

Horticulture Innovation Australia

Final Report

Optimising pollination of macadamia & avocado in Australia

The New Zealand Institute for Plant and Food
Research Ltd

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Summary

The New Zealand Institute for Plant & Food Research Limited commenced a project in late June 2014 to look at optimising the pollination of macadamias and avocados in Australia.

The project aims were to establish:

- The actual and potential rates of pollination in macadamia and avocado
- The floral biology of macadamia
- The key pollinators of macadamia and avocado
- Recommendations for optimising pollination.

In Australia, a significant amount of pollination occurs as a free service by feral honey bees and by other unidentified unmanaged species. The pollination services of feral honey bees will probably be significantly diminished should the varroa mite reach Australia. Therefore, it was important to understand the current rates of pollination within these crops, the relative contributions of honey bees versus other pollinators, including native bees, and which key pollinators could be better managed for pollination. It was also expected this research would highlight the importance of managed pollination for growers.

The goals of this milestone, in addition to previous milestones, were to have completed:

- All necessary reports complying with Horticulture Innovation Australia's requirements received and approved by Horticulture Innovation Australia Ltd
- Report on findings at one additional industry workshop on macadamia pollination
- Submit one additional article to a grower magazine on macadamia pollination.

Keywords

Pollination, macadamia, avocado, floral biology, bees, flies, beetles, *Apis*, Diptera, Coleoptera, crop yield, pollenizer, Sunraysia, Australia, Hass, Queensland, New South Wales, Tri-state

Pollination, macadamia, avocado, floral biology, bees, flies, beetles, *Apis*, Diptera, Coleoptera, crop yield, pollenizer, Sunraysia, Australia, Hass, Queensland, New South Wales, Tri-state

Introduction

1 Milestone achievements

1.1 Reports complying with Horticulture Innovation Australia's requirement completed

All field trials have been completed and key findings have been prepared in a series of reports given in section 3 (macadamias) and section 4 (avocados).

In summary, the project goals were to establish:

- The actual and potential for nut set due to pollination in macadamia
- The floral biology of both avocados and macadamia
- The key pollinators of avocados and macadamia
- Recommendations for optimising pollination

We have delivered numerous outputs throughout the programme, and these have been reported through earlier reports. During this milestone period (1 March 1 to 25 October 2016) we have reported on findings to industry and growers through:

- Howlett BG 2016. Macadamia Pollination, R&D Speed dating, chaired pollination session. Australian Macadamia Society Conference, Caloundra, 18–20 October 2016.
- Howlett B 2016. Pollination in Macadamias. Bundaberg Pollination Workshop, Macadamia's, Australia, QLD, 16 June 2016.
- Howlett B 2016. Pollination in macadamias; update from Plant & Food Research NZ. AMS – Consultants Meeting, Macadamia's Australia, Brisbane, QLD, 8–9 June 2016.
- Howlett B 2016. A different perspective on pollination of Avocado in Australia's Tri-State region. Qualicado Workshop Bundaberg, QLD, 11 August 2016.

We also prepared an article published in the AMS News Bulletin:

- Howlett B, Goodwin M, Cutting B, Cross S, Read S, Wilcox B, Pattemore D 2016. Does pollination between different Macadamia varieties improve yield? AMS News Bulletin, August 2016.

We provide a list of outputs delivered throughout the project in Section 1.1.3.

1.1.1 The actual and potential for nut set due to pollination in macadamia

Macadamia is considered partially self-incompatible and previous research indicates that for at least some varieties, cross-pollination can result in increased yields. A number of studies have drawn conclusions on improved yields from cross-pollination by examining initial nut set. However, this does not always reflect final nut set. Moreover, research has focussed on single raceme pollinations to draw conclusions on potential nut yields within macadamia, while detailed self-pollination experiments across the many varieties (nearly all identified as numbers) remain limited.

In summary, we demonstrated that cross pollinating single racemes within trees of various varieties increased final nut set within these racemes. This suggests a greater yield can be achieved when

macadamia is cross pollinated. However, we found that this effect declined in magnitude when we cross-pollinated multiple racemes per tree.

While increasing the number of racemes cross-pollinated per tree was associated with a decline in nut set in two varieties (indicating resource allocation within a tree) an assessment of nut yields in one of these varieties found that hand cross-pollination still led to significantly higher nut yields when we conducted quadrat counts of nut set to cover a higher proportion of racemes on the tree.

We therefore recommend that cross pollination be encouraged, in order to increase nut yields in the varieties we assessed.

Further details are provided in the accompanying report (Sections 3 and 4).

1.1.2 The floral biology of avocado and macadamia

Avocado

Avocado flowers open first as functionally female, then close before opening as functionally male on subsequent days. Pollination only occurs when female flowers are open and receptive, and when there is pollen available for transfer (either from male 'Hass' or other cultivars). Avocado cultivars are grouped in to those that flower as female in the morning and male in the afternoon (such as 'Hass') and those that flower as female in the afternoon and male in the morning. However, this description is highly simplified, as lower overnight minimum temperatures, along with other environmental factors, can delay the opening of female flowers. Furthermore, depending on the temperature, there can be considerable overlap between male and female stages in the middle of the day. Using a model that we developed which uses overnight temperature to predict when female flowers open, we evaluated temperature data collected from orchards in the Tri-State region along the Murray River. We found that the daily pattern of female flower opening times within orchards in the Tri-State region could be predicted based on the variation in minimum overnight temperatures. The presence of diverse wild pollinators that are active at different times and different weather conditions will maximise the chances of pollination in these orchards where the window period for pollination is highly variable.

Macadamia

We conducted an extensive literature review on the floral reproductive biology and pollination of macadamia that resulted in the scientific publication:

- Howlett BG, Nelson WR, Pattemore DE, Gee M 2015. Pollination of macadamia: review and opportunities for improving yields. *Scientia Horticulturae* 197: 411–419. DOI: 10.1016/j.scienta.2015.09.057

The studies undertaken in this project aimed to address some of the outstanding questions regarding floral reproductive biology of commercial macadamia grown in orchard conditions. We found that:

- Cross-pollination of single racemes within trees of various varieties resulted in increased yields in the majority of cases. This suggests a high degree of flexibility in mixing macadamia varieties within blocks to increase yields.
- A degree of self-pollination occurs in at least some varieties. However, this may vary with variety. Self-pollination is likely to explain why isolated blocks of a single variety still produce nuts. However, cross-pollination of racemes still resulted in increased nut set, pointing to potential yield increases in single variety orchards.
- Hand cross-pollinating larger numbers of racemes within trees of variety '741' and '842' resulted in a lower number of nuts set in these racemes than in trees where just one raceme was cross-pollinated. Despite this, our quadrat counts of final nut set still showed an increase in

number, on average, compared with trees that were not hand cross-pollinated (only '741' assessed). As cross pollination still results in increased nut set, we recommend orchard designs incorporate more than one variety to promote cross-pollination.

Determining the abundance and distribution of pollinating species is key for determining current and potential pollination within orchards. When the efficiency of a pollinating species has been determined, then knowledge of its abundance within the orchard will provide the measure of its effectiveness. Less efficient species can be more effective pollