollinators of the target crop. These involved studying the bees "on-flower" behaviour to see if they contacted the requisite parts of the flower to effect pollination, and testing how many pollen grains were deposited on stigmas with single or multiple bee visits, compared to unvisited control flowers.

In Phase 3, we examined several factors that might influence crop pollination by the stingless bees. These included spatial placement of hives relative to competing crops, timing of hive introduction relative to target crop flowering, density of hives, and the impact of recent hive "splitting".

WP2 Temperate Crops

Previous studies have shown Australian stingless bees to be good pollinators of some crops (Heard REF), but with a primary focus on tropical crops, which match well with the natural distribution of stingless bees. Since stingless bees will visit a very wide range of plants to forage, their potential as pollinators of crops in temperate cropping landscapes deserves more attention. There are two key aspects to this: a) which crops will the bees visit and pollinate, and b) how well do the bee colonies survive and perform in regions with climates that differ from those in their natural distributions. In particular, how do they deal with more extreme hot and cold weather than they would typically experience in their natural habitats?

Consequently, we investigated the potential of two Australian stingless bee species, *Tetragonula carbonaria* and *T. hockingsi*, to pollinate cucumber and watermelon crops in both open field and protected cropping (greenhouse or polytunnel) situations in NSW. We studied field dwarf cucumber in Cowra hosted by grower Ed Fagan, and field watermelons at the WSU Farm in Richmond. We established stingless bee hives at the edges and within fields of these crops and then conducted periodic surveys of insect visitation (stingless bees and other insects) to the crop flowers. As stingless bees seemed disinclined to visit cucurbit flowers in open field situations with other floral resources available, we also investigated protected cropping situations with only the crop available.

In addition to surveying insect visitors on the crop, we also monitored the hive weights and activity levels of the hives introduced to farms, and sampled small numbers of returning foragers to characterise the microbiomes of the colonies before, during and after field deployment in the Cowra farm, which is outside the natural range of the bees. In addition, we recorded temperature and humidity at hive sites continuously, using ibuttons.

WP3 Tropical Crops: Griffith University

The project involved experiments on 7 experiments on macadamia, lychee and avocado at 4 sites on the Sunshine Coast and 5 sites in the Bundaberg-Childers region, over 4 years. Key research themes were:

1) How best to managing stingless bees as pollinators in macadamia

2) The foraging behavior of stingless bees on avocado and lychee

3) Crop constancy and foraging behaviour of stingless bees in multi-crop environments.

We used 2 novel genetic approaches, DNA metabarcoding and SABER analysis of crop pollen to investigate these issues.

1. Managing stingless bees as pollinators in macadamia

There were three objectives:

a) Managing stingless beehive strength to increase pollination potential

b) Investigate the potential Impact of hive placement on the type/cultivar of pollen carried by stingless bees. This is a measure of their efficiency as pollinators, as macadamia requires cross pollen from a different cultivar rather than self-pollen to produce a commercial nut crop and improve kernel recovery.

c) Investigating management and maintenance strategies for stingless bees on macadamia farms in non-flowering periods, i.e. alternative food sources for stingless bees on macadamia farms

a) *Managing hive strength -How does "hive splitting" impact foraging behaviour of stingless bees in macadamia farms*
The increased demand for stingless bees has led to an increased need for beekeepers to propagate existing colonies. Hive "splitting" is a commonly used technique by hobbyists and commercial stingless beekeepers to multiply colonies. Splitting involves separating the hive box in two and transferring half of the brood, resource stores (pollen, nectar, resin) and foragers into a new hive box. We investigated how splitting effects both total foraging and individual foraged resources (pollen, nectar, resin) of colonies located in a macadamia plantation in Bundaberg, QLD. We installed 12 hives into a macadamia plantation and selected 6 hives to act as unsplit 'control' hives and split 6 hives to observe the effect of splitting. We counted returning foragers from each hive for 5 min in the morning and afternoon, recording the resource they were carrying (pollen, nectar, or resin). After 2 weeks of observations, we split 6 hives, giving us 12 treatment hives to compare to the 6 unsplit control hives. We then continued counts on all hives until the completion of flowering in the study site (45 days).

b) The impact of hive placement on cross pollen carried by stingless bees in macadamia
Macadamias are known to be highly dependant on cross pollination, i.e. pollen transfer between cultivars, to produce a commercial yield and high kernel recovery. This experiment was conducted to investigate how hive placement in a macadamia orchard affects the type of pollen carried by stingless bees. We placed stingless beehives in a pure block of single macadamia variety and also between the block of two varieties. The experiment was carried out in September 2021, at a Macadamias Australia farm, in Farnsfield, Queensland. The site consisted of two macadamia varieties including var. 741 and var. 816. Three stingless beehives were placed in the pure block of var. 816 and three hives were placed in between block of var. 741 and var. 816 . Ten returning forager bees were collected from each of the 6 hives (total 60 bees). The bees were sampled for 6 days on every 2nd day, over 2 weeks to capture the peak and late flowering season. We used SABER analysis to identify the pollen cultivars carried by returning foragers from each hive location. 12 macadamia cultivars including 741 and 816 were identified in the pollen samples using this method.

c) Alternative food sources for stingless bees on macadamia farms in non-flowering periods
Stingless bees that are maintained permanently on macadamia farms for pollination may not have sufficient diversity of food sources outside of macadamia flowering to sustain them and as a result may not survive in the long term. We investigated the food sources that stingless bees use on macadamia farms and in the neighbouring forest when macadamia is not flowering. A growers guide has been produced to recommend to growers' alternative food sources for bees (Rachele Wilson and Helen Wallace, Supporting stingless bees for pollination of macadamia. Australian Macadamia Society 2022). We sampled four forests and three orchards in Bundaberg and the Sunshine Coast Region. Pollens were sampled seasonally from four hives per site. Hives were split between the base and mid sections to gain access and a representative sample of pollen taken from three open or recently capped "pollen pots". Samples were collected in 1.5mL Eppendorf Safe-Lock Tubes® and stored at -18°C until DNA extraction. In total, 166 mixed pollen samples were collected. Plant sources of pollen samples were identified through pollen DNA meta-barcoding, using 2 markers, ITS2 and rbcL.

2) The foraging behaviour of stingless bees on avocado and lychee

a) Insect visitors to lychee and attractiveness of male and female lychee flowers to stingless bees
Lychee is known to be self-compatible, but lychee yields can be variable. Lychee produces two types of male flowers and one type of female flower at different times during flowering. If stingless bees are potential pollinators of lychee, they need to be frequent flower visitors, and they need to visit both male and female flowers to effectively move pollen from male to female flowers.

We investigated insect visitors to lychee at two sites in Queensland (Sunshine Coast and Bundaberg), and determined whether their behaviour would result in likely pollination (i.e. did insects visit different floral sex phases). We observed bee behaviour on lychee flowers over 3 seasons (2018, 2019, 2020). We counted visitors to lychee flowers for three to four days each year, at each site, during peak flowering. In 2019 and 2020 we observed visitors to different floral sex phases of c.v. Kwai May Pink. Here, observations were made on 12 randomly selected trees: six with panicles in female phase and six with panicles in male phases. Panicles of flowers were determined to be in "male 1" (M1), "female" (F), or "male 2" (M2) phase according to flower morphology.

b) Avocado: Floral phenology and attractiveness of male and female flowers to bees
Avocado has a complex floral biology where the flowers are functionally male and female at different times. The flowers of type A cultivars open as female in the morning and as a male in the afternoon of the next day. Type B cultivars open as

female flowers in the afternoon and male flowers in the morning of the next day. Pollen transfer of either self or cross pollen from male flowers to female flowers is necessary. Stingless bees will need to make contact with both male and female flowers to be effective pollinators of avocado. This study investigated the floral phenology and attractiveness of male and female flowers to insect visitors of Shepard and Hass avocado, the two main cultivars grown in the Australian avocado industry.

The study was conducted at farms on the Sunshine Coast and Childers in the Wide-Bay Burnett growing region, over 3 years on the cultivars Hass and Shepard. Insect visitors to flowers were observed in 2018, 2019 and 2020 during three-time intervals: 1) 0800-1100 hrs, 2) 1100-1400 hrs and 3) 1400-1700 hrs. Avocado flower phenology was recorded in 2020 by recording the number of flowers opening in either male or female phase for both cultivars at five intervals throughout each day from 0800-1000, 1000-1200, 1200-1400, 1400-1600 and 1600-1800h.

3) Managing stingless bees in multi-crop environments

a) Stingless bee constancy to crops and crop preference in macadamia, avocado,

We analysed stingless bee (Tetragonula carbonaria) pollen loads in three crops using DNA metabarcoding to determine crop constancy in macadamia, lychee and avocado during flowering and to examine bee behaviour in a multi-crop environment. Study crops included lychee, macadamia and avocado monocultures at three study sites in the Bundaberg and Sunshine Coast regions and one site at Beerwah with a mixed orchard of lychee, macadamia and avocado with patches of native vegetation. Bees returning to hives were sampled at each of the sites for a total of 4 days per site during peak crop flowering. We extracted DNA from bee-collected pollens, amplified the ITS2 gene region and sequenced this on a MiSeq.

b) How does distance to macadamia affect stingless bee foraging on avocado?

In part A above, we found that stingless bees were loyal to avocado in orchards where this was the only crop available, but in mixed orchards preferred macadamia. This experiment builds on these findings by testing whether the large distances to flowering macadamias can improve stingless bee foraging on avocado.

Our study was conducted in a site consisting of both macadamia and avocado. Five stingless bee hives were placed between macadamia cv. A203 and avocado rows, ("0 m hives"); and five hives were placed next to avocado but more than 200 m from the nearest flowering macadamia cv. A203 rows ("200 m hives)". The closest row of cv. 741 was 500 meters from 0 m hives and 700 meters from 200 m hives. We sampled stingless bees over the flowering period of both macadamia and avocado and identified their pollen loads using DNA metabarcoding.

WP3 Tropical Crops: Western Sydney University

1) The impact of timing of hive deployment on stingless bee crop fidelity

Despite the growing number of studies exploring the potential of stingless bees as pollinators, there is currently a lack of information regarding how to best manage stingless bees to optimise pollinator performance and produce the best outcomes in terms of crop yields and quality. There are often crop-specific guidelines regarding the timing of colony deployment, appropriate stocking rates, and best distribution of colonies in various crops for honey bees, but not for stingless bees. As stingless bees become an increasingly popular alternative pollinator, we need to establish similar guidelines for their effective management and deployment.

We explored how we can deploy colonies of the stingless bee species, *Tetragonula carbonaria*, to maximise pollination service provision by boosting the number of bees foraging on the target macadamia crop. We introduced colonies into orchards at different periods thought the flowering period and looked at the pollen collected by individual colonies each week.

2) The role of stingless bees in mango pollination

Mango research was carried out on Kensington Pride orchards in the Darwin and Katherine mango growing regions of the Northern Territory. We addressed aspects of three broad questions:

1) Do bees visit crop flowers and move pollen?
2) Are they effective pollinators of crops?
3) How are stingless bees best deployed in the crop?

During the 2019 mango flowering season we conducted floral visitor surveys on 6 farms in the Darwin growing region, and two locations in the Katherine growing region. At each farm we conducted 3 diurnal surveys on non-consecutive days during the flowering period. At each farm we surveyed Kensington Pride mango trees at 50 metre intervals from an edge of the crop adjacent to native vegetation, until up to 400m from the crop edge. We also captured mango floral visitors for insect identification and to quantify body pollen levels, and filmed mango panicles to quantify floral visitation rates and average time spent on flowers.

In the 2021 mango flowering season we searched the native vegetation surrounding mango farms in the Darwin and Katherine regions to locate *Tetragonula mellipes* stingless bee colonies and describe the nesting conditions. We also counted the number of pollen grains deposited by floral visitors on the stigma of mango flowers, and conducted a panicle bagging experiment on Kensington Pride mango trees to determine the role of different insect floral visitor treatment groups on fruit set. We had three treatment groups for the bagging experiment:

- Fine mesh panicle bags that excluded all insect visitors from panicles.
- Course mesh panicle bags that excluded pollinators larger than a stingless bee from panicles.
- Unbagged control panicles exposed to unrestricted insect visitation.

We monitored panicles from our 3 treatment groups to determine fruit set at 20, 30 and 45 days post flowering.

WP4 Glasshouse Crops

The use of protected cropping environments (PCEs) in horticulture has increased over recent decades. This is largely because PCEs such as glasshouses allow for more efficient use of key resources (such as growing substrates, nutrients and water), effective pest and disease management, and out-of-season production and supply. However, most fruit crops require pollination to improve yield and quality, yet the enclosed nature of the most sophisticated PCEs, known as controlled environment horticulture, prevents wild pollinators from accessing and pollinating flowers. One option to overcome this limitation is to introduce managed pollinators. Stingless are an excellent pollinator choice for PCEs due to their short foraging distances, inability to sting, large colony size, and rapid orientation to new environments. Many stingless bees are effective pollinators of crops in open field environments, and some are effective crop pollinators in PCEs. For example, it has been demonstrated that *T. carbonaria* and *Austroplebeia australis* can effectively pollinate capsicum crops in glasshouses (Greco et al. 2011) and raspberry crops in polytunnels. However, no study has investigated the pollination potential of stingless bees in glasshouse-grown strawberry crops.

The use of bees for crop pollination in PCEs may also impact colony health. Previous studies have reported that health parameters, such as colony weight, brood production and colony activity rate can decline when bees are deployed in PCEs. However, these health parameters have not been investigated for stingless bee pollination in glasshouse strawberry production. Understanding how bee health is impacted when used for crop pollination is important in ensuring their effective use and management. An important aspect of bee health is their gut microbiome, which contributes to various physiological processes of the host, including digestion and acquisition of nutrients from foods, provision of additional nutrients, detoxification of harmful compounds and protection against pathogens, and may also be important in maintaining bee health and pollination performance, even after their deployment in PCEs.

Here we investigated the pollination potential of *T. carbonaria* and *T. hockingsi* in glasshouse strawberry production, and how the glasshouse environment impacts bee colony performance and health, as well as their gut microbiomes. We first investigated the pollination effectiveness (i.e., the ability of bees to improve fruit yield and quality) of *T. carbonaria* and *T. hockingsi* in glasshouse strawberry production, and how the glasshouse environment impacted hive entrance activity rate and colony weight. We then investigated pollination efficiency (i.e., the number and duration of floral visits needed to achieve effective pollination) of the two stingless bee species, and how their on-flower foraging behaviour affected pollination efficiency. Finally, we investigated how the use of stingless bees for crop pollination in a glasshouse environment with limited floral resources affected their gut bacterial communities by comparing the bacterial community composition and structure before, during and after deployment in the glasshouse environment.

WP5. Understanding existing knowledge and use of stingless bees and translating knowledge

The use of stingless bees in crop pollination services is an emerging industry in Australia with various barriers to adoption and expansion. In addition to expanding knowledge through research (WP1-4), we conducted a range of activities to better understand the status quo for growers and stingless beekeepers, and to provide accessible information to growers through workshops, demonstrations, magazine articles and fact sheets. We also explored the potential of landscape mapping using interpretation of satellite images to reveal links between stingless bee habitats and crop plantings. In addition, a linked activity, funded separately, was to explore and develop links around stingless bees and crop pollination with industry and research partners in India.

Results and discussion

WP1. A review of the potential of native bees as pollinators for Australian horticulture

Below we summarise key findings of our literature survey, while a detailed review is presented in Appendix 1.

a) The role of Australian native bees in crop pollination

Australia has about 2000 native bee species and many of these do or can contribute to crop pollination. Many species have been recorded as visitors to the flowers of various crops, but the further steps of confirming whether they carry and deposit crop pollen and using experiments to test their efficiency in pollinating the target crop, are far less common. Nevertheless, native bees that visit crop flowers can generally be expected to make some contribution to crop pollination, so visitation data alone are a good start. We summarise existing knowledge in Appendix # and this shows that there are now extensive datasets on crop visitation for some major crops, e.g., apple, avocado and mango, but very little for some others, including some emerging industries. It is also clear, especially in a country as large as Australia, that there can be substantial regional variation in crop pollinator communities. In addition, temporal variation within and between years can also be substantial and can only be captured by time series of surveys. Overall, several bee species likely contribute to the pollination of many crops, but some bees may only be important in particular places or at particular times. Nevertheless, the robustness of the cropping system and stability of crop yield and quality will usually require a suite of bee (and other pollinator) species. Despite this, some crops, notably almond, essentially rely on one species, *Apis mellifera*, for pollination services, and this exposes them to high risk in case of decline in availability of managed honeybees due to Varroa or other factors.

b) Native bee species with potential to be further developed as managed pollinators

Australia's bee fauna is unusual in that > 50% of species belong to the family Colletidae, which is a much smaller component of bee faunas on other continents (Michener, 2007). This is significant, because many colletid bees specialise on a few native Australian plants (Slattery et al., 2023), particularly those in the Myrtaceae, and the bees are therefore unlikely to visit horticultural crops, which originated outside Australia and belong to a range of plant families. Moreover, while most types of bee carry pollen on the outside of their bodies, colletids such as *Hylaeus* spp. ingest pollen to carry it, and carry little pollen on their bodies, reducing their potential to pollinate plants. Despite this, the other 50% of Australian bees encompass species from several families and with a range of biologies more conducive to pollination of a various plants. Before considering these further we note that two important groups of crop pollinators in other continents – the genus *Bombus* (bumble bees) and the family Andrenidae – are not native to Australia, and the genus Apis includes only the introduced European honeybee (*A. mellifera*) and, in N. Queensland, the invasive *A. cerana.*

At the family level, Australian bee species are distributed mainly across 5 families: Apidae, Colletidae, Halictidae, Megachilidae, and Stenotritidae. Apidae includes the stingless bees (Meliponinae), which are the only Australian native bees to live in large colonies. The other bees are either solitary or have very small social groups; however, many ground-nesting bees can occur in large aggregations of up to hundreds of nests in favourable nesting locations, such as expanses of sandy earth exposed to a lot of sunlight. A huge advantage of honey bees and stingless bees is their life cycle involving large colonies that can be managed in boxes (hives) that are deployed for crop pollination. In all other bees, a significant challenge is associated with production of large numbers of bees for managed pollination. Nevertheless, this has been successfully negotiated in some cases, e.g., with the widespread use the alfalfa leafcutter bee *Megachile rotundata* in North America (Delaplane & Mayer, 2000) and various *Osmia* species in Asia, North America, and Europe (Bosch et al., 2008). In Australia, there are also promising candidates that could be managed for crop pollination services.

Within their native range, which overlaps with several key areas of horticultural production on mainland Australia, stingless bees are the only well-established alternative managed pollinators to honey bees. Stingless bee species can be kept in artificial hives that are easily propagated and transported, making them a desirable and cost-effective pollinator in terms of rapidly deploying high densities of foragers throughout target crops. They increase foraging activity in response to resource availability and mass flowering (Bartareau, 1996; Wallace, 1999) and are generalists, highly adaptable to non-native crop species, and have been recorded visiting many important crops in Australia. Despite being smaller, they can often deposit similar quantities of pollen to honey bees (Arachchige et al., 2022; Willcox et al., 2019). Stingless bees also take well to enclosed environments such as polytunnels and glasshouses when deployed correctly (Greco et al., 2011; Nacko et al., 2022; Nzie, 2022), and because they do not sting, they are much easier to manage.

Blue-banded bees (*Amegilla* spp.) and carpenter bees (*Xylocopa* spp.) are long-tongued bees that readily visit both native and introduced plants (Houston, 1992; Leijs et al., 2017). These bees display a key behaviour when visiting flowers, known as buzz-pollination. Honey bees do not perform this behaviour, but several horticultural crops require it to release pollen from their (poricidal) anthers; for example crops in the Solanaceae family, like tomato and eggplant. A number of studies suggest that blue-banded bees and carpenter bees are efficient pollinators of tomatoes in glasshouses (Bell et al., 2006; Hogendoorn et al., 2007; Hogendoorn et al., 2006; Hogendoorn et al., 2000). Currently, this pollination is largely done by hand, at great expense to growers due to labour costs. Therefore, blue-banded bees and carpenter bees are interesting candidates for glasshouse pollination of tomato and other glasshouse crops. Both types of bees can be captured in artificial trap-nests, which can then be transported and deployed in glasshouses (Cardale, 1968; Dollin, 2006; Hogendoorn et al., 2000), but more research into glasshouse rearing and management is necessary, especially to move their use up to commercial scale.

A complementary approach is to better manage nesting and floral resources present in agricultural landscapes to increase populations of bee taxa that are efficient crop pollinators. Reed bees (Allodapini spp.) are small, stem-nesting bees that have been observed foraging on many crops, particularly Rosaceous crops, where, despite their small body size, they are capable to depositing many pollen grains on stigmas (Bernauer et al., 2022). Coates et al. (2022) showed that an economically significant density of *Exoneura* reed bees can be achieved when there are sufficient nesting resources in the surrounding landscape. Sweat bees (Halictidae) are short-tongued, ground nesting bees that can aggregate in large densities (Danforth & Ji, 2001; Houston, 2018). They carry large amounts of pollen on their furry undersides and hind legs and can deposit large amount on stigmas whilst foraging (Bernauer et al., 2022). Sweat bees are good candidate pollinators for crops grown in landscapes with very little native vegetation in proximity, as they are considered to be 'open-adapted' and populations can be encouraged by leaving bare areas of soil in undisturbed areas (Arthur et al., 2010; Dollin et al., 2016; Hogendoorn & Keller, 2011) and avoiding tillage. Leafcutter and resin bees (*Megachile* spp.) have not been recorded as floral visitors in many crops in Australia, and when they are it is typically in low numbers, yet numerous studies from overseas in various crops suggest they could be efficient pollinators with better management (Balina et al., 2012; Cane et al., 1996; Hall & Avila, 2016; Koski et al., 2018; Sajjad et al., 2019; Singh, 2016; Stubbs & Drummond, 1996). They nest gregariously and some will use artificial nests such as drilled wood blocks or bamboo reed nests, commonly known as "bee hotels" (Gilpin et al., 2022; Hogendoorn & Keller, 2011, 2012; Prendergast, 2018).

c) Key knowledge gaps for stingless bees

Research on Australian stingless bee biology and pollination performance has focussed heavily on *T. carbonaria*, resulting in large knowledge gaps for the other 5 *Tetragonula* species. For *T. hockingsi*, this is surprising, as its natural range overlaps with many key areas of horticultural production (Dollin et al., 1997; Brito et al., 2014) and is also undergoing artificial expansion, possibly due to climatic or habitat change or anthropogenic hive movements (Cunningham et al., 2014). In addition, many hobbyists and commercial beekeepers keep *T. hockingsi* hives (Halcroft et al., 2013). While many aspects of biology and pollination performance may be similar across different *Tetragonula* species, we should also expect differences (e.g., in foraging behaviour), which may be highly relevant to their use in horticulture.

Limited evidence suggests that *T. hockingsi* may, at least sometimes, be a superior pollinator to *T. carbonaria*, due to slightly larger body size (Dollin et al., 1997) and/or colony size (Heard, 2016). Meanwhile, in a study of the crop fidelity of *T. carbonaria* and *T. hockingsi* in different horticultural crops, *T. carbonaria* appeared to show stronger loyalty to macadamia and lychee pollen than *T. hockingsi*, which showed higher fidelity to strawberry and avocado (Heard et al., 2014). Elsewhere, in the NT, wild colonies of *T. mellipes* appear to be the main pollinators of mango (Singh et al., 2022), but its wider biology and pollination effectiveness has been scarcely researched. Thus, the foraging behaviour and pollinator performance of a range of *Tetragonula* species warrants further research, in order to provide tailored pollination options for specific regions and crops. While research on *Tetragonula* species is biased towards one species, the situation is worse for Australia's other genus of stingless bees, *Austroplebeia*, which has received very little non-taxonomic study at all. Since *Tetragonula* and *Austroplebeia* belong to different major lineages of stingless bees (Heard 2016), they are likely to differ in many aspects of their biology.

There are many crop-specific guidelines regarding factors such as the timing of colony deployment, appropriate stocking rates, and best distribution of colonies, in various crops for honey bees, but there is a lack of evidence-based guidelines for Australian stingless bee species used for pollination. Developing management strategies for stingless bees will enable growers to maximise economic returns from pollinator-dependent crops, but to achieve this requires understanding how colony management strategies impact pollinator performance. Furthermore, management strategies must be crop-specific, for example, the correct density of hives to deploy will depend on factors such as the average number of inflorescences and the number of visits per flower needed to ensure full ovule fertilization (Evans et al., 2021). Best husbandry and management practises may also vary between stingless bee species and more research is necessary to encourage the commercial use of species such as *T. mellipes* and *Austroplebeia* species.

In addition to optimising the delivery of pollination services, management guidelines must also be balanced with methods to ensure the minimisation of harm, or loss of, colonies. It is now increasingly common to hire stingless bee colonies from commercial pollination service providers and deploy them in crops only for the period of crop flowering, as is typical of the way honey bees are managed. Bees used for crop pollination, especially those that are regularly moved between crops, are much more likely to experience stress, pesticide exposure, and reduced diversity and quality of forage plants. This is particularly relevant for colonies used for glasshouse pollination if they are deployed in a monoculture crop for an extended duration. Nzie (2022) found this can lead to a decline in both colony weight and foraging activity. Strong healthy colonies will have a larger active foraging population, which should provide better pollination services. In addition, they be better able to defend themselves against pests, and to withstand stressors associated with agricultural ecosystems, which promotes colony longevity. More research is necessary to establish clear threshold standards that indicate colonies are healthy and strong enough to be used for pollination, i.e., external measures, such as weight and foraging activity rate, and internal measures, such as brood production and signs of pathogens and pests.

Clear management guidelines will help further establish stingless bees as viable alternative pollinators and thus encourage the growth of the stingless bee pollination industry in Australia. A key factor limiting growth of the stingless bee pollination service industry is the number of hives available. Stingless bee hives can only be propagated once they are at a sufficient weight, approximately every one or two years (Heard, 2016) and following hive splitting events they may not perform most effectively as pollinators if they focus workers efforts on hive rebuilding. However, the number of hives will continue to increase in the future with the growth of the industry. Stingless bees are also limited in where they can be used; because they are primarily sub-tropical/tropical in their distribution, they are thermally adapted to forage within specific temperature ranges. For example, *T. carbonaria* does not forage below approximately 18°C (Heard & Hendrikz, 1993). Species with more constrained distribution ranges are likely to be even more limited by climate conditions, more research into the optimal foraging conditions for these species would be beneficial. Outside of their natural ranges, in regions such as Victoria, Sough Australia and much of West Australia, they could have a very restricted daily and seasonal active foraging period, which would be detrimental to the provision of pollination services, and furthermore, they would most likely not survive the winter. So, research efforts should focus on alternative pollinators in these regions.

WP2-4. An overview of trials of stingless bees in a range of cropping situations

As expected, we found that stingless bees were promising pollinators in some cropping situations but not in others (Table 1). Briefly, we found that stingless bees did not visit certain crops at all (or only in trivial numbers) due to lack of attraction to the crop or unsuitable climate during the flowering period. Where bees did visit the crop, they tended to carry and deposit pollen effectively and we thus proceeded to study effective deployment in several cases.

Trials	**Tropical**	**Temperate**	**Glasshouse**
Phase 1 *Bee visits*	Lychee - Yes Mango - Yes Avocado - Yes Macadamia - Yes	Dwarf cucumber – No (choice) Watermelon – No (choice) Almond – No (climate) Apple - Yes	Cucurbits – No (choice) Strawberry - Yes Capsicum - Yes
Phase 2 *Good pollination*	Lychee - Yes Mango - Yes Macadamia - Yes Avocado - Sometimes	Apple - Yes	Strawberry - Yes Capsicum - Yes

Phase 3 Deployment trial topics	- Lychee – competing crops, hive splits - Avocado – competing crops, hive splits - Macadamia - timing of hive deployment, competing crops, hive splits - Strawberry (glasshouse) – comparing bee species, numbers of visits, hive numbers

Table 1. Summary of results of studies of stingless bees in a range of crops and cropping situations. Traffic light colouring indicates cases where the bees are generally a good option (green), can be a good option (orange), or do not appear to offer good pollination options (red).

WP2 Temperate Crops: WSU Report

Cucurbits

Overall, we conducted 229 surveys of cucumber flowers and 144 surveys of watermelon flowers in field experiments where *T. carbonaria* colonies had been deployed, but no stingless bees at all were recorded visiting cucumber flowers, and only one was recorded visiting watermelon flowers. We observed that visitor assemblages in both crops were dominated by the introduced honey bee, *Apis mellifera*. However, on watermelon, other native bees were abundant in the form of ground-nesting halictid bees that made up about 30% of all visits.

As stingless bees seemed disinclined to visit cucurbit flowers in open field situations with other floral resources available, we also investigated protected cropping situations with only the crop available. In a no-choice experiment, stingless bee (*T. carbonaria* and *T. hockingsi*) visits to watermelon flowers in a polytunnel began to occur after four days but were low in number at first. Meanwhile, in a glasshouse experiment with different cucumber cultivars, visits to cucumber flowers occurred only after 22 days and resulted in poor fruit set. In fact, *T. hockingsi* bees more readily collected fungal spores than pollen from cucumbers. Overall, our results indicate that *T. carbonaria* and *T. hockingsi* are unlikely to be major contributors to the pollination of cucumber and watermelon, but other native flower visitors, notably halictid bees, may be important pollinators of these crops.

Since we completed our cucurbit studies (Nacko et al. 2022), a paper has been published (Subasinghe Arachchige 2022) that reports higher visitation of watermelon flowers by stingless bees in Queensland and the NT. Although these were wild bees and multiple species must be involved, they may well include *T. hockingsi* in Queensland. Stingless bees were the main native bees contributing at sites in south and central Queensland, while halictid bees were more numerous (as in our study in NSW) in the NT, northern QLD and Victoria. On balance, we suggest that further studies of stingless bees on melons are warranted in subtropical and tropical locations, while encouragement of ground-nesting halictid bees may be beneficial in most areas.

Stingless Bee Colony Performance in Cowra Cucumber Fields

We deployed nine managed hives of *Tetragonula carbonaria* for cucumber pollination experiments (see above) in Cowra (NSW), which is outside the bee's natural range. The climate in Cowra involves greater extremes of both hot and cold than would normally be experienced by these bees in the wild in their natural habitats in more coastal regions. After about 2 months of deployment, there was a heatwave of four consecutive days above 40 °C, and after this one colony was much weakened with few foragers. Consequently, this hive was removed from the site and returned to WSU campus for the colony to recover. After a second heat wave, another colony was found weakened and proved to be infested with small hive beetle (SHB), *Aethina tumida*, a well-known and damaging pest of honey bees. Inside the hive, we found 14 adult beetles and 133 larvae. In this case, extreme daily maximum temperatures and low humidity appear to have weakened the colony and made it vulnerable to SHB infestation, despite the generally strong defences of stingless bee hives (Halcroft REF; LeGros et al. 2022). This is the first documentation of infestation by SHB in a queenright colony of *T. carbonaria* and highlights the need for careful placement of managed hives, as SHB has potential to be a significant pest of stingless bees under adverse conditions.

It is now well known that maintaining beneficial interactions with microbial symbionts is vital for human and animal health. Yet, for social insects, the temporal stability of microbial associations in colonies is largely unknown. We therefore investigated temporal changes in the microbiomes of nine *T. carbonaria* colonies at seven timepoints across a 10-month period when moved between two climatically and florally different sites – the WSU campus in Richmond NSW and a cucumber farm in Cowra NSW. DNA metabarcoding of the bacterial 16S rRNA gene and fungal ITS amplicon showed that microbiomes varied considerably between colonies initially in Richmond, before deployment on the cucumber farm. However, following relocation to Cowra, there were considerable changes in each colony, and the microbiome composition became more similar across colonies. Notably, *Snodgrassella* disappeared and *Zymobacter* appeared as major components of the microbiome. Remarkably, bacterial microbiome within colonies continued to shift over time but remained similar across colonies, becoming dominated by *Acinetobacter* six months after returning to the original site. Our results indicate that the stingless bee microbiome can undergo major and quite rapid changes in response to the environment, and that these changes can be long-lasting. Such legacy effects have not been reported for corbiculate bees. Further understanding the microbial ecology of stingless bees should aid future management of colonies used in agricultural production.

Other temperate crops

Two major Australian temperate crops (almond, apple) rely heavily on honeybees for pollination. In the case of almond, managed honeybees are essentially the only pollinators and there is a huge demand for hives during almond flowering. Given this, it would be of great value to find alternative managed pollinators to complement the honeybees, and stingless bees are the only other managed pollinators currently available in large numbers in Australia. However, almond flowers in late winter (August) when temperatures are often low, while *T. carbonaria* only shows significant flight activity above about 18^{0}C. Despite this, we identified one almond grower in Victoria who was keen to trial stingless bees and obtained a few hives. One of our team visited the orchard and confirmed that, under suitable weather conditions, *T. carbonaria* will readily visit almond flowers (as they do apple flowers). However, suitable weather conditions (primarily temperature, but also wind and cloud cover) appear to only occur about 1-2 days within an almond flowering season in the Robinvale area, so there seems to be little scope for stingless bees unless other species are found that forage at lower temperatures. Given that they naturally occur further inland, where they are more likely to deal with cold conditions, and any further studies might be better directed at *Austroplebeia* than *Tetragonula* stingless bee species.

Apple was a major focus of PH15001, and we collected 3 years of data at apple orchards in Bilpin, NSW. This is within, but close to the edge, of the natural range of *T. carbonaria.* We found that two bee species - *T. carbonaria and A. mellifera* – consistently made up 90% or more of all crop flower visitors (Tierney et al. 2023) and both carried and deposited apple pollen well (Bernauer et al. 2022). Indeed, in one year, *T. carbonaria* was the most abundant visitor across all surveys and it was often the most abundant visitor on a given day. However, there was also a strong signal of temperature, with the stingless bee being relatively rare in surveys with ambient temperature below 20^{0}C. Stingless bees do not occur naturally in most other Australian apple production areas, but our results suggest they might be considered as additional managed pollinators if honeybees are limiting and weather conditions are often suitable for their activity.

WP3 Tropical Crops: Griffith University Report

1. Managing stingless bees as pollinators in macadamia

Managing hive strength -How does "hive splitting" impact the foraging behaviour of stingless bees in macadamia farms

We found the total amount of returning foragers in split hives reduced to less than a third of unsplit hives for 16 days, while returning nectar and pollen foragers reduced to less than a quarter of unsplit hives for 9 days following splitting. From day 15 to 23 of the experiment, split hives had less than half of the total foragers seen in unsplit control hives. Further, from day 37 until the end of the experiment, total foraging was still significantly lower in split hives, indicating that split hives had still not recovered from the splitting event. Split hives showed a dramatic drop in pollen foraging numbers than unsplit hives, from immediately after splitting (day 15) until the end of the experiment (day 45). Our results imply that after a split, stingless bees focus on repairing and defending the hive and allocate minimal effort towards foraging on floral resources for a significant period after splitting. This has implications for crop pollination and in turn crop yields, as split hives are unlikely to be effectively pollinating crops. For this reason, splitting should be carefully planned and executed well before (>31days), or after the macadamia or other crop flowering period for the best chance of successful pollination.

a) The impact of hive placement on cross pollen carried by stingless bees in macadamia

SABER analysis results showed that most of the stingless bees carried single-cultivar pollen in both the mixed-block and pure-block of macadamia. This indicates that most foraging trips are unlikely to result in transfer of the cross pollen that macadamia requires to produce a commercial yield and quality. Approximately 21-43 % of bees carried pollen that was neither of the two expected cultivars. This hidden variability is likely to be a major source of cross pollen in both mixed and pure blocks. We conducted further surveys of the orchard and found many seedling rootstocks and trees that did not resemble the surrounding cultivars that could be sources of this unknown pollen. The effects of these sources of cross pollen on kernel recovery is unknown, and likely to be variable.

3) Alternative food sources for stingless bees on macadamia farms in non-flowering periods

Our results show that stingless bee colonies in macadamia farms forage on many species other than macadamia located within their foraging range when given the opportunity. We identified 341 plant sources of stingless bee hive-pollens from 37 orders, 72 families, 218 genera and 302 species total from both orchards and forests. Macadamia spp. (Proteaceae) constitute between ~50% and 75% of pollen diets in spring, decreasing each season thereafter. In summer, Glochidion spp. (Euphorbiaceae), Corymbia spp. (Myrtaceae) and Cannabaceae (Cannabis sativa and/or Celtis paniculata) source plants increase in abundance. The highest diversity is observed in autumn, when Eucalyptus spp and Melaleuca spp. (Myrtaceae) are targeted. Finally, in winter, the native but also ornamentally planted Xanthostemon chrysanthus or “Golden Penda” (Myrtaceae) constitutes much of the pollen stores along with the introduced weeds Raphanus raphanistrum (Brassicaceae) and Ageratum spp. (Asteraceae), which are heavily utilised throughout the year.

Flowering trees are the most important source of food and nest materials for stingless bees, particularly if they exude resin, as colonies need plant resins to make structures within the hive. Stingless bees in our study foraged on many small rather than few large pollen sources regardless of the land use type they are located in. Stingless bees seemingly aim for diversity in their pollen diets, thus a variety of floral sources is required to sustain stingless bees outside of macadamia flowering season.

2) The foraging behaviour of stingless bees on avocado and lychee

a) Insect visitors to lychee and attractiveness of male and female lychee flowers to stingless bees

Honeybees, stingless bees, solitary bees (mostly Homalictus sp.) and other insects visited all lychee cultivars studied in all years. Stingless bees were the most frequent visitor to cultivars at all sites in all years on the Sunshine Coast, except in Mooloolah Valley in the second year. In contrast in Bundaberg, stingless bees were the least observed visitors, and honeybees were frequent visitors. The increased presence of stingless bee at the Sunshine Coast is thought to be driven by the presence of native forest within foraging range of the study sites. Native forest provides nesting sites for wild colonies and diversity of floral resources for stingless bees.

Stingless bees were observed to visit lychee flowers in all three sex phases, and to collect both nectar and pollen, although preferences varied between each year and site. Thus, they show potential as pollinators in lychee farms.

b) *Avocado: Floral phenology and attractiveness of male and female flowers to bees*

Hass flowers opened in the morning in female phase, peaking in abundance in the morning (0800-1000) and were all closed by 1200-1400. Male flowers began opening between 1000-1200 and panicles had both male and female open flowers during this time period. Shepard flowers remained in male phase from early morning (0800-1000), throughout the day until the afternoon where flowers began to open as female and remained as female (1600-1800). Shepard panicles had both male and female flowers between 1600-1800 h (Fig 2).

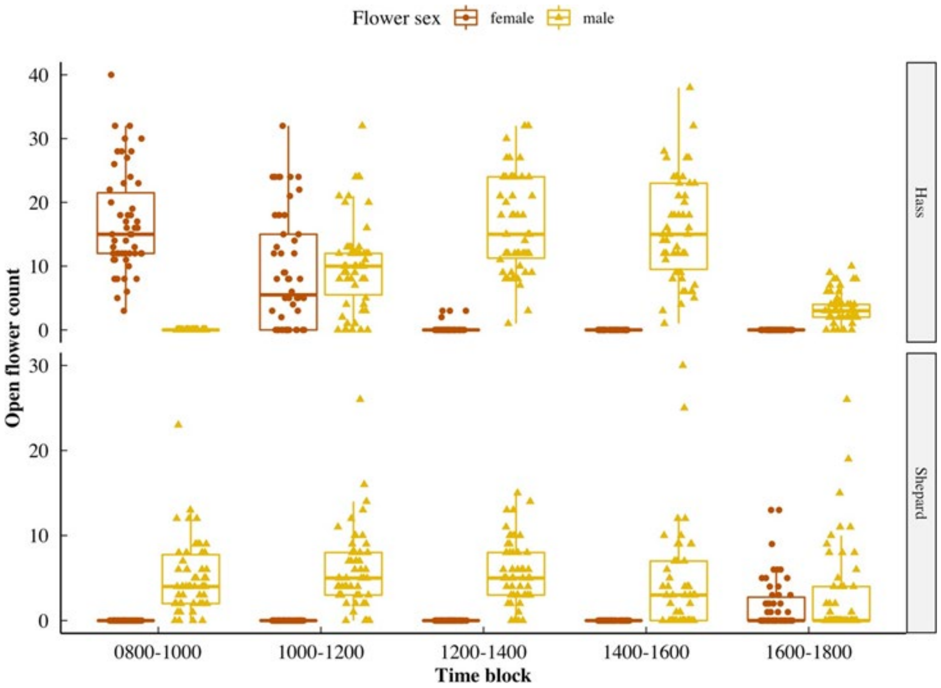


Figure 2. Number of open male and female Hass and Shepard avocado flowers at time intervals throughout the day.

We also observed stingless bees visiting avocado flowers in both Hass and Shepard trees demonstrating that stingless bees have potential as pollinators of avocado flowers. However stingless bee numbers were very low at some sites. Stingless bees and honeybees visited male flowers more than female flowers for both cultivars. Since bees are more attracted to male phase flowers, peak pollination is likely to occur during these crossover periods when both male and female flowers are open on the same panicle (Hass:10:00 -12:00, Shepard: 16:00-18:00).

3) Managing stingless bees in multi-crop environments

a) Stingless bee constancy to crops and crop preference in macadamia, avocado,

We identified 37 plant taxa in the pollen loads of returning stingless bee foragers in lychee (25 taxa), macadamia (17 taxa) and avocado (19 taxa) orchards. Crops pollens had the highest mean proportions in each orchard, complimented by ornamental shrubs and small trees like Viburnum sp. (Adoxaceae), Elaeocarpus sp. (Elaeocarpaceae) and Bauhinia × blakeana (Fabaceae), and introduced groundcover species such as clover (Trifolium sp., Fabaceae), indian weed (Sigesbeckia orientalis, Asteraceae) and panicgrass (Panicum sp., Poaceae).

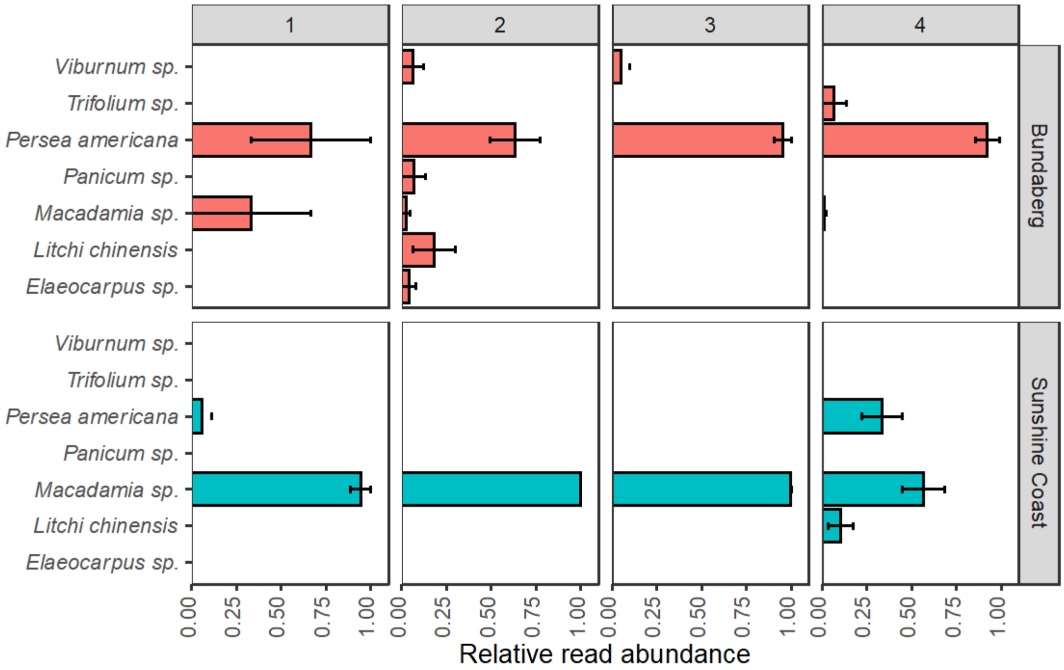


Figure 3. Plant sources of returning stingless bee forager pollen loads in avocado by day of sampling. Data are mean relative read abundance expressed as a proportion, excluding low abundance species (<5% of 19 taxa identified).

Our results show stingless bees show very high crop constancy to macadamia and a strong preference for lychee during lychee flowering. Stingless bees foraged almost exclusively on avocado where there were no other crops flowering nearby, however, when given a choice of avocado and macadamia, showed a strong preference for macadamia (Fig 3). This has implications for managing stingless bees as pollinators in avocado orchards when there are flowering macadamias nearby, as stingless bees are likely to forage on macadamia.

b) How does distance to macadamia affect stingless bee foraging on avocado?

Our results show that the type of pollen carried by stingless bees (macadamia, avocado, non-crop pollen) was strongly dependent on the distance of hives from macadamia orchard (Fig. 3). The bees foraged predominantly on macadamia when the hives were closer to macadamia. Initially, bees at the 200m site foraged mostly on non-crops when the hives were further away from macadamia however, the stingless bees shifted to foraging on macadamia when the nearby cultivar A203 started to flower (Fig. 4. Few bees foraged on avocados even though avocado flowers were available during the whole experimental period. These results suggests that stingless bees have clear preference on macadamia over avocado.

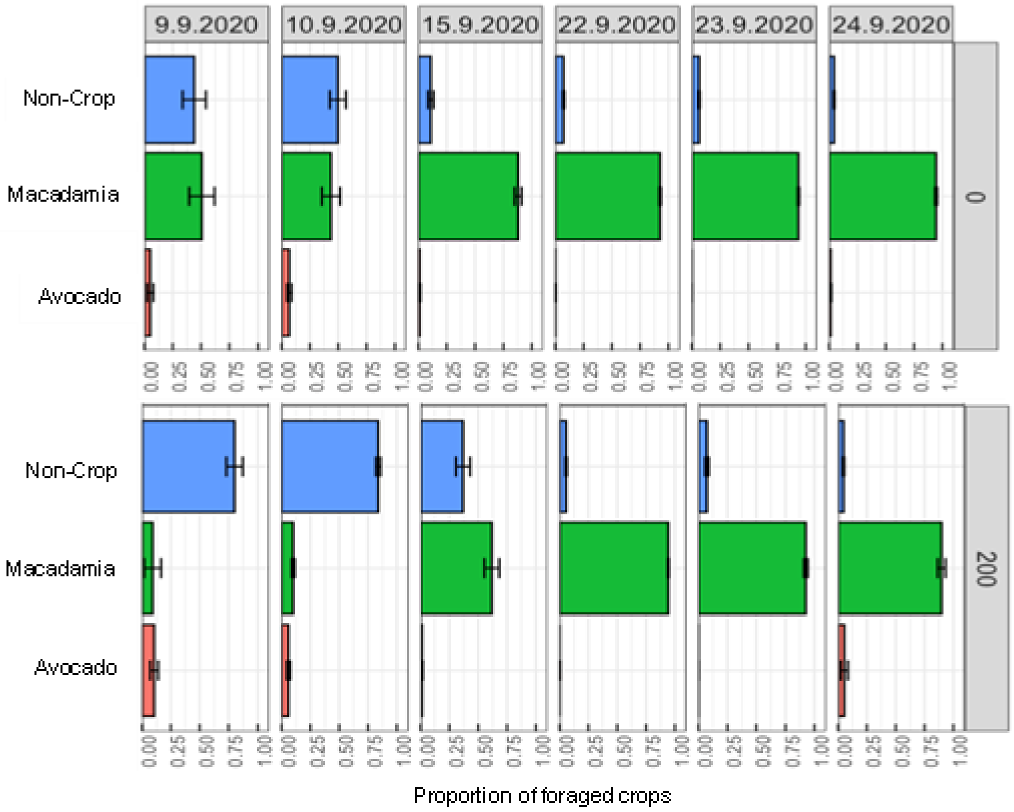


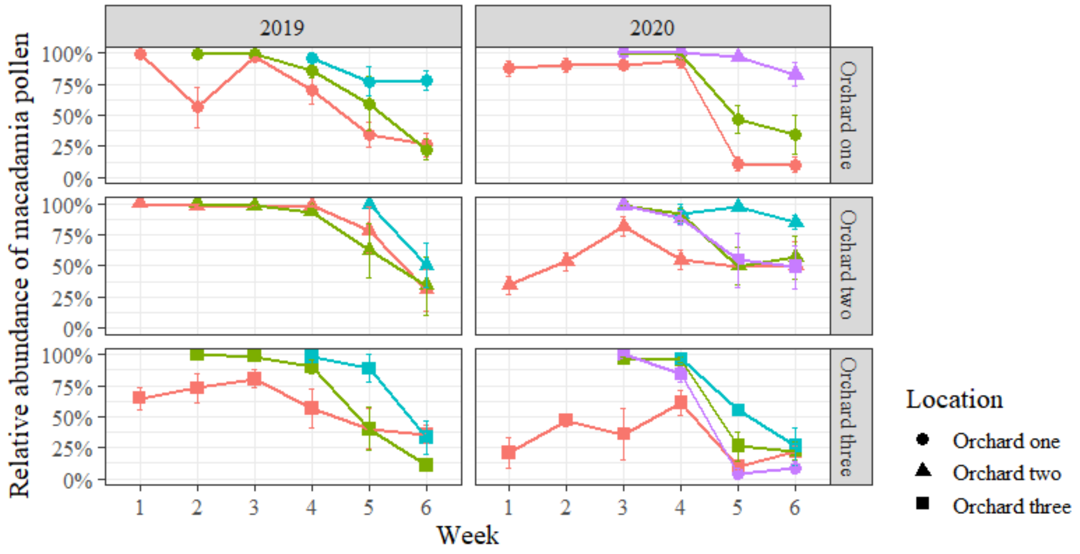
Figure 4: Type of pollen carried by stingless bees (proportion of macadamia, avocado or non-crop pollen) at site "0" (between macadamia and avocado) and "200" (in avocado 200m from macadamia) at 6 different dates during the flowering season of both crops. Peak flowering of macadamia occurs on 23.9.2020

WP3 Tropical Crops: Western Sydney University Report

The impact of timing of hive deployment on stingless bee macadamia crop fidelity

Despite the growing number of studies exploring the potential of stingless bees as pollinators, there is currently a lack of information regarding how to best manage stingless bees to optimise pollinator performance and produce the best outcomes in terms of crop yields and quality. We found that deploying colonies into orchards when macadamia was already in flower increased forager fidelity to the crop compared to colonies located in the orchard prior to flowering, indicating that sequential introduction of colonies into orchards could enhance the number of foragers on the target crop and increase the transfer of conspecific pollen (Fig. 5). Additionally, by looking at colony foraging rate and resource use we determined that the proportion of pollen foragers increased as diversity of pollen species they collected increased. This suggests that stingless bees will allocate a larger percentage of their foraging population to pollen collection in order to maximise the diversity of resources that they collect.

(a) Microscopic pollen counts



(b) ITS2 amplicon reads

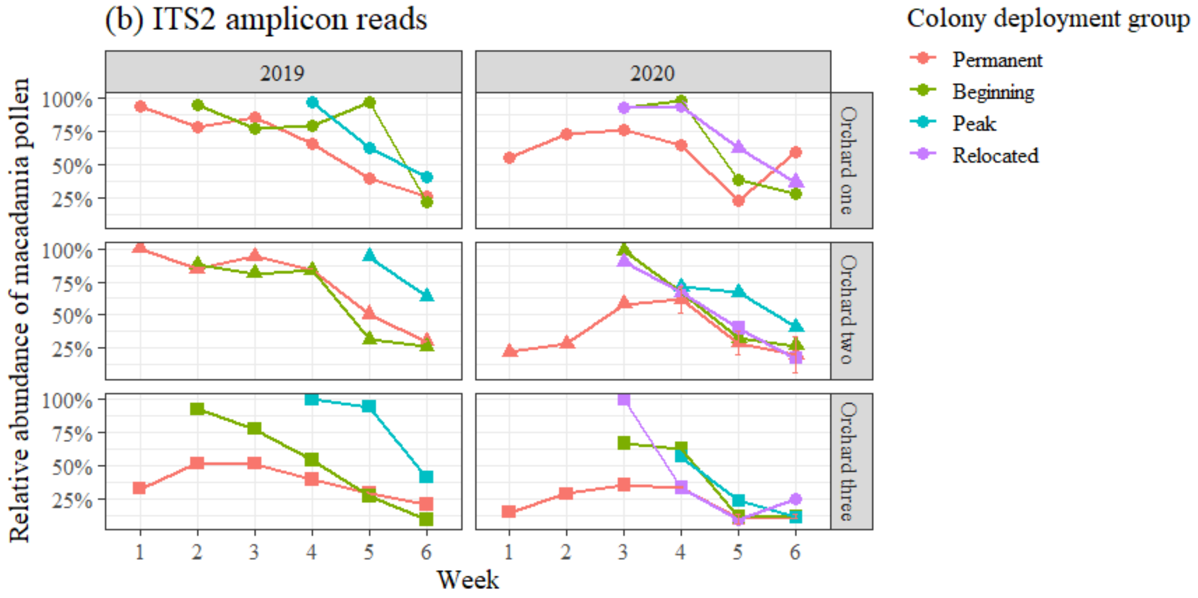


Figure 5. Mean values (±SE) of the relative abundance of macadamia pollen, based on the relative abundance of pollen grains identified as macadamia by light microscopy (a) and the relative abundance of reads identified as macadamia by ITS2 metabarcoding (b) per week of the macadamia flowering period, for each year of the experiment across the three orchards. In the case of the relocated colonies, the plot is faceted by the location the colonies are originally from, and symbols indicate which orchard colonies were located in. Orchard one (●), Orchard two (▲) and Orchard three (■).

We then explored how we can ensure that *T. carbonaria* colonies are in good condition when using them for crop pollination, by looking at how colonies of different strengths varied in their diurnal foraging patterns and responded to changes in climatic conditions. Foragers exhibited clear diurnal foraging patterns of nectar and pollen collection, regardless of colony strength, but colonies of different strength were differentially affected by climate variables. In particular, a higher proportion of the total forager population was assigned to pollen collection in stronger colonies. We found that hive weight, a commonly used measure of colony strength, did not necessarily predict foraging activity of colonies and suggest that better assessment of colony strength prior to pollination events should incorporate multiple metrics, such as both foraging rate and hive weight (Fig. 6).

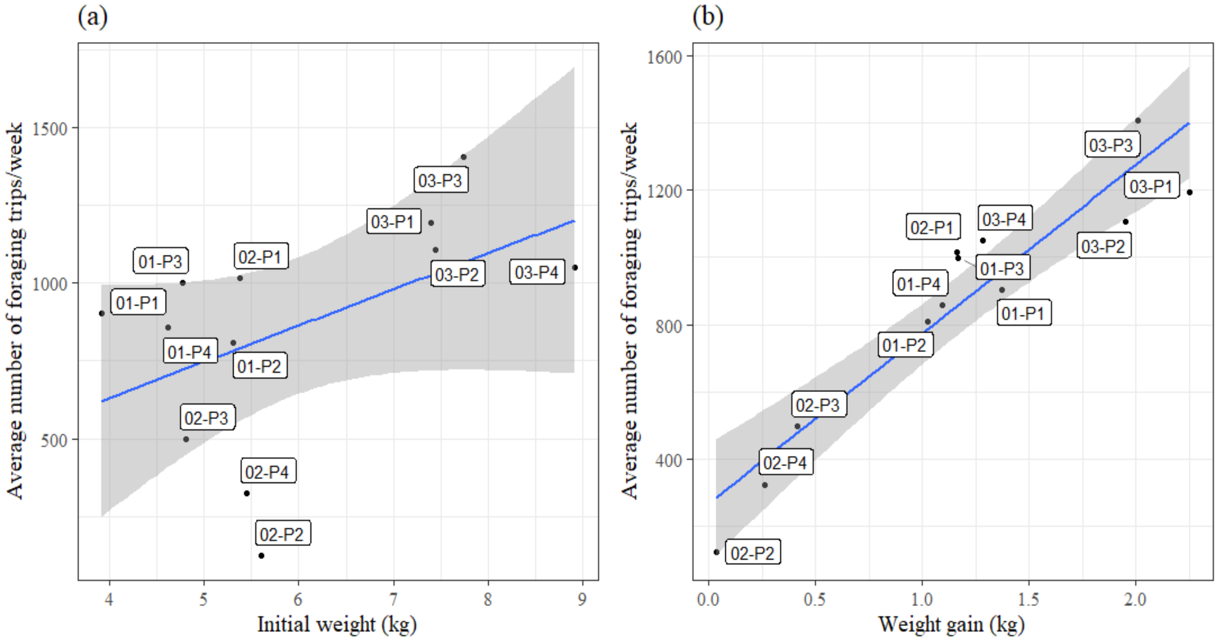


Figure 6. The relationship between average weekly foraging rate and initial weight (a) and weight gained (b) over the course of the macadamia mass flowering event.

Mango

Do bees visit crop flowers and move pollen?

In 2019 we recorded 7,605 insect visits to Kensington Pride mango flowers from 148 insect species during 12,960 minutes of diurnal floral visitor surveys. The stingless bee species *Tetragonula mellipes* was the most common floral visitor in both Darwin and Katherine farms, being found at 6 out of 8 study locations and making up about half of all insect visits (46% and 58% in Darwin and Katherine respectively) (Figure 7a-b). The hoverfly species *Mesembrius bengalensis* was the second most abundant floral visitor in Darwin farms, making up 19% of visits (Figure 7a). In contrast, hoverflies were entirely absent from the two Katherine locations, and instead ants were the 2nd most common floral visitors, making up 26% of visits. *Braunsapis* and *Homalictus* bees, as well as *Chrysomya* and *Eristalinus* flies, were also frequently observed visiting mango flowers (Figure 1a-c). Interestingly, remarkably few honey bees (*A. mellifera*) were observed visiting mango flowers on any of the surveyed farms (only 7 visits, 0.09%).

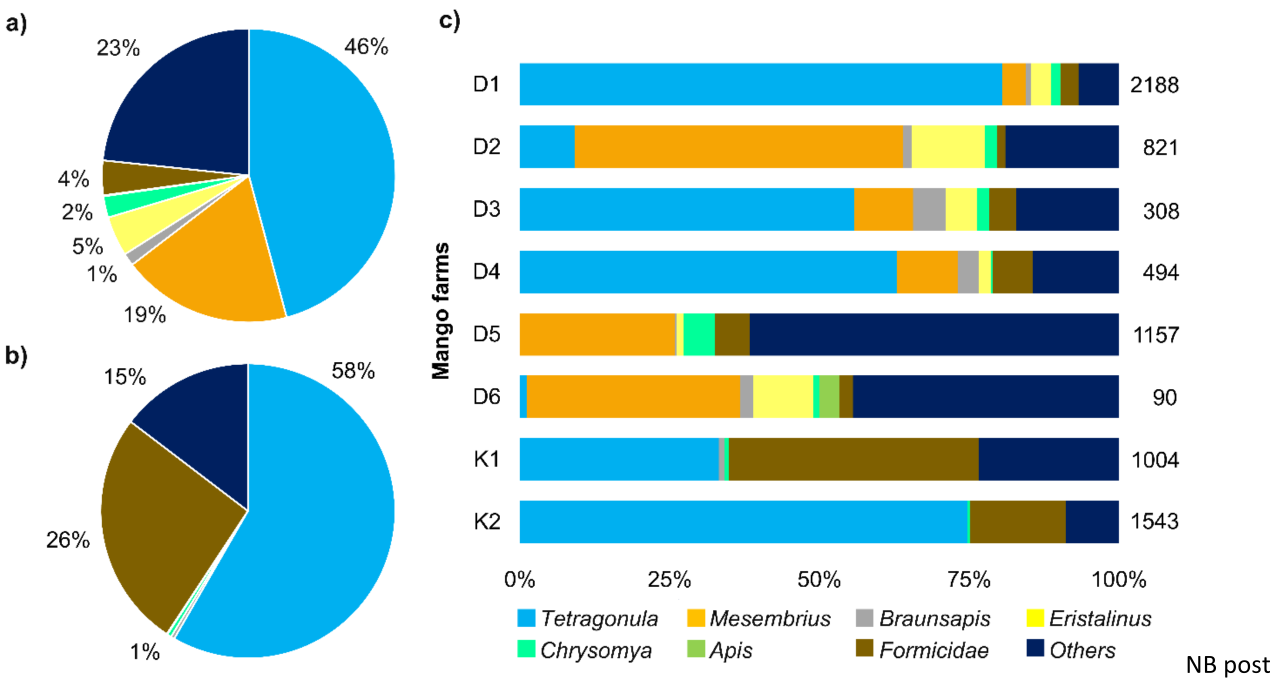


Figure 7: Relative abundance of diurnal Kensington Pride mango floral visitors in Darwin (a) and Katherine (b) mango farms. Figure 1c shows the relative and total abundance (numbers on the right of the bar) of dominant floral visitors in each study site (n=8). D1-6 denote Darwin farms, while K1-2 denote Katherine locations. Total abundance is not directly comparable between farms as the number of different 50m interval survey points varied with farm size (see methods).

We noticed a striking difference in the spatial distribution of different pollinators within the orchards, with around half of the stingless bee visitations recorded on the edge trees alone and no stingless bees recorded beyond 300m into the crop In strong contrast to the stingless bees, fly species such as hoverflies (Syrphidae) and blowflies (Calliphoridae) were fairly evenly distributed over all the distances from the crop edge.

Loose body pollen analysis of floral visitors collected in 2019 and 2021 revealed that *T. mellipes* stingless bees and honey bees, *Apis mellifera,* carried the significantly more loose mango pollen grains than other mango floral visitors ($\bar{x}$ ± S.E. = 803.2 ± 61.2, n=72 and 668.5 ± 173.3, n=6).

Our results from 2019 demonstrate that the stingless bee *Tetragonula mellipes* is the most frequent floral visitor on NT mango farms, and also carries the most pollen on its body, demonstrating that they do indeed visit crop flowers and move pollen around. One factor limiting their effectiveness as a pollinator in mango orchards though is the fact that the majority of wild *T. mellipes* crop visitation occurs on edge trees in the block, and no stingless bees were found greater than 300m into the block.

Are they effective pollinators of crops?

In 2021 we counted mango pollen grains on a subset of Kensington Pride mango flower stigmas visited by the two most common floral visitors in 2019, *T. mellipes* and *M. bengalensis*, as well as unvisited control stigmas. *T. mellipes* deposited significantly more mango pollen grains per visit (mean ± S.E= 3.14±0.49, n=128) compared to *M. bengalensis* (0.56±0.20, n=25) and the unvisited control stigmas (0.29±0.09, n=179).

In our 2021 panicle bagging experiment we found that stingless bee *T. mellipes* was the most frequent flower visitor on unbagged open panicles (71.1% visits, n=864), followed by the hoverfly *M. bengalensis* (11.6%, n=141) and blowflies (*Chrysomya* spp.) (2.9%, n=35). No larger insects such as hoverflies or blowflies could enter our coarse mesh bags, and we found *T. mellipes* to make up the overwhelming majority of insect visitors to coarse mesh bagged panicles (96.1% visits, n=447) with rare visits from ants (2.4%, n=11), *Braunsapis* bees (0.7%, n=3) and other insects (0.7%, n=3). No floral visitors were recorded visiting panicles bagged with fine mesh bags.

We recorded fruit set in our 3 panicle treatment groups at 20, 30 and 45 days after bagging. The number of fruits per panicle at 20 days was significantly higher in open panicles (x̅ ± S.E. = 21.9 ± 1.4 fruits) compared to panicles bagged with a fine mesh (7.0 ± 0.6 fruits); however, it was similar to panicles bagged with a coarse mesh (16.7 ± 1.5 fruits) (Figure 8a). Similarly, the number of fruits on open panicles and coarse mesh bagged panicles at 30 and 45 days were not significantly different, but were significantly higher than panicles bagged with a fine mesh (Figure 8 b and c). KP mango also produces a high number of fruits without seeds, called nubbins, which usually abort before maturity. The proportion of nubbins was significantly higher in panicles bagged with a fine mesh (0.57 ± 0.05, n=90) compared to open panicles (0.36 ± 0.04, n=89 panicles) and coarse mesh bagged panicles (0.38 ± 0.04, n=89) (Figure 8d).

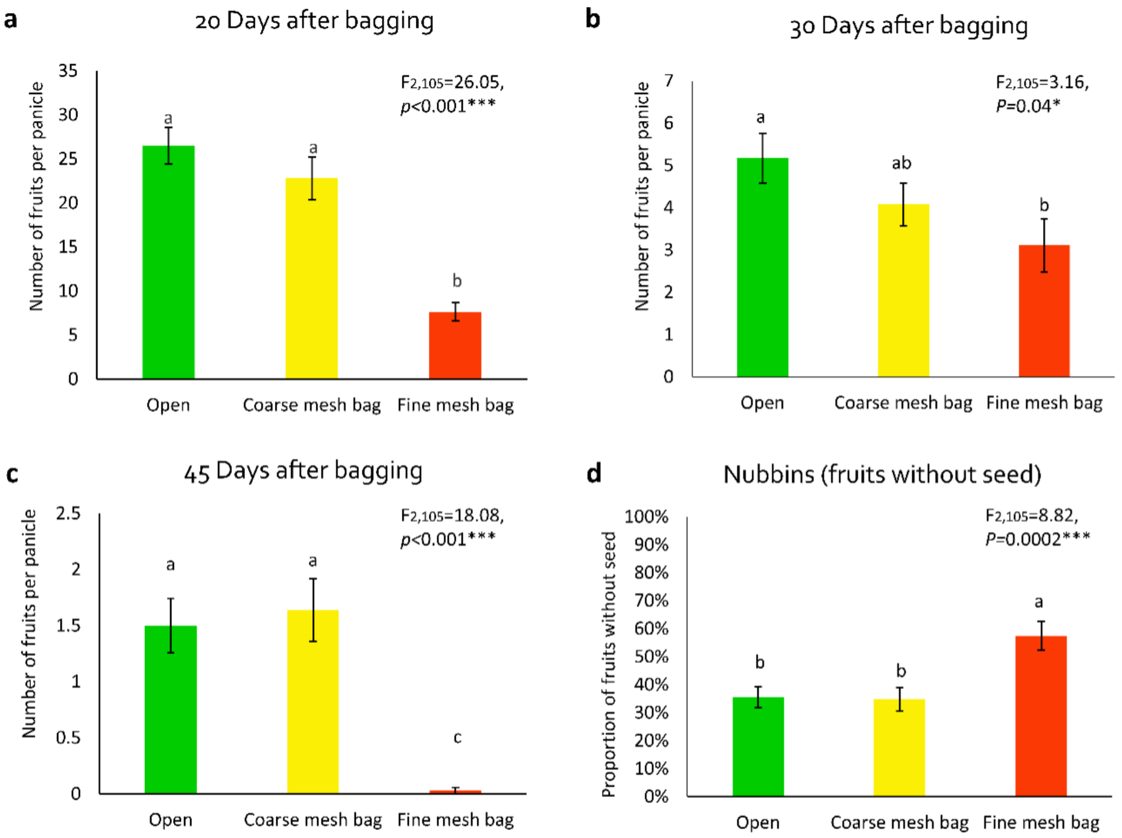


Figure 8. Fruit set on tagged Kensington Pride mango panicles at 20 days after bagging (a), 30 days after bagging (b), and 45 days after bagging (c). Figure (d) shows the formation of nubbins in different exclusion treatments.

Taken together, our single visit pollen deposition and panicle bagging experiments demonstrate that the stingless bee *Tetragonula mellipes* is effectively pollinating Kensington Pride mango flowers. This holds true even when they are essentially the sole floral visitor to the mango flowers, as seen the course mesh bagging experiment.

How are stingless bees best deployed in the crop?

The stingless bee *Tetragonula mellipes* is not commonly kept in managed hives in the Northern Territory. For this component of our mango research, we therefore instead searched for wild colonies near mango orchards. In 2021, 50 *Tetragonula mellipes* colonies were found nesting up to 460m from the crop in bushland adjacent to mango orchards. Of these 50 colonies, 37 (74%) were found nesting in Ironwood (*Erythrophleum chlorostachys*), 7 (14%) in Darwin woollybutt (*Eucalyptus miniata*), and the remaining 6 (12%) in a range of other tree species. All trees containing *Tetragonula* hives displayed active or past termite damage, with 22 (44%) of colonies nesting within the termite mound itself. Mango pollen was found in the entrances of all but 4 of the colonies, up to the furthest distance of 460m from the crop.

Our 2021 colony survey identified the nesting requirements of wild *Tetragonula mellipes* colonies around Darwin farms, and illustrated the importance of mature stands of native trees. Despite being the predominant floral visitor in Darwin and Katherine mango farms, stingless bees are only found up to 300m into the crop, and little work has been undertaken to manage the species in hive boxes for pollination services. Our results therefore highlight the need for further work on how to manage this bee species and effectively deploy it in crops for improved pollination services.

WP4 Stingless bees as glasshouse pollinators

Overall, our results suggest that stingless bees provide an excellent option for pollination of strawberries in glasshouses. We found that pollination by stingless bees substantially improved strawberry yield and quality when compared to flowers that had been bagged to prevent bee visitation (Fig. 9).

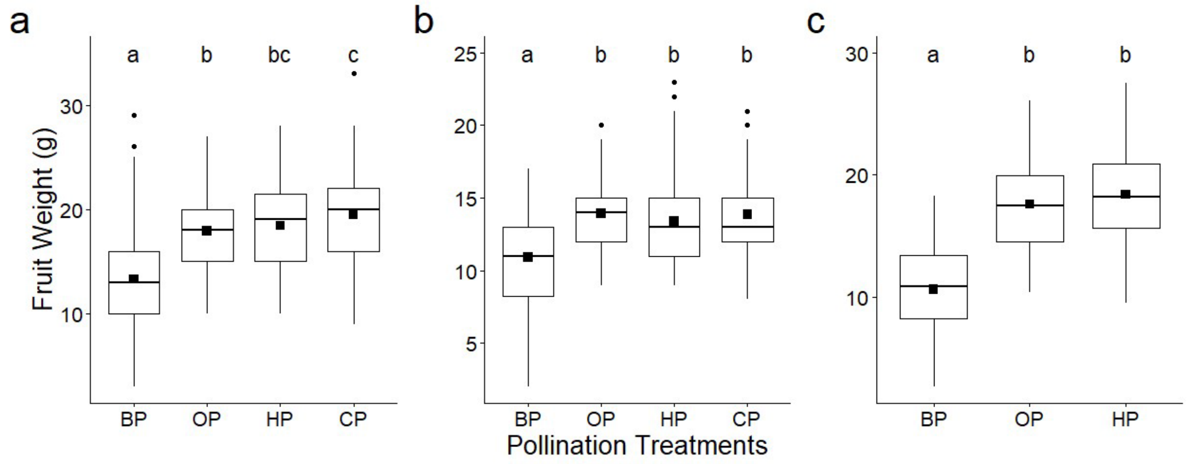


Figure 9: Treatment effect on fruit weight: (a) Tetragonula carbonaria in Year 1, (b) T. hockingsi in Year 1, (c) T. hockingsi in Year 2. Treatments are bagged pollination (exclusion, BP), open pollination (no exclusion, OP), hand-pollination with the same variety (HP) and hand-pollination with a different variety (cross pollination, CP). Boxes show the interquartile range, the median is indicated by a horizontal line, whiskers indicate the data range, the small points are outliers and the black square dot in the middle of the box shows the mean. Different letters above boxes indicate that means are significantly different from each other.

We also demonstrated that stingless bee colony weight and hive entrance activity rate were reduced after colonies were introduced into a glasshouse, but subsequently increased rapidly when returned to an open field environment (Fig. 10).

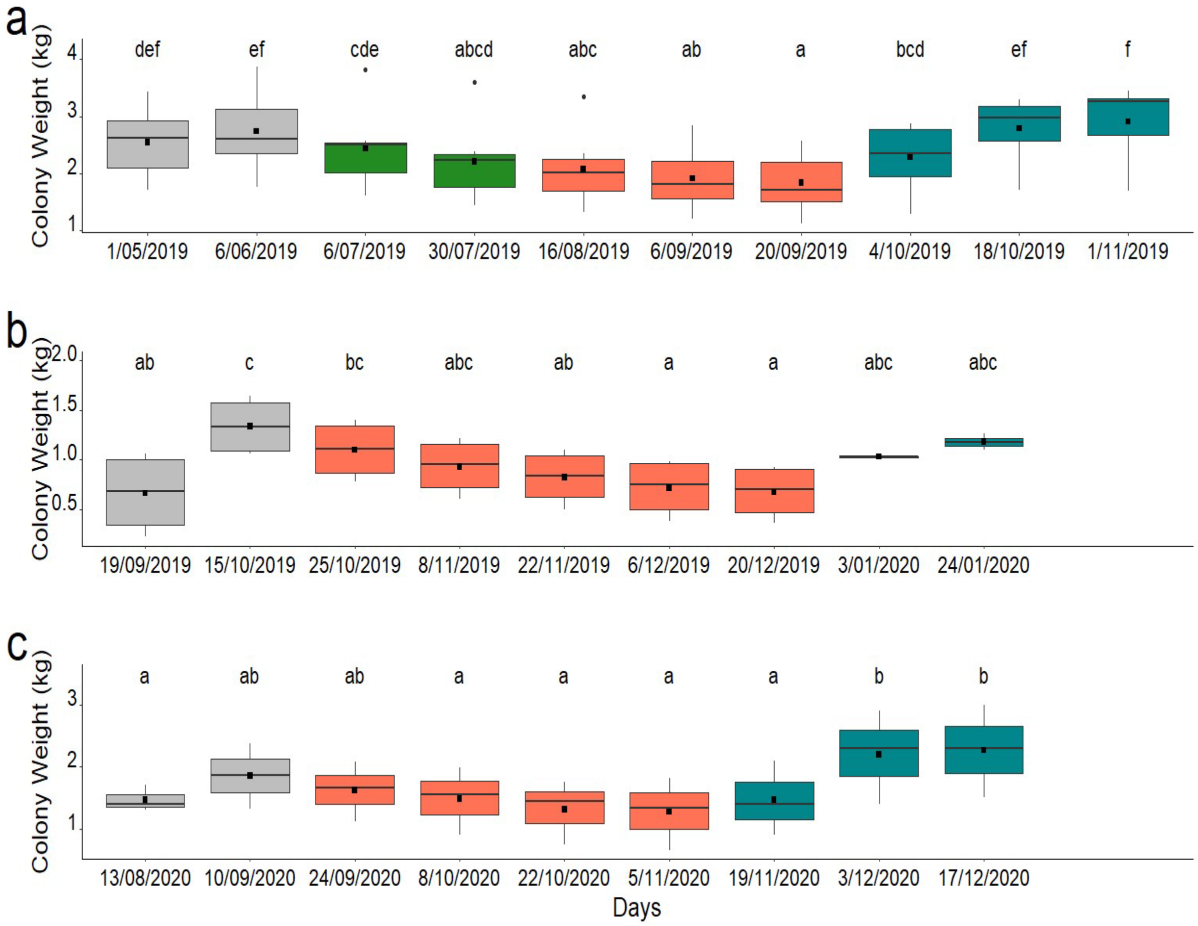


Figure 10: Change in the colony weight as colonies were moved from the open environment into the glasshouse for strawberry pollination and then moved back to the open environment. (a) Tetragonula carbonaria in Year 1, (b) T. hockingsi in Year 1, (c) T. hockingsi in Year 2. Glass- refers to colonies in the glasshouse. Boxes show the interquartile range, the median is indicated by a horizontal line, whiskers indicate the data range, the small points are the outliers and the black square dot in the middle of the box indicate the mean. Different letters above the boxes indicate that means are significantly different from each other.

We then investigated pollination efficiency (i.e., the number and duration of floral visits needed to achieve effective pollination) of the two stingless bee species, and how their on-flower foraging behaviour affected pollination efficiency. We found that two visits by *T. carbonaria*, and two to ten visits by *T. hockingsi* led to superior fruit weight and marketable grades, at similar levels to those obtained by open pollination and hand pollination controls.


Extra Class
Class 1
Class 2
Class 3
Class 4

Figure 11: Examples of strawberry fruit classified under each quality grading: "extra class" fruits are well formed and has no deformity. "Class 1" fruits are similarly well formed except for very minimal ridge. "Class 2" fruits are reasonably well formed but has more ridges than class 1 fruits. "Class 3" fruits have several unfertilized achenes making it deformed. "Class 4" fruits are strongly deformed.

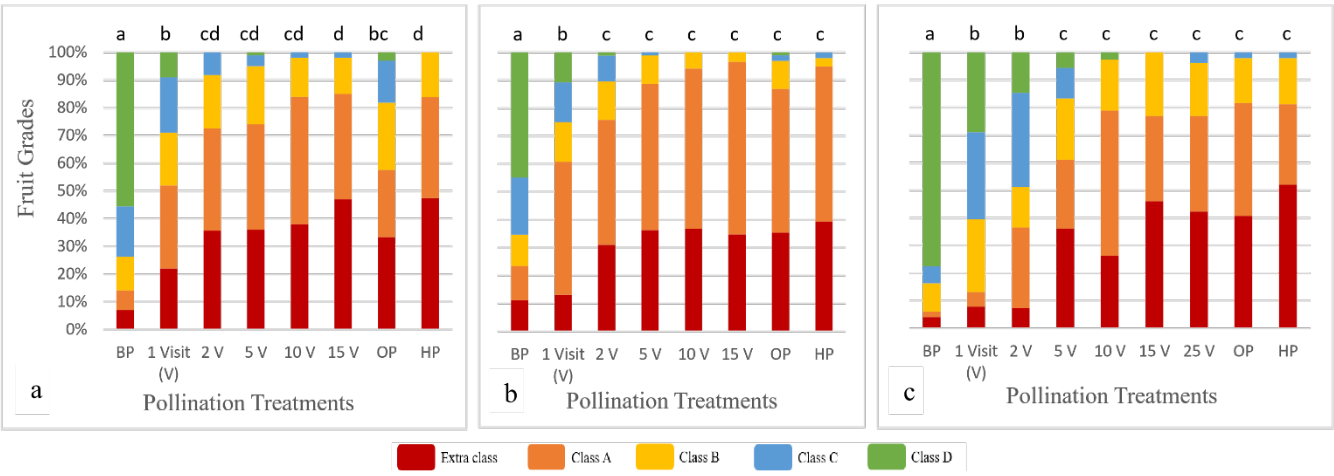


Figure 12: Treatment effect on grade quality of fruits: (a) Tetragonula carbonaria in Year 1, (b) Tetragonula hockingsi in Year 1, (c) Tetragonula hockingsi in Year 2. Treatments are bagged pollination (exclusion, BP), controlled number of bee floral visits (1 visit (1V), 2V, 5V, 10V, 15V, 25V), open pollination (no exclusion, OP) and hand-pollination (HP). Different letters above each pollination treatment indicate that means are significantly different from each other based on estimated marginal means.

With regard to flower visit duration, *T. carbonaria* spent more time on flowers than *T. hockingsi*. In terms of type of resources collected and on-flower foraging behaviour, *T. carbonaria* foragers mostly collected pollen or mixed resources (pollen and nectar) and both types of foragers spent a similar time on flowers. In contrast, *T. hockingsi* had pollen, nectar and mixed resource foragers, and both nectar and mixed foragers spent more time on flowers than pollen foragers. Pollen and/or nectar foraging by bees led to similar fruit quality, because despite bees having different on-flower foraging behaviours, both were effective in transferring sufficient pollen across the disparate flower stigmas.

Finally, we investigated how the use of stingless bees for crop pollination in a glasshouse environment with limited floral resources affected their gut bacterial communities by comparing the bacterial community composition and structure before, during and after deployment in the glasshouse environment. We found that deployment of *T. hockingsi* and *T. carbonaria* colonies in the glasshouse for strawberry crop pollination affected their gut bacterial community when compared to colonies of both bee species that were kept in the open field environment. Additionally, we also found that the colonies in the glasshouse became similar in their degree of bacterial community re-ordering, which persisted even after they were returned to the open field environment.

WP5. Compiling and analysing data from existing horticultural use of stingless bees

Surveys of Australian beekeepers and growers

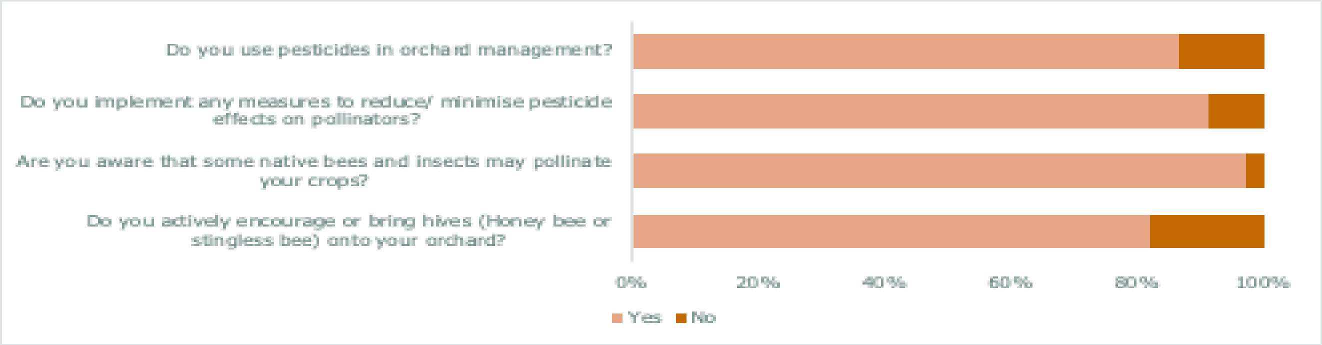
A survey was conducted in 2019-2020 to assess the nature and size of Australia’s stingless bee industry. This was a follow-up survey to two previous surveys conducted by Heard and Dollin (1999) and Halcroft et al. (20130. The survey received responses from 1,158 bee keepers who kept more than 11,900 stingless bee colonies. The geographic focus of the industry continues to be SE Queensland – NE New South Wales, with a slow rise in the number of bee keepers extending down southwards to Sydney. When comparing the stingless bee (SB) industry with the honey bee (HB) industry, based on the number of bee keepers, the annual growth rate of the SB industry is higher by 3%. However, almost half of the stingless bee keepers have less than a year’s experience, while a similar number have engaged in some form of hive manipulation through splitting or transferring nests.

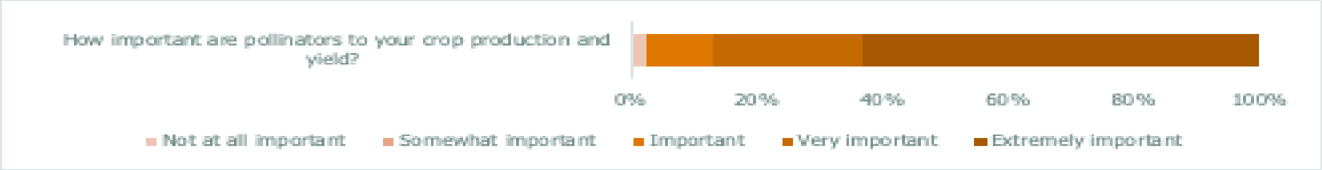
We conducted an additional survey targeted specifically at commercial bee keepers (defined as those owning >90 hives) who engaged in hive sales, honey production and pollination of crops. The 21 commercial bee keepers who responded managed nearly twice as many (7,800) stingless bee colonies as the 1137 hobby bee keepers. About 47% of the hives produced by commercial bee keepers are sold, while the remainder are used for activities such as honey production, pollination, and research.

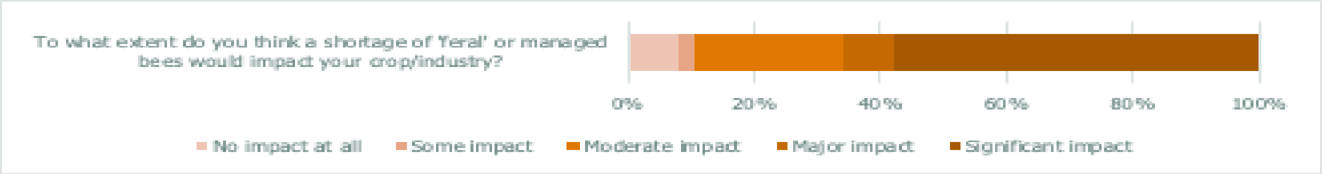
A survey was also conducted among growers from selected horticultural crop industries in SE Queensland. Questions were designed to better understand the current knowledge base of growers regarding native pollinators. Overall, the survey respondents represented 8% (38 growers) of the total industry landholding for macadamia and 3% (14) for avocado. Most respondents were business owners (macadamia 76%, avocado 86%), representing a range of orchard sizes (macadamia 7.52 ha – 120 ha, avocado 0.4 ha – 117 ha).

Most macadamia growers (77.78%) perform regular insect surveys, mainly for pest identification and management, while only 36% of avocado growers perform the same insect surveys. It is clear from responses that owners in both crops are aware of the importance of both managed and wild bee populations for pollination services, and most take measures to limit or mitigate pesticide use to encourage those pollination services. However, mitigation methods vary significantly across growers and could benefit from better information for more efficient implementation.

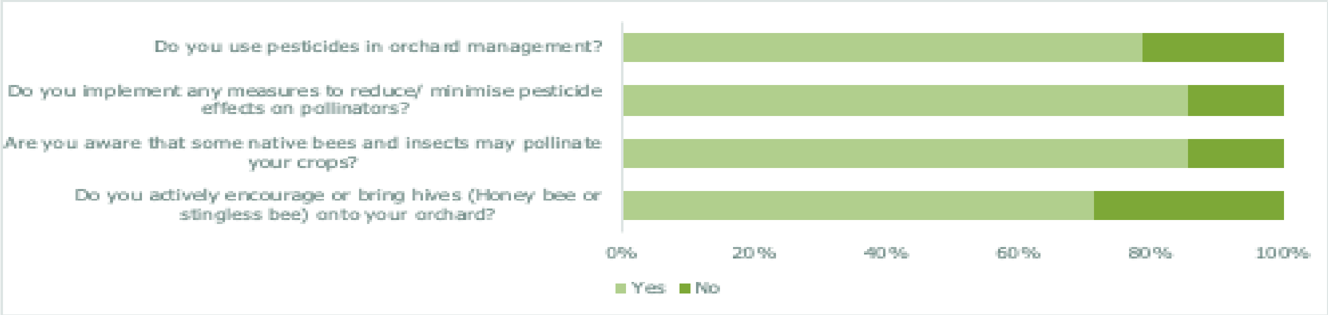
Industry Grower Survey – Macadamia (38 respondents)

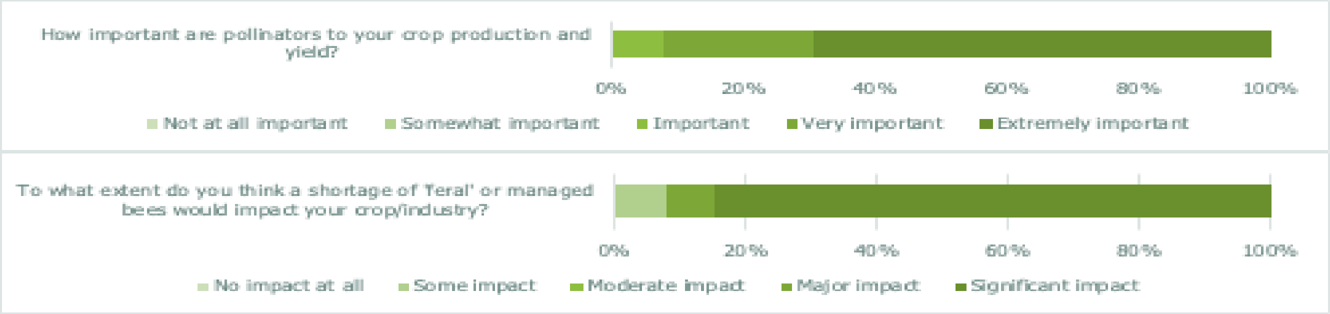






Industry Grower Survey – Avocado (14 respondents)





Developing links with India

From 2017-2023 a wide range of activities took place to develop links around stingless bees and crop pollination between Australia and India. These are reported in detail in Appendix 5 and include, grower, beekeeper and researcher exchanges, conferences, research collaborations, and grants. In summary, the commencement of Australia-India collaboration on pollination in horticultural crops recommended and supported by Hort Innovation, initially with PH15001 Healthy bee populations for horticultural pollination services but fully developed in the current project, has led to a strong and on-going series of activities within and beyond these projects, which have supported research, development and extension to farmers in both countries, as well as mentoring junior staff and PhD training, which has been achieved despite the intervention of the COVID-19 epidemic.

One major outcome of these links is the development of ongoing dual PhD studentship schemes between WSU and a number of Indian Agricultural Universities. A second exciting outcome is a grant from the Australia-India Council for ajoint project between WSU and Tamil Nadu Agricultural University investigating the impact of climate change on mango pollination. The Australia-India project, with Ms Venkatesh as PhD student, will continue until 2024. We will explore other opportunities for additional Australia-India collaboration in horticultural crop pollination both within opportunities under the recently established Centre for Australia-India Relations, as well as with our AICRP (H&P) colleagues. We have worked successfully with three AICRP Coordinators, Dr R Thakur, Dr P Chakrabarty, and Dr B Singh, and expect similar good relationships with the newly appointed Coordinator, Dr S Suroshe.

Outputs

Given the size and length of this project, there is a long list of project outputs and extension activities. In the output table below (Table 3), we summarise different categories of outputs. A detailed table of individual outputs is provided in Appendix 6 while examples of industry articles are provided in Appendix 7 and scientific publication that have already been published in Appendix 8.

Table 3. Output summary

Output	Description	Detail
Overall	193 outputs	See Appendix 6
Grower Engagement	38 examples of grower engagement	This included farm visits, onsite demonstrations, outreach meetings, and surveys to capture grower data
Published Grower Articles	41 articles released in established grower and/or beekeeper publications	See Appendices 6 and 7 These include articles in Australian Macadamia Society Mango Matters Australian Berry Journal

		Australian Tree Crop Organic Gardener Magazine The Cross-pollinator
Media engagement	19 promotional interviews with established broadsheet and conventional media organisations	See Appendix 6 This included radio interviews, podcasts, TV interviews, and interest pieces for web/print
Grower Conferences	48 presentations and talks given at industry-led conferences	See Appendix 6
Demonstrations	7 Demonstrations and workshops	See Appendix 6

Outcomes

Table 4. Outcome summary (following Project M & E Plan)

Outcome	**Alignment to fund outcome, strategy and KPI**	**Description**	**Evidence**
Intermediate outcome: New knowledge on native stingless bees as crop pollinators in: • Tropical crops • Temperate crops • Glasshouse environments Implementing BMP recommendations	The project outcomes progress two of the three strategic investment themes of the Hort Innovation Pollination Fund EAC: a) Optimising crop pollination efficiency b) Identifying alternate crop pollinators.	We have made excellent progress to better understanding of the potential for native stingless bees to function as crop pollinators in: • Tropical crops – the bees appear to be good pollinators of mango, macadamia and lychee in orchards. Their potential in avocado is lower as they show low attraction to this crop if others e.g. macadamia are also available • Temperate crops – we tested Australian stingless bees with a range of cucrbits, in fields and in glasshouses, and found that the bees are not pollinators of these crops. • Glasshouse environments – we have shown that stingless bees are excellent	• Updates on progress with all these activities are provided with each milestone report • Two completed modules of work on cucurbit pollination and stingless bee health have already been published as scientific research articles

		pollinators of glasshouse strawberries - Glasshouse environments – we have found that stingless bee hives can be used for several weeks in glasshouse environments but do lose condition. Careful monitoring of hive activity and provision of non-crop floral and sugar range of cucurbit crops	
Intermediate outcome: Translating research findings for Hort. growers and levy payers through final workshops / training and monitoring uptake of research / benefits.	The project outcomes progress two of the three strategic investment themes of the Hort Innovation Pollination Fund EAC: a) Optimising crop pollination efficiency b) Identifying alternate crop pollinators.	- These activities have been ongoing throughout the project with particular emphasis early (launch and aims) and late (final results and recommendations) in the project cycle. - We have held a range of grower workshops in NSW and QLD. We have also presented at industry conferences and in grower magazines, as well through online media and research publications. These activities are summarized in the Table 3 and listed in Appendix 6	- Updates on progress with these activities are provided with each milestone report - Several project updates with preliminary findings and recommendations have been published in industry magazines and communicated at grower workshops
End-of-project outcomes: Has this research changed grower practices to utilise native stingless bees for pollination?	The project outcomes progress two of the three strategic investment themes of the Hort Innovation Pollination Fund EAC: a) Optimising crop pollination efficiency b) Identifying alternate crop pollinators.	- Many growers have attended our specific workshops targeted at e.g. avocado, macadamia or protected cropping growers and/or read industry magazine articles with recommendations for deploying stingless bees - Macadamia and almond growers have adopted on-farm	- Attendance records at grower workshops - Grower magazine articles published - On-farm plantings

		plantings to support bees used in pollination services	
Has crop productivity changed?		- Our research has shown how stingless bees can increase fruits set and/or quality in e.g. orchard mangoes (Appendix 3) and glasshouse strawberries (Appendix 4)	- Milestone and final reports - Appendices 2-4 - Publications

Monitoring and evaluation

Table 5. Key Evaluation Questions

Key Evaluation Question	**Project performance**	**Continuous improvement opportunities**
Has new knowledge been generated on native stingless bees as crop pollinators in: - Tropical crops - Temperate crops - Glasshouse environments	Yes, substantial new knowledge has been generated for each of these cropping environments. This is evidence by regular updates in milestone reports, conference presentations, 6 PhD theses, and several scientific publications (both published and pending). These activities and results are summarised in other parts of this report and its appendices.	- Testing stingless bees with further crops, especially tropical ones and emerging industries - Better understanding of pollination potential of Australia's other stingless bee species (beyond *T. carbonaria* & *T. hockingsi*) - How to monitor hive strength and maintain bee health in cropping environments
Have issues and research findings been translated for Hort. growers and levy payers through workshops, training and monitoring uptake of research benefits	Yes, issues and research findings been translated through grower workshops and training days, grower and beekeeper surveys, industry magazine articles and industry conference talks and displays. These activities are summarised in other parts of this report (e.g. outputs table) and its appendices.	- Continued dissemination of key findings and recommendations in a range of formats – workshops, field days, online and magazine articles, mini-videos, fact sheets
Has this research changed grower practices to utilise native stingless bees for pollination?	Yes, we have seen some direct outcomes: a) planting of non-crop flora to support stingless bees by growers of almonds and macadamias; b) modification of hive introduction timings by macadamia	- Greater uptake of stingless bees in protected cropping, where our work suggest they are an excellent option - More consideration of landscape management around farms where

	growers; c) greater consideration of chemical spray timings during flowering by mango growers; overall greater awareness and interest in using stingless bees as managed pollinators in various settings	wild stingless bees nest and from which they visit the crop • Optimising on-farm practices to support stingless bee health and activity

Recommendations

- Stingless beehives used for pollination should have a standard weight (2 – 4 kg) (Heard 2016) and foraging rate which indicates a strong, healthy hive (30 - 60 returning foragers per/min) (Heard 2016). This weight and foraging rate indicate the best opportunity to contribute effective pollination services to the chosen crop.
- Hives should not be split for at least a month prior to flowering in agricultural crops, due to it significantly reducing the number and proportion of pollen foragers. This reduction in foraging for floral resources may have significant negative impacts for pollination within the target crop.
- Techniques to increase the amount of cross pollen carried by stingless bees, such as dusting bees with cross pollen, need to be developed to improve the number of bee visits that result in cross pollination.
- The hidden variability of unexpected cultivars and seedling rootstocks in macadamia orchards is likely to be a frequent source of cross pollen, with variable effects on kernel recovery. Kernel recovery of the crop could be increased by providing sources of cross pollen known to produce high kernel recovery.
- Growers should aim to retain diverse floral resources all year round, particularly in lower diversity landscapes.
- In the short-term, growers and other land managers could conserve existing riparian zones, windbreaks and other trees and retain certain fast-growing annual herbs, including some weeds, between crop rows until just before seed development.
- Long-term strategies could include planting flowering trees and shrubs along creeks, beside dams or as windbreaks.
- Stingless bees are attracted to all flower phases of lychee, collect both nectar and pollen and thus show potential as good pollinators of this crop, suggesting further study would be valuable.
- Stingless bees have potential as pollinators of avocado, but stocking rates would need to be high, as stingless bee visit numbers were low, especially at sites with few stingless bee hives.
- Stingless bees, like honeybees, are more likely to visit avocado during male phase. Thus pollination is most likely to occur during the crossover phase between 10:00 and 12:00 for Hass and 16:00 to 18:00 for Shepard when both male and female flowers are open on the same panicle. Growers should avoid sprays during these times.
- Stingless bees demonstrate crop constancy during flowering with most bees carrying crop pollen. Stingless bees show very high crop constancy to macadamia and lychee at all sites and show promise as pollinators.
- Stingless bees will readily forage on both avocado and macadamia, however, stingless bees will fly large distances to macadamia (> 500m) and ignore avocado when both are available and flowering together. Stingless beehives installed into avocado orchards for pollination should be placed as far as possible from sources of macadamia pollen to avoid loss of pollination services and potential impacts on the avocado crop.
- A mixed deployment strategy featuring multiple introductions of managed hives may work best for orchard pollination, with colonies located in orchards prior to flowering to ensure that earlier opening flowers receive pollination, and later introductions to boost the ongoing number of foragers collecting macadamia pollen throughout the flowering season. Predicting when peak flowering will occur is difficult, so we suggest hive introductions two weeks apart, as we found that after two weeks crop fidelity began to decline.
- If there are no colonies already in an orchard and the grower is relying on rented colonies, hives can be introduced even when there are very few flowers open, as newly introduced colonies typically showed very high crop fidelity, irrespective of the level of crop flowering. Waiting too long before introducing colonies means that early flowers will not benefit from visitation by foragers from rented colonies.
- We found that relocating colonies mid-way through flowering did not increase crop fidelity again. Therefore, we would not suggest moving colonies between orchards once flowering is underway.
- In the second year of our study, one of the reasons the peak colonies in one orchard did not have higher crop fidelity than other colony groups when initially deployed is because in they also began collecting from which was highly abundant at that site. Removing competing non-crop resources (e.g. wild radish (*R. sativus*)) just before

crop flowering may improve crop fidelity, particularly with permanently deployed colonies, by encouraging foragers to switch to the crop from other on-farm plants to which they have established constancy.

- When planting or re-planting orchards, selecting synchronously flowering cultivars should increase levels of cross-pollination and produce shorter overall flowering periods, which may be beneficial as the crop fidelity of bees to macadamia declines after a couple of weeks.
- Wild stingless bees are key pollinators of mangoes in the Northern Territory and nest in the surrounding forest, so maintaining healthy forests around farms is essential for wild stingless bee pollination services. Landscapes containing large mature trees of ironwood (*Erythrophleum chlorostachys*) and Darwin woollybutt (*Eucalyptus miniata*) appear to be particularly important role in Darwin and Katherine.
- Managed stingless bee hives should be placed within blocks of the mango crop to increase stingless bee visitation throughout the orchard, and not just on edge trees. There is a strong drop off in visitation from wild bees beyond 50 m from the crop edge.
- Creating a managed stingless bee pollination industry in the Northern Territory requires: a) developing a methodology to rear stingless bee colonies of the local bee species *Tetragonula mellipes*; b) establishing optimal hive deployment strategies for stingless bee colonies in mango (including optimal hive densities, spacing and deployment times); c) determining the importance of cross pollination in different Australian mango varieties and whether stingless bees are capable of providing effective cross pollination in mango.
- The stingless bees *T. carbonaria* and *T. hockingsi* provide an attractive alternative to laborious hand pollination of glasshouse strawberries techniques, producing similarly high-quality fruits with a faster development time, which may lead to economic benefits to the grower because fewer resources (nutrients, pesticides, energy) will be required to grow them and more can be grown in shorter time frames.
- Our results suggest that *T. hockingsi* may be a more efficient pollinator than *T. carbonaria* for glasshouse strawberry pollination, but both species are good options.
- It is important to deploy a high enough density of stingless bees in glasshouses to adequate pollination without excess visitation that may damage flowers. The correct density will depend upon the number of flowers within a given space of a glasshouse, but we suggest that one strong colony is adequate to pollinate the 360 to 480 plants we used in each 8 m x 13 m experimental chamber.
- Positioning of stingless bee hives within glasshouses should be given careful consideration. Some colonies in our research faced air circulation fans and these colonies were not actively foraging when the fans were on. This is similar to reports of bees not flying on windy days (Heard & Hendrikz, 1993; Leonhardt et al., 2014). Orientation of the hive entrances to morning sun could have also affected their foraging but this was not investigated.
- We observed that the rate of decrease in weight and activity rates of colonies on introduction to the glasshouse varied considerably. Stronger colonies (based on activity rate and weight before to introduction to the glasshouse) had a slower decrease in performance compared to weaker colonies. Therefore only strong colonies should be deployed in glasshouses for pollination and further investigation is warranted to establish a benchmark foraging rate and weight for a strong colony.
- We suggest supplying additional floral resources or a source of polyfloral pollen within glasshouses so that bees are not forced to use up pollen and honey stored in the hive and can maintain healthy brood production.
- Sugar syrup should also be supplied, as it is important to keep the forager bees active, especially for crops which may have low nectar production. Our study, as well as others, indicates that providing bees with sugar syrup can influence the type of resources which the forage for (Goodwin, 1997). This may be useful in crops where a certain type of foraging behaviour results in better pollination.
- Resin should also be supplied in glasshouses as it is needed for building new brood cells, pollen pots and honey pots, and the absence of resin could limit colony growth.
- Provisioning bees with additional floral resources or supplementary pollen, sugar syrup and resin may also aid the maintenance of microbial communities which support colony health and the foraging performance of stingless bees.
- Further research is recommended on maintaining the health and vigour of stingless bee colonies used in

pollination, especially in glasshouses. For now, we suggest monitoring the weight and hive entrance activity rate of hives weekly to provide early warning of weakening hives. We further suggest that hives should not be used for more than 4-6 weeks in a glasshouse before returning them to a benign outside environment.

- Stingless bees are very promising for use in glasshouses with controlled conditions. However, in open cropping environments, their use is limited by prevailing weather, e.g. *T. carbonaria* does not forage at ambient temperatures below 18^{0}C, and heat-stressed hives may be more susceptible to pests like small hive beetle. Hence attempts to use them outside their natural range should consider carefully the likely weather conditions during the planned period of use.

Refereed scientific publications

Hall, M. A., Brettell, L. E., Liu, H., Nacko, S., Spooner-Hart, R., Riegler, M., & Cook, J. M. (2020). Temporal changes in the microbiome of stingless bee foragers following colony relocation. *FEMS Microbiology Ecology*, *97*(1). https://doi.org/10.1093/femsec/fiaa236

Le Gros, K. S., Makinson, J. C., & Spooner-Hart, R. N. (2022). Hygienic behaviour in the Australian stingless bees Tetragonula carbonaria and T. hockingsi. *Journal of Apicultural Research*, *61*(5), 578–590. https://doi.org/10.1080/00218839.2022.2109915

Liu, H., Hall, M. A., Brettell, L. E., Wang, J., Halcroft, M., Nacko, S., Spooner-Hart, R., Cook, J. M., Riegler, M., & Singh, B. K. (2023). Microbial diversity in stingless bee gut is linked to host wing size and influenced by the environment. *Journal of Invertebrate Pathology*, *198*, 107909. https://doi.org/10.1016/j.jip.2023.107909

Nacko, S., Hall, M., Duncan, M., Cook, J., & Riegler, M. (2020). Scientific note on small hive beetle infestation of stingless bee (Tetragonula carbonaria) colony following a heat wave. *Apidologie*. https://idp.springer.com/authorize/casa?redirect_uri=https://link.springer.com/article/10.1007/s13592-020-00799-6&casa_token=jdpUgj4DEO4AAAAA:1CCb2wpwL9zl0_gLjDY-_c-sxkwchFz1QO4-XkdtOzwClb2vZ67wyYKyNWJKcNLWg-Lxp6PpOoldjGeW

Nacko, S., Hall, M., Spooner-Hart, R., Cook, J., Bernauer, O., & Riegler, M. (2022). Cucurbit crops in temperate Australia are visited more by native solitary bees than by stingless bees. *Journal of Apicultural Research*, *61*(5), 675–687. https://www.tandfonline.com/doi/full/10.1080/00218839.2022.2110742

Several further scientific publications will arise from the work carried out for this project, but due to the lengthy processes in scientific publishing these are not yet published

References

Anderson, D.L., Sedgley, M., Shortland, J.R.T., & Allwood, A.J. (1982) Insect pollination of mango in Northern Australia. Australian Journal of Agricultural Research, 33, 541-8.

Arachchige, E. C. W. S., Rader, R., Cutting, B. T., Keir, M., van Noort, T., Fale, G., Howlett, B. G., Samnegård, U., & Evans, L. J. (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.

Arthur, A. D., Li, J., Henry, S., & Cunningham, S. A. (2010). Influence of woody vegetation on pollinator densities in oilseed Brassica fields in an Australian temperate landscape. Basic and Applied Ecology, 11(5), 406-414.

Balina, P. K., Sharma, S. K., & Rana, M. K. (2012). Diversity, abundance and pollination efficiency of native bee pollinators of bitter gourd (Momordica charantia L.) in India. Journal of Apicultural Research, 51(3), 227-231.

Bartareau, T. (1996). Foraging behaviour of Trigona carbonaria (Hymenoptera: Apidae) at multiple-choice feeding stations. Australian Journal of Zoology, 44(2), 143-153.

Bell, M., Spooner-Hart, R., & Haigh, A. M. (2006). Pollination of greenhouse tomatoes by the Australian bluebanded bee Amegilla (Zonamegilla) holmesi (Hymenoptera: Apidae). Journal of Economic Entomology, 99(2), 437-442.

Bernauer, O. M., Tierney, S. M., & Cook, J. M. (2022). Efficiency and effectiveness of native bees and honey bees as pollinators of apples in New South Wales orchards. Agriculture, Ecosystems & Environment. https://www.sciencedirect.com/science/article/pii/S0167880922002122

Bernauer, O. M., Tierney, S. M., & Cook, J. M. (2022). Efficiency and effectiveness of native bees and honey bees as pollinators of apples in New South Wales orchards. Agriculture, Ecosystems & Environment, 337, 108063.

Bosch, J., Sgolastra, F., & Kemp, W. P. (2008). Life cycle ecophysiology of Osmia mason bees used as crop pollinators. Oxford University Press.

Brito, R. M., Francisco, F. O., & Ho, S. Y. W. (2014). Genetic architecture of the Tetragonula carbonaria species complex of Australian stingless bees (Hymenoptera: Apidae: Meliponini). Biological Journal of the Linnean Society. Linnean Society of London. https://academic.oup.com/biolinnean/article-abstract/113/1/149/2415957

Cane, J. H., Schiffhauer, D., & Kervin, L. J. (1996). Pollination, foraging, and nesting ecology of the leaf-cutting bee Megachile (Delomegachile) addenda (Hymenoptera: Megachilidae) on cranberry beds. Annals of the Entomological Society of America, 89(3), 361-367.

Cardale, J. (1968). Nest and nesting behaviour of Amegilla (Amegilla) pulchra (Smith)(Hymenoptera: Apoidea: Anthophorinae). Australian Journal of Zoology, 16(4), 689-707.

Coates, J. M., Brown, J., & Cunningham, S. A. (2022). Wild bees nest in the stems of cultivated Rubus plants and act as effective crop pollinators. Agriculture, Ecosystems & Environment, 325, 107741.

Cunningham, J. P., Hereward, J. P., Heard, T. A., De Barro, P. J., & West, S. A. (2014). Bees at war: interspecific battles and nest usurpation in stingless bees. The American Naturalist, 184(6), 777–786. https://doi.org/10.1086/678399

Cunningham, S. A., FitzGibbon, F., & Heard, T. A. (2002). The future of pollinators for Australian agriculture. *Australian Journal of Agricultural Research*, *53*(8), 893–900. https://doi.org/10.1071/ar01186

Danforth, B. N., & Ji, S. (2001). Australian Lasioglossum and Homalictus form a monophyletic group: resolving the “Australian enigma”. Systematic Biology, 50(2), 268-283.

Delaplane, K. S., & Mayer, D. F. (2000). Crop pollination by bees. CABI Publishing. https://doi.org/10.1002/mmnz.20020780120

Dollin, A. E., Dollin, L. J., & the late Shôichi F. Sakagami. (1997). Australian stingless bees of the genus Trigona (Hymenoptera: Apidae). *Invertebrate Systematics*, *11*(6), 861–896. https://doi.org/10.1071/it96020

Dollin, A., Hogendoorn, K., Heard, T., Cunningham, S., Rader, R., Saunders, M., Latty, T., Threlfell, C., Smith, T., & Halcroft, M. T. (2016). Australian native bees. NSW Department of Primary Industries.

Dollin, L. (2006). How to make nest blocks for blue banded bees. Aussie Bee Online.

Evans, L. J., Jesson, L., Read, S. F. J., Jochym, M., Cutting, B. T., Gayrard, T., Jammes, M. A. S., Roumier, R., & Howlett, B. G. (2021). Key factors influencing forager distribution across macadamia orchards differ among species of managed bees. Basic and Applied Ecology, 53, 74-85.

Gilpin, A. M., Brettell, L. E., Cook, J. M., & Power, S. A. (2022). The use of trap-nests to support crop pollinators in agricultural areas. Ecological Research, 37(6), 768-779.

Greco, M. K., Hoffmann, D., Dollin, A., Duncan, M., Spooner-Hart, R., & Neumann, P. (2010). The alternative Pharaoh approach: stingless bees mummify beetle parasites alive. *Die Naturwissenschaften*, *97*(3), 319–323. https://doi.org/10.1007/s00114-009-0631-9

Greco, M. K., Spooner-Hart, R. N., Beattie, A. G. A. C., Barchia, I., & Holford, P. (2011). Australian stingless bees improve greenhouse Capsicum production. *Journal of Apicultural Research*, *50*(2), 102–115. https://doi.org/10.3896/IBRA.1.50.2.02

Greco, M. K., Spooner-Hart, R. N., Beattie, A. G. A. C., Barchia, I., & Holford, P. (2011). Australian stingless bees improve greenhouse Capsicum production. Journal of Apicultural Research, 50(2), 102-115.

Halcroft, M. T., Haigh, A. M., Holmes, S. P., & Spooner-Hart, R. N. (2013). The thermal environment of nests of the Australian stingless bee, Austroplebeia australis. *Insectes Sociaux*, *60*(4), 497–506. https://doi.org/10.1007/s00040-013-0316-4

Halcroft, M. T., Spooner-Hart, R., Haigh, A. M., Heard, T. A., & Dollin, A. (2013). The Australian stingless bee industry: a follow-up survey, one decade on. *Journal of Apicultural Research*, *52*(2), 1–7. https://doi.org/10.3896/IBRA.1.52.2.01

Halcroft, M., Spooner-Hart, R., & Dollin, L. A. (2013). Australian Stingless Bees. In P. Vit, S. R. M. Pedro, & D. Roubik (Eds.), *Pot-Honey: A legacy of stingless bees* (pp. 35–72). Springer New York. https://doi.org/10.1007/978-1-4614-4960-7_3

Halcroft, M., Spooner-Hart, R., & Neumann, P. (2011). Behavioral defense strategies of the stingless bee, Austroplebeia australis, against the small hive beetle, Aethina tumida. *Insectes Sociaux*, *58*(2), 245–253. https://doi.org/10.1007/s00040-010-0142-x

Hall, H. G., & Avila, L. (2016). Megachile sculpturalis, the giant resin bee, overcomes the blossom structure of sunn hemp (Crotalaria juncea) that impedes pollination. Journal of Melittology(65), 1-11.

Heard, T. A. (1994). Behaviour and pollinator efficiency of stingless bees and honey bees on macadamia flowers. *Journal of Apicultural Research*, *33*(4), 191–198. https://doi.org/10.1080/00218839.1994.11100870

Heard, T. A. (1999). The role of stingless bees in crop pollination. *Annual Review of Entomology*, *44*, 183–206. https://doi.org/10.1146/annurev.ento.44.1.183

Heard, T. A. (2016). The Australian Native Bee Book: Keeping stingless bee hives for pets, pollination and sugarbag honey. Sugarbag Bees.

Heard, T. A., & Hendrikz, J. K. (1993). Factors influencing flight activity of colonies of the stingless bee Trigona carbonaria (Hymenoptera, Apidae). Australian Journal of Zoology, 41(4), 343-353.

Hogendoorn, K., & Keller, M. (2011). Development of native bees as pollinators of vegetable seed crops (0734126999).

Hogendoorn, K., & Keller, M. (2012). Native Australian Bees as Potential Pollinators of Lucerne. RIRDC.

Hogendoorn, K., Coventry, S., & Keller, M. A. (2007). Foraging behaviour of a blue banded bee, Amegilla chlorocyanea in greenhouses: implications for use as tomato pollinators. Apidologie, 38(1), 86-92.

Hogendoorn, K., Sedgley, M., Gross, C., & Keller, M. (2006). Increased tomato yield through pollination by native Australian blue-banded bees (Amegilla chlorocyanea Cockerell). J. Econ. Entomol, 99, 828-833.

Hogendoorn, K., Steen, Z., & Schwarz, M. P. (2000). Native Australian carpenter bees as a potential alternative to introducing bumble bees for tomato pollination in greenhouses. Journal of Apicultural Research, 39(1-2), 67-74.

Houston, T. (2018). A Guide to Native Bees of Australia. CSIRO Publishing.

Houston, T. F. (1992). Biological observations of the Australian green carpenter bees, genus Lestis (Hymenoptera: Anthophoridae: Xylocopini). Records of the Western Australian Museum, 15(4), 785-798.

Koski, M. H., Ison, J. L., Padilla, A., Pham, A. Q., & Galloway, L. F. (2018). Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant–pollinator mutualism. Proceedings of the Royal Society B, 285(1880), 20180635.

Le Gros, K. S., Makinson, J. C., & Spooner-Hart, R. N. (2022). Hygienic behaviour in the Australian stingless bees Tetragonula carbonaria and T. hockingsi. *Journal of Apicultural Research*, *61*(5), 578–590. https://doi.org/10.1080/00218839.2022.2109915

Leijs, R., Batley, M., & Hogendoorn, K. (2017). The genus Amegilla (Hymenoptera, Apidae, Anthophorini) in Australia: A revision of the subgenera Notomegilla and Zonamegilla. ZooKeys(653), 79.

Malagodi-Braga, K. S., & de Matos Peixoto Kleinert, A. (2004). Could Tetragonisca angustula Latreille (Apinae, Meliponini) be effective as strawberry pollinator in greenhouses? *Australian Journal of Agricultural Research*, *55*(7), 771–773. https://doi.org/10.1071/AR03240

Nacko, S., Hall, M., Spooner-Hart, R., Cook, J., Bernauer, O., & Riegler, M. (2022). Cucurbit crops in temperate Australia are visited more by native solitary bees than by stingless bees. *Journal of Apicultural Research*, *61*(5), 675–687. https://doi.org/10.1080/00218839.2022.2110742

Michener, C. D. (2007). The bees of the world (2nd ed.). Johns Hopkins University Press.

Nacko, S., Hall, M., Spooner-Hart, R., Cook, J., Bernauer, O., & Riegler, M. (2022). Cucurbit crops in temperate Australia are visited more by native solitary bees than by stingless bees. Journal of Apicultural Research, 61(5), 675-687.

Nzie, O. P. (2022). Pollination efficacy, foraging behaviour and colony health of Australian stingless bees as pollinators in glasshouse strawberry production Western Sydney University]. NSW, Australia.

Prendergast, K. (2018). Nesting biology of 'Megachile ignita' Smith, 1853 (Hymenoptera: Megachilidae) in artificial nesting blocks in urbanised southwestern Australia. TheAustralian Entomologist, 45(2), 139-148.

Sajjad, A., Akram, W., Ali, S., Farooqi, M. A., Mujtaba, G., Ali, M., & Ahmad, A. (2019). Pollination of Grewia asiatica (Malvaceae) by Megachile cephalotes (Hymenoptera: Megachilidae): male vs. female pollination. Sociobiology, 66(3), 467-474.

Singh, A. (2016). Pollinating efficiency of native bee pollinators of pigeonpea (Cajanus cajan) in Nagaland. Russian Journal of Ecology, 47, 310-314.

Singh, G., Cook, J.M., Spooner-Hart, R.N. and Makinson, J.C. 2023 Pollinators on the Edge? The spatio-temporal distribution of diurnal and nocturnal floral visitors in mango orchards and its effect on fruit set. *In review.*

Slattery, P. S., Parslow, B. A., Lee, M. S. Y., Batley, M., Walker, K. L., & Schwarz, M. P. (2023, 2023/02/28). Disparate continental scale patterns in floral host breadth of Australian colletid bees (Colletidae: Hymenoptera). Apidologie, 54, 17.

Stubbs, C. S., & Drummond, F. A. (1996). Pollination of wild lowbush blueberry, Vaccinium angustifolium by the alfalfa leafcutting bee, Megachile rotundata. VI International Symposium on Vaccinium Culture 446,

Subasinghe Arachchige, E. C. W., Rader, R., Cutting, B. T., Keir, M., van Noort, T., Fale, G., Howlett, B. G., Samnegård, U., & Evans, L. J. (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). https://doi.org/10.1002/2688-8319.12189

Trinkl, M., Kaluza, B. F., Wallace, H., Heard, T. A., Keller, A., & Leonhardt, S. D. (2020). Floral Species Richness Correlates with Changes in the Nutritional Quality of Larval Diets in a Stingless Bee. *Insects*, *11*(2). https://doi.org/10.3390/insects11020125

Wallace, H. M. (1999). Bees and the pollination of macadamia. Australian Macadamia Society Technical Papers, 26, 71-73.

Willcox, B. K., Howlett, B. G., Robson, A. J., Cutting, B., Evans, L., Jesson, L., Kirkland, L., Jean-Meyzonnier, M., Potdevin, V., Saunders, M. E., & Rader, R. (2019). Evaluating the taxa that provide shared pollination services across multiple crops and regions. Scientific Reports, 9(1), 1-10.

Wilson, R. S., Keller, A., Shapcott, A., Leonhardt, S. D., Sickel, W., Hardwick, J. L., Heard, T. A., Kaluza, B. F., & Wallace, H. M. (2021). Many small rather than few large sources identified in long-term bee pollen diets in agroecosystems. *Agriculture, Ecosystems & Environment*, *310*, 107296. https://doi.org/10.1016/j.agee.2020.107296

Intellectual property

‘No project IP or commercialisation to report’

Acknowledgements

We acknowledge the traditional custodians of all the lands on which we have worked. We also thank the many growers who supported this project in a range of ways and permitted access to their orchards and properties to perform surveys and experiments.

Appendices

*Appendix 1. Native bees and crop pollination review
*Appendix 2. Tropical orchard crops report and technical guide – Griffith University
*Appendix 3. Tropical orchard crops report and technical guide – WSU
*Appendix 4. Glasshouse report and technical guide
Appendix 5. Report on India activities
Appendix 6. Detailed list of outputs and engagement activities
#Appendix 7. Examples of industry magazine articles
#Appendix 8. Examples of scientific publications

* Material in appendices 1-4 is embargoed till 30 June 2024 due to scientific publication requirements

Appendices 7 and 8 include several articles published in grower magazines and science journals. Copyright for at least some of these lies with their respective publishers.

REPORT ON AUSTRALIA-INDIA LINKS VIA THIS PROJECT

Prepared by Robert Spooner-Hart

This project envisaged from the outset “liaison and research exchange” with separately funded efforts in India and Thailand- two key countries for stingless bee use. And particularly “the data collection, demonstration and extension program will be implemented in collaboration with ongoing multimillion dollar projects…. such as the All India Coordinated Research Program on Honeybees and Pollinators (AICRP H&P) … with a view to data collection and sharing”. In India, the importance of honey bees and their role in enhanced agricultural production and productivity was realised by the National Commission on Agriculture in 1976 that recommended establishment of the "All India Coordinated Project on Honeybee Research & Training", launched by the Indian Council of Agricultural Research (ICAR) in 1980-81. Realising that apart from honey bees, many non-Apis bees, insects and animals contribute to potential yield enhancement of cross pollinated crops, the project was renamed the All India Coordinated Research Project on Honey bees and Pollinators (AICRP H&P) in July, 2007.

Activities in 2017

Prior to commencement of the current project, a number of activities associated with this initiative were organised by Western Sydney University and Horticulture Innovation Australia with ICAR, including an Australian study visit and workshop by key ICAR staff and the Hort Innovation project team Healthy bee populations for horticultural pollination services, 6-12 November 2016, followed by a study visit organised by AICRP (H&B) Coordinator Dr RK Thakur to Himachal Pradesh apple-growing areas by Robert Spooner-Hart and Markus Riegler (WSU) and Bill Shields (collaborating grower and member, Project Management Committee) in April 2017, and meetings at ICAR, Delhi.

As the current project was being developed, a Stingless bee study group tour of Southern India (Kerala, Tamil Nadu) April 2017, including representatives from Hort Innovation, staff from Western Sydney University and horticultural industry leaders selected by Hort Innovation (see final report for PH15001) was organised for April 2017 by Western Sydney University and Horticulture Innovation, together with ICAR and Kerala Agricultural University. At the conclusion of the study tour, key WSU (Prof Ian Anderson and Dr Nisha Rakhesh) and Hort Innovation staff (Dr David Moore) visited ICAR Delhi, where a MOU was developed to support collaborative activities in horticultural crop pollination. This was finalised following further meetings with WSU senior staff and Indian researchers from across the country at ICAR HQ in Delhi in December 2017 and a plan of action (“The workplan”) was developed and approved. Links with India further progressed significantly in with the signing of a MOU with ICAR for parallel Indian studies of some crops (e.g. mango & cucurbits), following meetings in India attended by Prof Anderson and Dr Rakhesh.

Activities in 2018

A follow-up meeting with key ICAR/AICRP (H&P) staff, including DDG ICAR Dr AK Singh and Dr PK Chakrabarty (ADG and Coordinator AICRP (H&P)) (Table 1) occurred at Hort Innovation offices in Brisbane, following a study visit to Western Sydney University, and participation in the First Australian Native Bee Conference (which showcased stingless bees) in July 2018 on the Gold Coast. This meeting led to firming up of Indian commitment and funding to conduct parallel work both on stingless bee industry surveys as well as on pollination studies of selected crops of high significance in both countries- notably mango, lychee and apple.

Table 1. Attendees of Australian study tour as part of stingless bee project, July 2018.

Name of the Scientists	Designation and Address
Dr. Anand Kumar Singh	Deputy Director General (Horticulture Sciences and Crop Sciences, ICAR, Krishi Bhawan, New Delhi-110001
Dr. Pranjib Kumar Chakrabarty	Assistant Director General (Plant Protection and Biosafety), ICAR, Krishi Bhawan, New Delhi-110001 Acting Coordinator AICRP (H&P)
Dr. Hemant Kumar Singh	Professor and Principal Investigator, AICRP (H&P), Department of Entomology, School of Agricultural Sciences and Rural Development, Nagaland University, Medzhiphema, Nagaland
Dr. V. S. Amritha	Assistant Professor and Principal Investigator, AICRP (H&P), College of Agriculture, Vellayani, Kerala Agricultural University, Thiruvanthapuram, Kerala (Western Ghats)
Dr. M.R. Srinivasan	Professor and Principal Investigator, AICRP (H&P), Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu (Eastern Ghats)
Dr. Ataur Rahman	Professor and Head cum Principal Investigator, AICRP (Honey Bees and Pollinators), Department of Entomology, Assam Agricultural University, Jorhat, Assam (North East)
Dr. Lalit.V. Ghetiya	Associate Professor and Principal Investigator, AICRP (Honey Bees and Pollinators), Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari (Western India)
Dr. Deepak Nayak	Scientist, Central Institute of Sub tropical Horticulture-Regional Research Station, Malda, West Bengal (Eastern India)

In addition, MOUs were signed in 2018 with four ICAR-affiliated Agricultural Universities for joint studentships with WSU to pursue pollination (and other horticulture) studies.

Activities in 2019

Robert Spooner-Hart conducted WSU/privately funded research visits/meetings associated with WSU-India collaboration and the stingless bee project in June-July, travelling to key AICRP (H&P) centres in Delhi, Udaipur (Rajasthan), Kochi (Kerala), Coimbatore (Tamil Nadu) and Malda (West Bengal).

These Indo-Australian activities were further progressed in November-December 2019, when four WSU staff (Simon Tierney, James Makinson, Amy Gilpin and Robert Spooner-Hart and one PhD student (on joint stingless bee project/WSU scholarship) contributed to a ICAR-WSU organised Pollination Symposium as part of the XIX International Plant Protection Congress in Hyderabad. The symposium was followed by a two-day workshop in Delhi with senior ICAR staff and key members of AICRP (H&P) researchers to firm up specific details for collaborative research (some involving PhD students) at different ICAR research stations around India (e.g. with mango, apple, cucurbits and protected cropping)

Activities in 2020

As part of the collaborative pollination research in apples and cherries, Drs Simon Tierney and Robert Spooner-Hart visited Himachal Pradesh and met with key pollination researchers at YS Parmar University for Horticulture and Forestry, Solan, and conducted field visits and farmer interviews in apple-growing areas in March-April. They also interacted with a joint WSU/ Sher-e-Kashmir University of Agricultural Sciences and Technology, working on almond pollination, in Delhi.

Following the world-wide outbreak of COVID-19, further travel by Indian or Australian collaborators was curtailed, until mid-2022.

Activities in 2021

James Makinson and Robert Spooner-Hart conducted video meetings between September and December) with one of our AICRP (H&P) partners Tamil Nadu Agricultural University regarding submitting a bid for joint project on mango pollination under DFAT Australia-India Council Grant. This application was a direct outcome of our collaborations initiated under the two Hort Innovation-funded pollination projects.

Robert Spooner-Hart gave a virtual plenary talk on “Beekeeping and Pollination in Australia: Life after the introduction of honey bees” at the International Conference on Global Perspectives in Crop Protection for Food Security, Dec 8-10, 2021 TNAU, Coimbatore. Robert was also on the Conference International Advisory Committee.

Activities in 2022

In March 2022, we received notification from DFAT of our successful Australia-India Council bid for the project “How will climate change impact mango cultivars and their pollinators”, with James Makinson, Robert Spooner-Hart and James Cook the WSU collaborators, and TNAU staff as Indian partners. As part of this AIC project, and under our MOU agreement, WSU sponsored an Indian student scholarship for a joint TNAU-WSU PhD. Subsequent meetings were conducted between WSU and TNAU to finalise activities associated with the AIC project.

Robert Spooner-Hart, as part of our AICRP collaborations with India, delivered an invited virtual seminar on “Pollination management in horticultural crops” at the 2nd Indian Horticulture Summit 27-29 April 2022 at Navsari Agricultural University, Navsari, Gujarat, India.

In November 2022, James Makinson, Robert Spooner-Hart together with our TNAU colleagues Profs SV Krishnamoorthy and SK Srinivasan co-convened and chaired a two-day international symposium “How will climate change impact mango cultivars and their pollinators?” at TNAU Coimbatore as part of DFAT project activities. Participants included scientists from AICRP (H&P) as well as other mango scientists and mango farmers. In addition to the symposium, eight prospective students were interviewed for the PhD scholarship, with Ms Dharini Venkatesh being selected. She was successfully admitted to her studies at WSU. Additionally, visits were made to other major areas conducting mango pollination studies: in Navsari Gujarat, Bengaluru Karnataka, and Malda West Bengal.

Activities in 2023

James Makinson returned to TNAU in January-February 2023, where he supervised Ms Venkatesh in conducting pollination studies in mango, around Coimbatore and Bengaluru.

Dharini will visit Australia in March 2023 for approximately six months to conduct mango pollination studies in the Darwin area.

Future planned activities

The Australia-India project, with Ms Venkatesh as PhD student, will continue until 2024. We will explore other opportunities for additional Australia-India collaboration in horticultural crop pollination both within opportunities under the recently established Centre for Australia-India Relations, as well as with our AICRP (H&P) colleagues. We have worked successfully with three AICRP Coordinators, Dr R Thakur, Dr P Chakrabarty, and Dr B Singh, and expect similar good relationships with the newly appointed Coordinator, Dr S Suroshe.

In summary, the commencement of Australia-India collaboration on pollination in horticultural crops recommended and supported by Hort Innovation, initially with Healthy bee populations for horticultural pollination services but fully developed in the current project, has led to a strong and on-going series of activities within and beyond these projects, which have supported research, development and extension to farmers in both countries, as well as mentoring junior staff and PhD training, which has been achieved despite the intervention of the COVID-19 epidemic.

The CROSS-POLLINATOR

Newsletter of Australian Native Bee Association
Issue 3, August 2019

Australian Native Bee ASSOC

We aim to pack our monthly newsletter, The CROSS-POLLINATOR, with information and news about native bees and to keep our members updated with the workshops, meetings, demonstrations and any events that are happening in the native bee world.

FEATURE ARTICLE

A holistic approach to pollination research

by James Cook, Mark Hall & Simon Tierney

Prof James Cook, left, surveying insect pollinators in an apple orchard

Inside this issue

FEATURE ARTICLE

Each monthly issue of CROSS-POLLINATOR includes an original feature article. This month's summarizes the bee and pollination investigations currently underway at Western Sydney University. A dynamic team has formed at this institute to conduct a broad and deep research agenda into many of the really important questions that we need to answer to ensure that our food production systems are not limited by a lack of pollination.

ANBA
Australian Native Bee ASSOC

The **Australian Native Bee Association Inc** promotes the conservation and sustainable use of all Australian native bees. ANBA achieves that by providing resources, disseminating information, supporting members and communicating with stakeholders.

For more information and to join, please go to our website: https://australiannativebee.org.au

Facebook: https://www.facebook.com/Australian.Native.Bee.Association/

Contact: Secretary: Trevor Weatherhead, 0427 960735, Email: sec@australiannativebee.org.au

A holistic approach to pollination research

by James Cook, Mark Hall & Simon Tierney

Hawkesbury Institute for the Environment (HIE),
Western Sydney University (WSU)

Bee research has a long history at WSU Hawkesbury Campus, which for a century was the *Hawkesbury Agricultural College*. This has continued since WSU was formed 30 years ago, aided by our campus apiary and extensive grounds for field studies. But bee research has really expanded in the last few years, as concerns over the resilience of crop pollination services have translated into more funding for research on bees and pollination. Currently, we have three projects through the Hort Frontiers Pollination Fund (https://bit.ly/2KB4uKN), with co-funding from Hort Innovation, Universities and Industry. Our projects are led by three HIE entomologists (James Cook, Robert Spooner-Hart, Markus Riegler) with strong interests in bees and other pollinators, aided by other HIE academic staff with expertise in plant ecology and microbiology. The "**Healthy bees**" project explores various issues regarding bee biology and crop pollination, including aspects of climate change, a research focus for the wider HIE community. Meanwhile the "**Stingless bees**" project focuses on Australian stingless bees, including their basic biology and husbandry, and potential as managed pollinators for different crops and cropping situations. Finally, a third project, not strictly ANBA fare, focuses on flies as pollinators.

A really exciting part of all this is that we have been able to recruit several dynamic PhD students and Postdoctoral Fellows to research specific topics within this broad program. In addition, we benefit from the hard work and enthusiasm of Masters students, interns and glasshouse staff, such as Kayla Le Gros, Goran Lapitiki and Eliette Reboud. Our scientific teams maintain close ties with crop growers and beekeepers, who facilitate our research. We aim to provide tangible solutions for management issues and often involve growers in experimental trials and provide full disclosure of results.

Markus Riegler, Kayla Le Gros, Goran Lopatiki, Robert Spooner-Hart and Onyeka Nzie with strawberry plants in the greenhouse facility

We also seek to engage the public, scientific community and industry as much as possible to create real links between our research and pollinator conservation and commercial application.

Project - Healthy bee populations for sustainable pollination in horticulture

The first WSU research project focuses on themes related to insect pollination of fruit orchards, predominantly undertaken by bees, including:

- behavioural ecology
- crop pollination
- floral resources and climate change
- diseases and microbiomes
- knowledge transfer

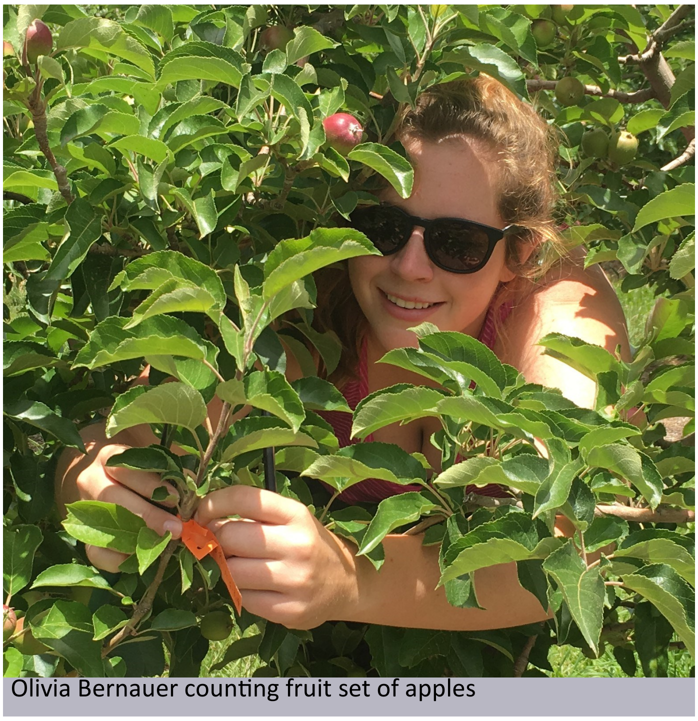
Our main aims are to study the natural history of native bees that visit crops, investigate whether they are acting as effective pollinators, and understand how to better support them in changing landscapes. We are also interested in whether native bee species have the potential to augment the pollination services provided by honeybees (both managed and feral colonies), should their Australian populations exhibit similar declines to those recently documented overseas. The predominant focus is on apple crops, which plug into broader collaborative efforts at the national level involving *Australian National U.*, *U. Adelaide*, *U. New England*, and *U. Sydney*. The core research compares an area surrounded by native bush (Blue Mountains) with areas subjected to land clearing and with reduced native habitat to support bees (Central West and South West Slopes regions of NSW).

Behavioural Ecology and Crop Pollination

Simon Tierney, Olivia Bernauer and Lisa Vella have been investigating bee foraging behaviour, combining: broad-scale visitation rates with fine-scale individual bee behaviours on flowers; natural history; bee-mediated pollen transport using advanced genetic tools; assessments of functional morphology at the community level; and the effect of habitat at the landscape-scale.

Simon Tierney observing pollinators of apple flowers

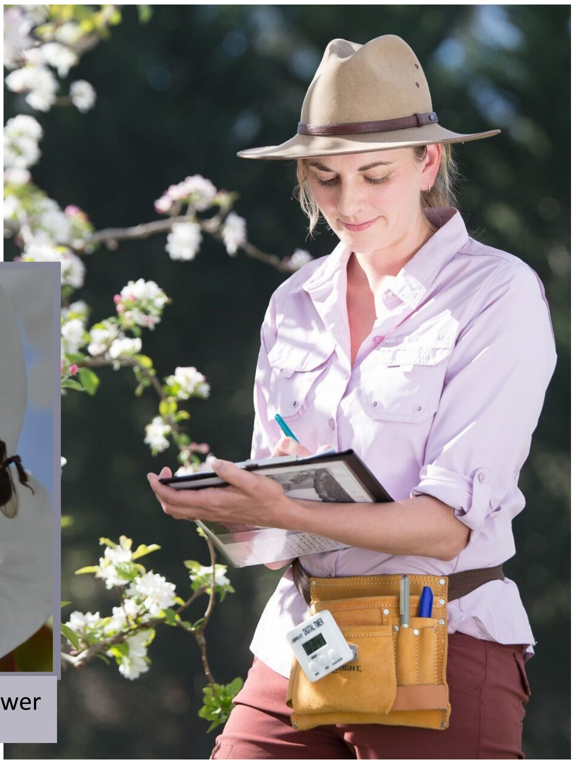
Stingless bees crowd an apple flower



Olivia Bernauer counting fruit set of apples

Floral Resources and Future Climates

Amy-Marie Gilpin and Lena Schmidt are interested in extra-crop floral resources, namely what weed and native species are available to pollinating insects that may compete with crops during spring and provide sustenance in other seasons. Key activities include: Year-round plant flowering surveys; experimental trials of supplemental floral resource plantings; and experimental tests of drought and heatwave effects on floral resources. Meanwhile, Lea Hannah is trialling supplemental floral plantings in almond orchards. Another exciting activity is using our major EucFACE experimental platform to see how elevated CO_2 influences eucalypt flowering and bee foraging.



Amy-Marie Gilpin recording bee visitations in orchards

Disease

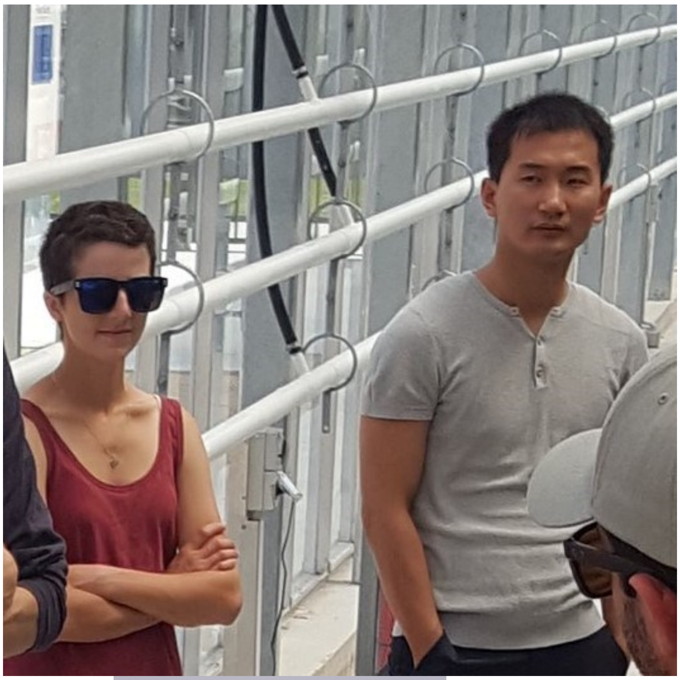
Laura Brettell and Lea Hannah are exploring bee pathogens (parasites and viruses): using community-level screening surveys to assess the health of Australian bees, and disease-transfer within and between insect species. For instance, Australia is the only continent not to harbour a parasitic mite that transmits deformed wing virus, which cripples honey bee populations worldwide. Meanwhile, Bronwen Roy is focussing more specifically on stingless bee diseases.

Laura Brettell (above) and Lea Hannah (below) recording pollinators in crops

Bronwen Roy collects samples from a diseased stingless bee hive

Microbiomes

Hongwei Liu is interested in bee terroir: he explores the microbial communities inside bees, the plants that bees visit and the soil that flowering plants grow in. Human gut bacterial communities can have dramatic affects and human health and similarly Hongwei's research explores how environmental microbiota might influence bee health.



Lisa Vella and Hongwei Liu

Project - Stingless bees as effective managed pollinators for Australian horticulture

We are very interested in the pollination efficiency of stingless bees and other wild pollinators in and around commercial crops. This project is being conducted with research partners at the University of the Sunshine Coast. It is examining the suitability of Australia's native stingless bees as alternative pollinators to honey bees.

Mark Hall and Eliette Reboud surveying pollinator visits

The overall aim is to explore the potential of stingless bees as managed crop pollinators, by testing their effectiveness for selected field crops and their suitability as glasshouse crop pollinators. This also involves exploring various colony health and husbandry considerations associated with their use in such environments.

We are currently working on temperate field vegetables in the Sydney region, tropical fruits and nuts in NSW and the NT, and in protected cropping environments (glasshouses and polytunnels) on our campus. Specific crops include: avocado, cucumber, eggplant, lychee, macadamia, mango, strawberry, and watermelon. Our current research team consists of two postdoctoral research fellows (Mark Hall and James Makinson) and five PhD candidates (Scott Nacko, Claire Allison, Sunyana Sajith, Onyeka Nzie and Gaurav Singh).

Temperate and tropical crops

We are investigating the efficiency and behaviour of stingless bees on certain crop species. For instance, Scott is exploring pollination in cucurbit crops (cucumber, melon, squash), both in field conditions and in glasshouses/polytunnels, while Claire and Gaurav are working on tree crops, such as mango, avocado and macadamia. Their work includes visitation surveys, where stingless bees and other pollinators are allowed to visit flowers a certain number of times, then the resulting fruit set is analysed. This allows us to determine how well stingless bees can produce marketable fruits. James is also looking at how landscape structure affects bee foraging behaviour and floral choice.


Gaurav Singh and James Makinson inspecting mango flower visitors


Stingless bee hives introduced to cucumber crops to test foraging behaviour


Scott Nacko

Pollination under protected cropping

Glasshouses and polytunnels are increasingly being used to produce year-round produce. However, these are unnatural foraging conditions for stingless bees. We are again looking at foraging efficiency here, as well as the effects of the glasshouse conditions on bee health, orientation and longevity. Onyeka is exploring strawberry pollination, while Mark is investigating how microclimatic conditions and spatial arrangement of crops in polytunnels affect foraging behaviour.


Claire Allison

Farmer attitudes toward, and use of, stingless bees

Sunyana Sajith is conducting surveys on farmer attitudes to stingless bee keeping both here in Australia and in India. Her research will investigate how willing farmers would be to utilise the potential of stingless bees as crop pollinators.


Sunyana Sajith in a stingless bee yard in India

Glasshouse strawberry pollination using native stingless bees

Project update from 'Stingless bees as effective managed pollinators for Australian horticulture' PH16000

Onyeka Nzie, Mark Hall, Robert Spooner-Hart, James Cook and Markus Riegler, Western Sydney University

Meeting the high demand for strawberries entails measures to ensure year-round production, including in protected cropping environments. Most varieties require insect pollination to increase yield and fruit quality, which is achievable in a field setting, but more challenging in polytunnels and glasshouses that exclude wild pollinators, and therefore suffer from a pollination shortfall.

Honeybees (*Apis mellifera*) are not well adapted to use in protected cropping environments and the use of managed bumblebees (*Bombus* spp.) is not available to Australian growers because they are not native to Australia. In addition, both honeybees and bumblebees often pose a health risk to workers in such environments, and honeybees themselves may suffer if the mite *Varroa destructor* ever establishes in Australia.

> **Australia has 11 species of stingless bees, so named due to their lack of a stinger, commonly also known as sugarbag bees. At least 3 species can be managed in man-made hives and have proved effective in pollinating multiple fruit, vegetable and nut crops (including raspberry and blueberry).**

However, we know less about the effectiveness of Australian stingless bee species in improving the yield and marketability of strawberry fruits, nor whether this can be achieved under protected cropping.

One strawberry variety commercially grown in Australia is 'Red Rhapsody', a cross between the Department of Agriculture and Fisheries (DAF) breeding line '2005-063' and 'Suncoast Delight'. This variety has superior taste and vigour and is resistant to pests and fruit bruising. In the open field, it produces early season crops (May-August) and in a protected environment can also produce a late season crop (September-December).

We are investigating how two species of native stingless bee, *Tetragonula carbonaria* and *Tetragonula hockingsi*, perform in a glasshouse facility on the Western Sydney University Hawkesbury campus in Richmond, NSW, where we are growing 'Red Rhapsody' strawberries. We tested whether visitation by either of these species can improve yield and fruit quality, and the number of visits required to produce the highest quality fruits.

We grew crops in two experimental glasshouse chambers (88 m^2; 8 m x 13 m x 6 m) with 480 strawberry plants in each chamber. We introduced hives of *T. carbonaria* into both chambers. After sufficient trials were conducted, we removed these and introduced *T. hockingsi*. Here, we present some preliminary results from our glasshouse experiment.

First, 400 primary flowers were bagged, prior to opening, with organza bags, which still allow airflow and light to the flowers. Bags were removed from 100 flowers once they opened to allow unlimited access to bees during the receptive period (open pollination, OP).

As a negative control (BP), another 100 flowers remained bagged to exclude all foragers from accessing the flowers. As a positive control, an additional 200 flowers were hand pollinated, 100 with pollen from the same variety (HP), and 100 crossed with a different variety ('Valor', CP). This polliniser variety was developed in California, USA. Fruits are conical shaped and reasonably large, with firm texture, attractive red colouration and prominent seeds. Red Rhapsody flowers that were used for hand pollination were re-bagged immediately after treatment to prevent the bees from pollinating these flowers. All bags were removed from flowers ten days later, when flowers were no longer receptive to pollination, to reduce any bag effects on fruit development.

Second, we wanted to test how many visits were needed to ensure most flowers produced high quality, marketable fruits. We allowed both stingless bee species (*T. carbonaria* and *T. hockingsi*) different numbers of visits to strawberry flowers: 1, 2, 5, 10 and 15 visits. A total of 500 flowers (100 for each number of visits) were used for this. Flower buds were again bagged. When the flowers opened, bees were then allowed to land on a flower for the required number of visits and then we re-bagged immediately to prevent further pollination. Bags were again removed ten days later when flowers were no longer receptive to pollination.

Fruits were harvested when ripe (i.e. when more than 90% of the fruit surface was red). Physical fruit characteristics were then recorded in a number of ways. Fresh weight, basal circumference and length were taken to determine fruit size.

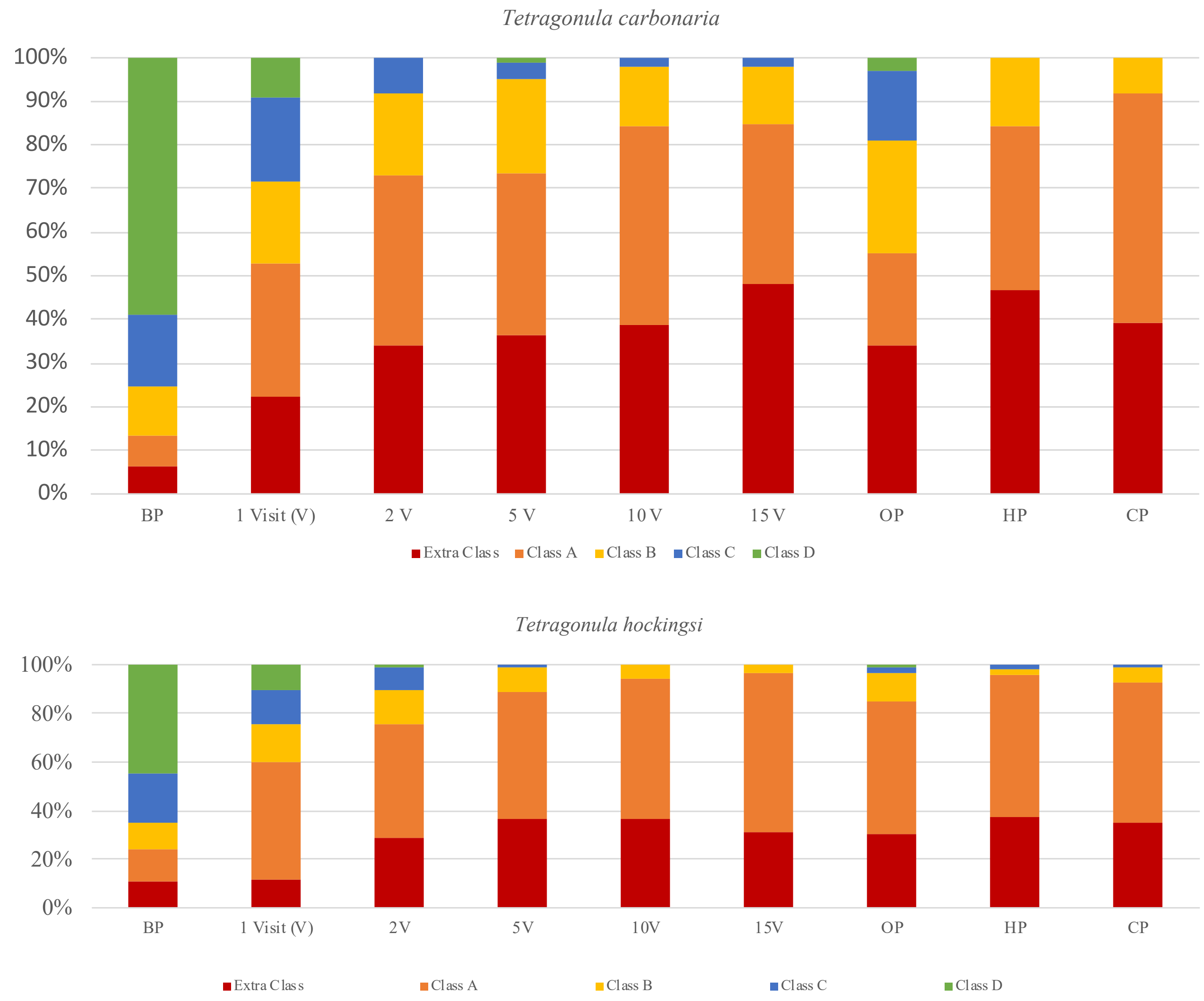
Fruits were also graded by their uniformity of shape, based on industry standards: Grade Extra, Grade A, Grade B, Grade C and Grade D. Grade Extra and Grade A constitute highly marketable fruits, while Grade C were of lowest marketable quality and Grade D were non-marketable (Figure 1).

Early signs indicate that stingless bee visitation to glasshouse strawberry crops clearly improves fruit quality. For both stingless bee species, the open pollination treatment (OP) had a higher percentage of high-quality fruits than negative controls (BP, Figures 2 & 3).

Hand pollination (positive control), both with the same variety (self-pollination, HP) and crossed with the polliniser variety 'Valor' (CP), produced the highest quality fruits from these trials. Fruit quality also increased with a greater number of bee visits, with 15 visits yielding 84% high quality fruits (Grade 'Extra class' and Grade 'A') from *T. carbonaria* (Figure 2) and 96% high quality fruits from *T. hockingsi* (Figure 3). The percentages were similar for both hand pollination treatments.

EXTRA CLASS: No ridge or deformity
CLASS A: Very little/minimal ridge or deformity
CLASS B: Some pronounced ridge/deformity
CLASS C: Much more pronounced deformity
CLASS D: Other deformities

Figure 1. Examples of strawberry fruit classified under each quality grading
Photo credit: Onyeka Nzie



Figures 2 & 3: The percentage of fruit of different quality grades for each treatment type: bagged pollination (BP), 1, 2, 5, 10 and 15 bee visits, open pollination (OP), hand pollination treatments with the same strawberry variety (HP) and cross-pollinated with variety "Valor' (CP). Records presented for visits by *Tetragonula carbonaria* (Figure 2) and *Tetragonula hockingsi* (Figure 3).
Photo credit: Onyeka Nzie

Our results also indicated that the quality of fruit from open pollination (unlimited bee visits) was lower than the quality of fruit from 10 and 15 visits (Figures 2 & 3). We believe this is a result of excessive bee visitation causing flower damage. We will repeat surveys this year with fewer hives to test optimal hive stocking rates in such environments. We are also unsure what the optimal number of visits is, so will increase the number of visits allowed in the next trial, to determine if fruit quality continues to improve, or we reach a saturation or even a tipping point. Finally, our results also indicate that the sugar content of strawberry fruits is higher with bee visitation compared with no bee visits. We will continue to investigate this and other chemical properties of strawberry fruits under glasshouse pollination conditions.

This research was conducted under the project "Stingless bees as effective managed pollinators for Australian horticulture", which is funded by the Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Western Sydney University and the Hawkesbury Institute for the Environment, Syngenta Asia-Pacific, OLAM, Griffith University and contributions from the Australian Government.

Mark Hall is a Postdoctoral Research Fellow, Robert Spooner-Hart & Markus Riegler are Associate Professors, James Cook is a Professor and Onyeka (Peter) Nzie is a PhD student; the glasshouse experiments form part of his PhD project supervised by the other authors.

SDGs: 2 15

PLAN BEE

In making plans to withstand a globally devastating bee parasite, researchers are finding other ways to help crops thrive.

Australia is the final stronghold against the Varroa mite (*Varroa destructor*). This microscopic parasite has sucked the life out of honey bee hives the world over, prompting headlines urging action, and global pro-bee campaigns. In addition to making honey, bees pollinate crops, and plants such as clover that add nutrients to pasture.

While Australia is still Varroa-free, it has been intercepted twice in the last five years coming in on ships. “Pretty much everyone thinks it’s a case of when we get it, not if we get it,” says Professor James Cook, leader of the Plants, Animals and Interactions research theme at Western’s Hawkesbury Institute for the Environment. He is leading a five-year, $19 million research push to better understand bees’ role in crop pollination, so that when Varroa arrives, Australia is prepared to minimise the damage.

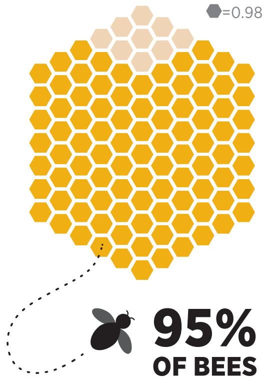
“We expect a rapid drop-off in honey bee colonies and that’s what we need to be ready for. But the good news is Varroa is really specific to honey bees so it’s not going to affect stingless bees, or our many species of solitary bees,” says Cook.

Contrary to popular understanding, European honey bees (*Apis mellifera*) are the exception when it comes to bees. Of the world’s 20,000 species of bees, only seven species live in large colonies, have stingers, and make honey. Around 95% of bees live on their own. There are also about 500 species of stingless bees, which also live in colonies, but most don’t make enough honey for humans to harvest. Many of these lesser-known bees, along with some other insects, such as flies, are consummate pollinators, and they could be the insurance policy Australian farmers are looking for. But much needs to be learned before their role as a safeguard against honey bee losses can be assured.

NEED TO KNOW

- Australian stingless bees are being tested as crop pollinators.
- Stingless bees are not affected by the devastating Varroa mite.
- Stingless bee knowledge could be exported to help farmers globally.

BUZZ OFF AND LEAVE ME ALONE



95% OF BEES live on their own

STING LIKE A BEE?

There are 500 SPECIES of stingless bees

This multi-pronged project is funded by a partnership initiative developed by Hort Innovation, with co-investment from Bayer CropScience, Syngenta Asia-Pacific, the food and agri-business company, OLAM, and Greening Australia, along with contributions from the Australian Government.

Ashley Zamek is the manager of the programme at Hort Innovation. She says the research is about protecting pollination. “We have 35 member organisations at Hort Innovation and 25 of them depend on pollination. We don’t want to put all our eggs in one basket so we’re exploring stingless bees, flies, and honeybees and the pollination services they provide.

“WSU proved to be the best at delivering this type of research. Their state-of-the art facilities including greenhouses and polytunnels meant they have the infrastructure to work with us.”

Of the many avenues Professor Cook is exploring, one is deploying stingless bees into glasshouses containing commercial crops. In this regard, they may be superior to the honey bee.

“Honey bees don’t like being in glasshouses and polytunnels and they sting people who are working there. So, having a stingless bee is good from that point of view,” says Cook.

His team have moved hives of *Tetragonula carbonaria*, a small black Australian stingless bee commonly known as the sugarbag bee, into glasshouses to see how the bees coped. They weighed the bees before and after they were put in the greenhouse, took note of honey and pollen stocks in the hive, and monitored their behaviour for any unusual signs.

They found that many bees spent the first two weeks pressed up against the glass trying to get to their usual feeding fields, but then the colonies settled down and began to enjoy the blossom banquet before them.

Kayla Le Gros is a masters student working on the bee project. She looked specifically at whether the native bees can navigate successfully in glasshouses, and whether a UV-blocking film on the glass affected the bees. Honeybees navigate using the ultraviolet end of the light spectrum, but it was not known whether *T. carbonaria* bees also do.

“With the native bees we found there was absolutely no difference between the two claddings, so that meant they were using colour vision or landmarks to find their way to and from the hive,” says Le Gros. With or without the film, the bees found their way around the glasshouse with no trouble.

Her finding is a sweetener for the case for stingless bees, as UV-blocking glass is preferred by horticulturalists.

Now other members of Cook’s team are looking at finer details such as whether the stingless bees do better with pollen, sugar or floral supplements to their diets and examining their adequacy as pollinators for specific crops.

The first test is with strawberries, usually grown in long polytunnels. Individual strawberry plants are covered with a small muslin bag to prevent accidental pollination, then uncovered to let the bees in. Researchers observe bees visiting and then re-bag the plant after a flower has had one, two, or three visits. They then monitor the plants and record the number of fruits produced, and their quality, corrrelating this with the number of bee visits.

The lessons learned on Australian stingless bees could very easily be applied to stingless bees native to other locations around the world; Indonesia, Malaysia, India and Thailand have very closely related stingless bees and are also keen to make more use of them. Cook expects there will be transfer of methods, equipment and knowledge between countries.

With insect species around the world threatened by pesticide use, loss of habitat and growing urbanisation, a deeper understanding of the unseen hand of the pollinators is essential for our own species. As Cook puts it: “Our fate is very intertwined with that of pollinators.”

©James Cook

This research was funded by Hort Innovation through the Hort Frontiers Pollination Fund.

> “OUR FATE IS VERY INTERTWINED WITH THAT OF POLLINATORS.”

Stingless bees, such as *Tetragonula carbonaria* are not susceptible to the Varroa mite.

AUSTRALIAN MACADAMIA SOCIETY

PRINT POST APPROVED: 100000824 ISSN 0811 - 3471

WINTER 2020 | VOLUME 48 | NUMBER 2

NEWS BULLETIN

A profitable and sustainable industry supplying quality macadamias for a global market

Macadamia pollination

Research shows us that pollination – whether by European honey bees, native stingless bees or other insects – is vital for nut set and ultimately productivity.

The photos these authors have offered us for this special feature are absolutely stunning – enjoy!

Photo: Brian Cutting

The importance of macadamia pollination: an overview

Stephen Trueman, Professor of Plant Science, & **Helen Wallace,** Professor of Agricultural Ecology, Griffith University

Macadamia yields are limited by a harmful combination of cultivar self-sterility and limited movement of pollen by bees. Self-pollination produces very few nuts in some cultivars. Recent research has shown that nut production in these self-sterile cultivars is almost totally dependent on pollinators transporting cross-pollen from the flowers of one cultivar to the flowers of another cultivar. This process is often occurring inadequately, leading to poor pollination success and reduced yield. This article describes what we currently know about pollination in macadamia, which cultivars are self-sterile, how self-sterility affects yield, and what to look for in the orchard for signs of poor pollination.

AUSTRALIAN MACADAMIA SOCIETY

AusMac2020

20-22 October Sunshine Coast Convention Centre QLD

Stephen Trueman and Helen Wallace will be hosting the **How can growers improve pollination for better yields?** workshop at AusMac2020 on Wednesday 21 October. This is a must-attend event for growers needing answers to these questions:

- What is self-pollination?
- Which cultivars require cross-pollination?
- How does more effective pollination improve yield?

Tickets on sale now via the AMS website.

Macadamia trees can produce about 2,500 racemes each year, with each raceme having about 200 flowers. That's about half a million flowers. Each tree would produce 3,500 kg of nuts if every flower produced a 7-gram nut. More realistically, each tree could produce 35 kg of nuts if just 1% of the flowers produced nuts. Why are we not achieving this sort of yield? Average industry productivity is currently around 8–9 kg/tree. Is poor pollination limiting nut production?

Pollination and nut set

Macadamia flowers have four petal-like segments, four stamens (male parts), one pistil (the female part, made up of stigma, style and ovary), and nectaries to attract insects (Figure 1). The anthers release their pollen before the flower opens and so self-pollen is already sitting on top of the pistil when the flower opens. The stigma only becomes sticky 1 or 2 days after the flower opens, by which time pollinators may have deposited fresh self-pollen (from the same cultivar) or fresh cross-pollen (from a different cultivar).

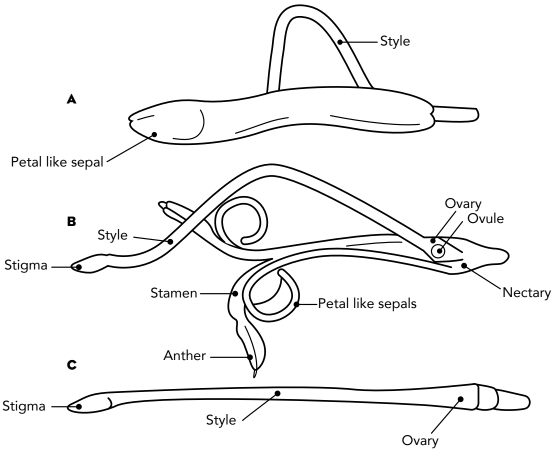


Figure 1. The parts of a macadamia flower showing (B) the stamens and (C) pistel (made up of stigma, style and ovary). (Image: www.apiservices.biz)

Honey bees and stingless bees are the main pollinators in Australian orchards, but other insects and birds are sometimes seen on the flowers and may also transfer pollen. The stigma is too small for significant wind pollination and the pollen does not disperse well in the wind. Racemes that are bagged with mesh that allows air movement, but excludes insects, produce very few nuts. Racemes that are bagged during the day, but not at night, also produce very few nuts, demonstrating that the major pollinators of macadamia are not active at night.

We have known for a long time that macadamia flowers are partially self-incompatible, with self-pollination resulting in much less pollen tube growth and initial nut set than cross-pollination. Many of the developing nutlets fall during premature nut drop in spring. It has not always been clear whether yield is limited by poor cross-pollination, because a successful pollination season might simply be followed by a heavy premature nut drop. For example, premature nut drop might also be affected by other factors such as soil moisture levels, crop nutrition, photosynthesis, carbohydrate reserves, pests or diseases. However, there is growing evidence that self-sterility and poor cross-pollination are major factors that limit yield in Australian orchards.

Figure 2. Trees of cultivars 816 (rows on the left) and Daddow (row on the right) with hives of honey bees. Almost all nuts of 816 were pollinated by Daddow and almost all nuts of Daddow were pollinated by 816.

Self-sterility in macadamia cultivars

Recent advances in DNA analysis mean that we can now identify a nut's father (the pollen parent) just as DNA analysis can be used to identify a person's father. We have used this forensic approach to determine whether nuts in commercial orchards are produced by self-pollination or cross-pollination. DNA analysis is most informative when nuts are sampled in the middle of large blocks of a single cultivar, where levels of self-pollination should be highest.

CSIRO found in the late 1990s that 86–90% of nuts were cross-pollinated in the middle of a 27-row block of A16 trees (MC98027 *Maximising the benefits of cross-pollination in macadamia orchards*). Recently, our team has found that at least 83–88% of nuts were cross-pollinated in the middle of a 42-row 816 block or 48-row Daddow block, respectively (*AMS News Bulletin,* Winter 2019, pp. 72–73). These results strongly suggest that A16, 816 and Daddow are self-sterile and require pollen from another cultivar to produce a good nut set. Similar results from a narrower 5-row block suggest that A4 might also be self-sterile.

Cathy Nock's team at Southern Cross University has also found that nuts from single trees of A4, A16, 246, 344 and 800 in a regional variety trial were mostly or totally cross-pollinated (*AMS News Bulletin,* Autumn 2020, pp. 70–72). This was expected because trees of the different cultivars were interplanted closely. However, 20–40% of nuts from individual 660, 741, 791 and 842 trees were self-pollinated. This suggests that some cultivars might be self-fertile. We are now undertaking a wider screening program to identify cultivars that are self-sterile and cultivars that are self-fertile.

Self-sterility effects on yield

Macadamia yields might be limited by the amount of pollen that bees transport across the orchard from one cultivar to another cultivar. If this were true, then yields would decline as we move away from a cross-pollen source and into the middle of large blocks of a single cultivar. This is definitely the case in large blocks of A16 and 816 trees.

Yields were 20–42% lower in the middle (14th row) of the 27-row block of A16 than in the first five rows near the adjacent block of A4 (MC98027). Yield was 54% lower in the middle of the 42-row block of 816 than in the row nearest the adjacent block of Daddow (*AMS News Bulletin,* Spring 2019, pp. 68–70).

Low yields in the middle of the large blocks did not show definitively that poor cross-pollination was the problem. These rows could somehow have had soil moisture, crop nutrition, pest or disease problems that were limiting the trees' nut carrying capacities. We tested whether cross-pollination was the limiting factor by cross-pollinating entire trees in the middle of the large blocks of 816 and Daddow to see whether these trees could produce more nuts. We also cross-pollinated entire trees in the rows next to the other cultivar (Figure 2), with the idea

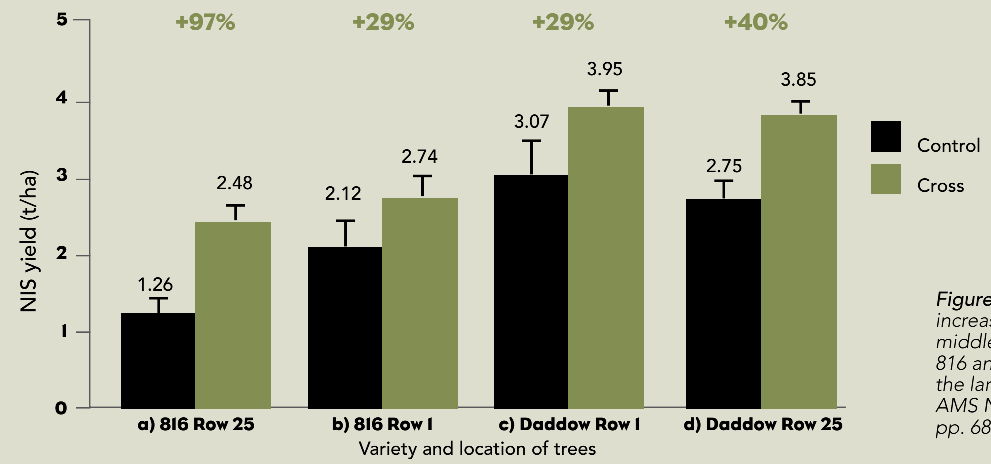


Figure 3. *Cross-pollination increased yields by 97% in the middle of the large blocks of 816 and 40% in the middle of the large blocks of Daddow. See AMS News Bulletin, Spring 2019, pp. 68–70.*

that, even here, yield might be limited by poor cross-pollination.

Cross-pollination increased yields by 97% in the middle of the large blocks of 816 and 40% in the middle of the large blocks of Daddow (Figure 3). This showed that the trees could produce many more nuts, and that poor cross-pollination was the cause of lower than expected yield. Furthermore, cross-pollination increased yields by 29% in both the 816 row and the Daddow row that immediately faced each other (Row 1). Here, too, yield was limited by the amount of cross-pollen that the flowers were receiving.

Research shows us cross-pollination of racemes increases:

- **pollen tube growth**
- **fruit set**
- **nut-in-shell and kernel mass**
- **kernel recovery**

Figure 4. *Bees quickly remove self-pollen from the tips of macadamia flowers. Here, many flowers on the left- and right-hand sides of the photograph still retain clumps of self-pollen on the tops of the pistil (see orange circles), whereas bees have removed self-pollen from many flowers in the centre of the photograph (white circle).*

Classic signs of poor cross-pollination in your orchard

There are some classic signs that poor cross-pollination could be a problem in your orchard. These include:

1. Flowers should not have large clumps of self-pollen remaining on them in the middle of the day. Macadamia flowers have clumps of self-pollen sitting on the top of their pistil when they open in the late afternoon or early morning. This self-pollen is quickly removed during the morning when there are large numbers of pollinators in the orchard (Figure 4). However, self-pollen sitting on flowers throughout the middle of the day is a sure sign that the flowers have not been visited by pollinators.
2. Most racemes should still have nutlets at 2–3 weeks after flowering. We observed that cross-pollinated trees had much higher initial nut set than control trees. Cross-pollinated racemes were loaded with nutlets whereas many racemes on control trees were bare, or almost so (Figure 5). The cross-pollinated trees were not able to carry all of these nutlets to maturity, but they did have 29–97% higher yields than control trees.
3. Yields should not decline with increasing distance from another cultivar, or with increasing distance from introduced or feral bee hives. Yields that decline in this manner are a sign that poor cross-pollination is a problem, unless there is some other underlying effect such as changing soil type, soil moisture, crop nutrition, or pest or disease incidence across the orchard. Detailed yield records of individual rows, if not individual trees, are the best way to identify pockets of poor cross-pollination in the middle of single-cultivar blocks or at long distances from bee hives.

Figure 5. Heavy initial nut set on trees that were cross-pollinated (top left and top right) compared with very low initial nut set on trees that were not cross-pollinated (bottom left and bottom right).

What we still don't know

There are still some major gaps in our understanding of macadamia pollination. We don't know at what distances from another cultivar the yields begin to decline, and how best to interplant cultivars in orchards to maximise cross-pollination. Further yield observations are required in different orchard designs to determine how wide each single-cultivar block can be before nut production decreases. We also don't know how many bees we need, how they move through the orchard, and how best to place them in the orchard, to make sure we have enough cross-pollination.

Ongoing work by numerous researchers is attempting to answer these questions to ensure macadamia pollination rates, nut set and yield continue to grow.

About the authors

Stephen Trueman is Professor of Plant Science and Helen Wallace is Professor of Agricultural Ecology at Griffith University, Nathan Campus, Brisbane.

Acknowledgements

Project PH16001 is funded by the Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Griffith University, University of the Sunshine Coast, Plant & Food Research Ltd and contributions from the Australian Government.

MC302 and MC98027 were funded by Hort Innovation, using the macadamia research and development levy and contributions from the Australian Government.

We thank Duane Joyce, Johan Oosthuizen, Ngarie Myers and Ray Norris for assistance and orchard access, and Chris Searle for valuable advice and assistance.

Bee foraging behaviour and pollen transport: what does it mean for pollination?

Dr Mark Hall, The Hawkesbury Institute for the Environment at Western Sydney University

A native stingless bee, Tetragonula carbonaria, *carrying a full pollen load in the pollen baskets, or corbiculae, on its hind legs. Photo: Ian Watson*

A key consideration for any grower of fruits, vegetables or nuts that rely on pollination, is how to get the best pollination possible to maximise yield and quality. Bees are arguably the best option, as they have physical features specifically designed for the transport of nectar, and more importantly, pollen. Bees rely on these food sources for their sugar and protein, while plants benefit by having an efficient vector to transport pollen from one flower to another, thus achieving pollination. But are some bees better pollinators than others? And if so, which features of the different species best promote pollination?

The European honey bee (*Apis mellifera*) is often the first option used by growers, as they visit most flower types, have large colonies, undertake almost year-round foraging and are relatively easy to manage. These characteristics make them a good choice for many growers.

Other characteristics of honey bees also ensure good pollination. First, like most bees, they are quite hairy, which is good for picking up pollen from flowers. They also let their nest mates know where a good resource is, through the well-known 'waggle dance'. If at least some of the foragers find your macadamias, they will likely let their sisters know.

The sheer numbers of this species ensure many will visit macadamia, but they aren't the most efficient pollinators out there. Honey bees scrape much of the pollen from their bodies and transport it in special pollen baskets (corbiculae) on their back legs. This means not as much pollen rubs off on the next flower they visit, but through repeated visits, they achieve adequate pollination.

There are a group of native bees that likely already visit your macadamia flowers and can be easily managed if desired – stingless bees. Australia is home to at least 11 species of stingless bees; so named because they don't have a functional stinger, unlike the honey bee which can cause major health concerns. These bees also form large colonies and carry pollen in corbiculae (see picture) but they are much smaller than honey bees. They have soft grey hairs clumped mostly in the middle of their bodies (thorax) but have branched hairs on their legs and abdomen that are ideal for carrying pollen. Being small, their behaviour on flowers differs from honey bees.

In fact, all bees can behave differently on flowers while collecting nectar and pollen. Some species (or even individuals) visit quite quickly, only making brief contact with the reproductive parts of flowers. Being small, stingless bees tend to spend time walking around the whole flower and make lots of contact with pollen and the stigma, which makes them effective pollinators. Sometimes species like the honey bee will hang off flowers and only put their head in for a drink, or others

A native leaf-cutter bee (Megachile sp.) carrying large amounts of pollen on its abdomen and some on its legs and body. Photo: Michael Duncan

A native blue-banded bee (Amegilla sp.) showing pollen attached to its hairy hind legs. Photo: Michael Duncan

may rob nectar by puncturing the bottom of the flower and not even touch the pollen.

Of the stingless bees, two species (*Tetragonula carbonaria* and *T. hockingsi*) are most commonly managed for their pollination service. And being native to the growing region of macadamia, they are well adapted to conditions. To date, both species have been successful at pollinating macadamia.

As well as the colonial (or Eusocial) bees, Australia is estimated to have 1,600 to 2,000 solitary native bee species. These other wild bees, such as blue-banded, leaf-cutter or sweat bees mostly carry pollen on their immensely hairy legs or abdomen (see pictures above). This mode of pollen transport means more pollen can be moved between flowers. Thus, wild bees are great pollinators on a per-visit basis as more pollen gets where it needs to go, but because honey bees and stingless bees live in large colonies, their sheer numbers make them great managed pollinators.

However, another consideration for bees is whether they can access the nectar provided by flowers. Bee tongues are either short or long, and this dictates which flowers they will visit. So while stingless bees and honey bees will happily visit macadamia flowers, some other wild species may not because they don't receive any sugar reward.

To understand pollination, watch your macadamia flowers closely. See what is visiting the flower and how they are collecting pollen and moving it between flowers. Also watch how they behave on flowers and you will soon get a sense of how different pollinators play a role in increasing the productivity of the plants.

Don't get discouraged if not all bees visit your macadamias. The social bees, such as honey bees and stingless bees, often visit the same type of flower in any foraging bout, something we call floral constancy. But because they live in large colonies, not all individuals visit the same type of flower. Some will visit the macadamia, while others will look in the surrounding landscape. In fact, bees are much healthier if they can feed from a diverse range of floral sources, so having some other flowering plants close to the crop may take a few bees away from the crop in the short term, but will keep the colonies much healthier in the long term, so they can continue to deliver great pollination to your macadamias for many seasons to come.

About the Author

Dr Mark Hall is a pollination ecologist with The Hawkesbury Institute for the Environment at Western Sydney University. His research focusses on stingless bee pollination in temperate and glasshouse crops, conducted under the *Stingless bees as effective managed pollinators for Australian horticulture project,* funded by Hort Innovation Australia. He is also a board member of Wild Pollinators Oceania, who host the bi-annual Wild Pollinator Count.

Acknowledgements

Stingless bees as effective managed pollinators for Australian horticulture (PH16000) is funded by the Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Syngenta Asia-Pacific, OLAM, Griffith University and contributions from the Australian Government.

The potential role of stingless bees in mango pollination

Gaurav Singh, James Makinson, Robert N. Spooner-Hart and James M. Cook

Stingless bees (Hymenoptera: Apidae) are eusocial bees (Michener, 2000) and closely related to honeybees, bumble bees, carpenter bees and orchid bees (Roubik, 1989). There are over 500 species of stingless bees worldwide, belonging to 36 genera, found in most tropical and subtropical parts of the world; including Africa, Australia, Southeast Asia and tropical America (Michener, 2000). Stingless bees are common flower visitors to at least 90 crop species and have been reported as effective pollinators of nine cultivated food and fruit crops, including mango (Heard, 1999). They can be one of the most common mango flower visitors (Willcox *et al.,* 2019; Anderson *et al.,* 1982) and are the only bees, other than honeybees, that can be effectively managed in hives and deployed into mango crops in tropical regions.

Mango (*Mangifera indica* L.) is one of the most widely grown and important fruit crops worldwide (FAOSTAT, 2018). A typical mango tree has hundreds of pyramidal panicles, which can grow up to 30 cm long and produce thousands of tiny flowers of about 5-10mm in diameter (Figure 1a & b). Mango has two types of flowers: male (staminate) and hermaphrodite (or perfect) flowers (Figure 1c).The flower sex ratio varies between cultivars, environmental conditions and even among trees of the same cultivar (Ramírez & Davenport, 2016). The importance of insects in mango pollination has frequently been demonstrated and a number of insects, including different species of honeybees, stingless bees and flies, frequently visit mango flowers (Ramírez & Davenport, 2016; Huda *et al.,* 2015; Dag & Gazit, 2000).

The European honeybee, *Apis mellifera* Linnaeus, is considered an effective mango pollinator in some parts of the world; however, in many countries, it often plays a negligible role in mango pollination and only visits mango flowers occasionally(Ramirez and Davenport, 2016). Native stingless bees and/or flies are reportedly more effective pollinators than honeybees in Australia (Willcox *et al.,* 2019; Anderson *et al.,* 1982), India (Reddy, 2010) and some other countries (Huda *et al.,* 2015; Sung *et al.,* 2006).

Our research at Western Sydney University focuses on investigating the pollination efficiency of stingless bees and other wild pollinators in mangoes. In 2019, we performed floral visitor surveys on plantations of cv. Kensington Pride mangoes at eight field sites in the Northern Territory (NT), Australia. Our surveys show that a stingless bee, *Tetragonul amellipes* (Friese), is the dominant flower-visiting insect, followed by a hoverfly, *Mesembrius bengalensis* (Wiedemann), and then blowflies, *Chrysomya* sp. (Singh *et al.,* 2019).

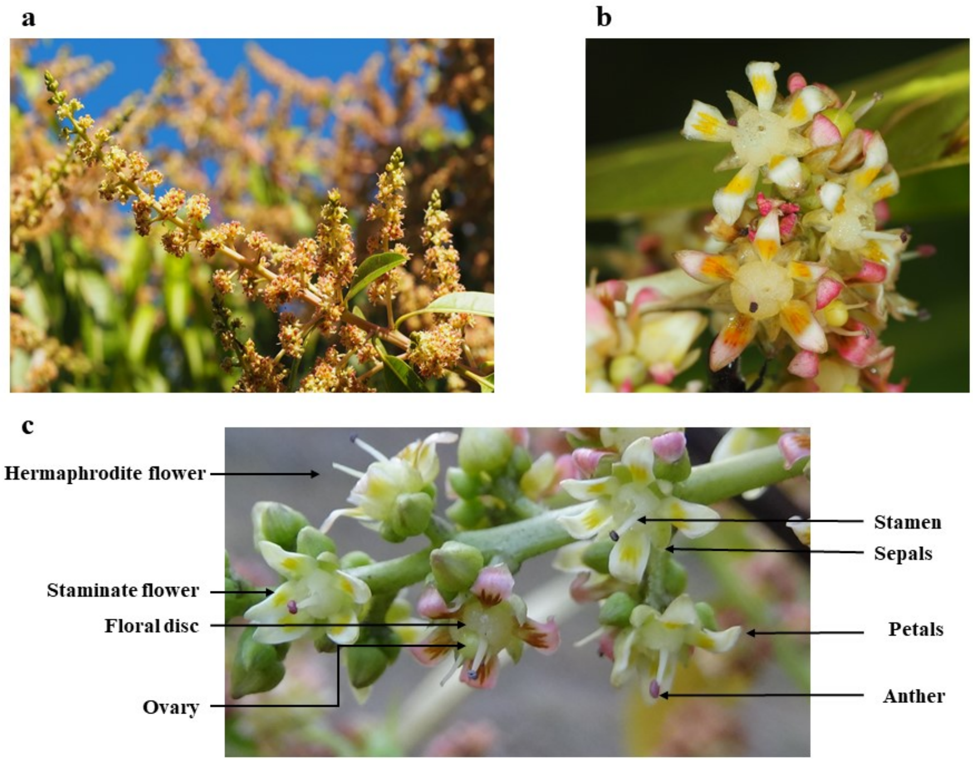


Fig. 1. Typical structure of a panicle (a) and flower (b) and, different types of flowers and floral morphology (c) of Kensington Pride mango variety (Photo: James Makinson and Gaurav Singh)

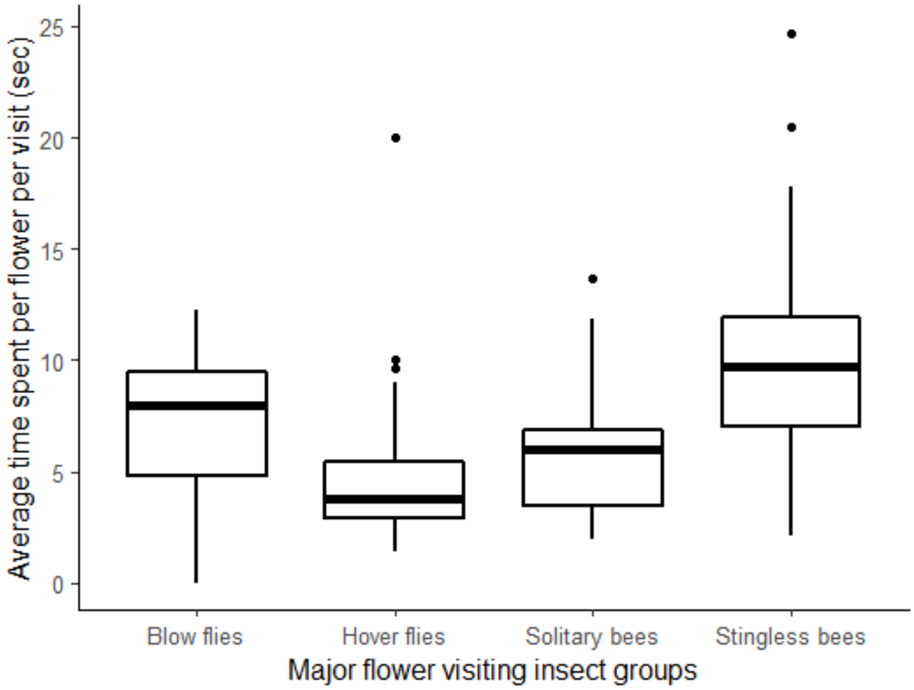


Fig. 2. Average time spent per flower by major floral visitors in mango orchards

Stingless bees actively forage for both nectar and pollen and spend more time on mango flowers than flies and honeybees (Figure 2). Owing to their small size, stingless bees fit completely in the middle of a mango flower (Figure 3) and repeatedly walk around and across the flowers with their abdomen and legs actively contacting the flower's reproductive parts. Stingless bees carry pollen grains distributed widely over their bodies and deposit more pollen grains per single visit compared to other pollinators (Willcox *et al.,* 2019; Anderson *et al.,* 1982). Surprisingly, only a few *A. mellifera* visits were recorded during our surveys at any of the eight farms in the NT, which suggests that mango flowers are not a first-choice food for honeybees.

Fig. 3. A stingless bee, *Tetragonula mellipes* on a mango flower (Photo: James Makinson)

Our observations show that stingless bees frequently visit mango flowers in the NT of Australia, but occur in low numbers towards the centre of orchard blocks of trees. Therefore, moving stingless bee hives into the mango crop is likely to increase their abundance and ability to provide pollination services across the orchards, potentially increasing the productivity of mango farms.

Acknowledgements:

"Stingless bees as effective managed pollinators for Australian horticulture" is funded by the Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Syngenta Asia-Pacific, OLAM International, Western Sydney University, Griffith University and contributions from the Australian Government.

We would like to express our sincere thanks to the staff and management of Cheeky Farms, Jenko's Mangoes, Skliros Produce, Tou's Garden Pty Ltd, Manbullo Ltd, and PTA Docking Pty Ltd, for allowing researchersto work and stay on their properties.

References

Anderson D L, Sedgley M, Short JRT, Allwood A J.1982. Insect pollination of mango in Northern Australia. Australian

Journal of Agricultural Research 33(3): 541–548.

Faostat. 2018. Statistical databases. Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat.

Heard T A. 1999. The role of stingless bees in crop pollination. Annual Review of Entomology 44(1): 183–206. https://doi.org/10.1146/annurev.ento.44.1.183

Huda A N, Salmah M R C, Hassan A A, Hamdan A, Razak M N A. 2015. Pollination services of mango flower pollinators. Journal of Insect Science 15(1):113 https://doi.org/10.1093/jisesa/iev090

Michener C. 2000. The bees of the world. Baltimore, London: John Hopkins University Press. 953 pp.

Ramírez F, Davenport T L. 2016. Mango (*Mangifera indica* L.) pollination: A review. Scientia Horticulturae 203: 158–168. https://doi.org/10.1016/j.scienta.2016.03.011

Reddy P V R, Verghese A, Dinesh M R. 2010. Diversity and foraging behaviour of pollinator fauna in mango. Proceedings. National Symposium on Perspectives and Challenges of Integrated Pest Management for Sustainable Agriculture, Nauni, Solan, Himachal Pradesh.

Roubik D W. 1989. Ecology and natural history of tropical bees. Cambridge University Press, Cambridge.

Singh G, Spooner-Hart R N, Cook J M, Makinson J C. 2019. Impact of distance from native vegetation and pesticide spray regime on potential pollinators diversity and abundance in mango farms in Northern territory, Australia. Proceeding. XIX International Plant Protection Congress IPPC 2019, Hyderabad, Telangana, India. pp. 56.

Sung I H, Lin M Y, Chang C H, Cheng A S, Chen WS, Ho KK. 2006. Pollinators and their behaviors on mango flowers in Southern Taiwan. Formosan Entomologist 26: 61–170.

Willcox B K, Howlett B G, Robson A J, Cutting B, Evans L, Jesson L, Kirkland L, Jean-Meyzonnier M, Potdevin V, Saunders M E, Rader R. 2019. Evaluating the taxa that provide shared pollination services across multiple crops and regions. Scientific Reports 9(1): 13538. https://doi.org/10.1038/s41598-019-49535-ws

AUTHORS

Gaurav Singh, James Makinson, Robert N. Spooner-Hart & James M. Cook - Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW, Australia, 2751. (Corresponding author: G. Singh) Email: g.singh4@westernsydney.edu.au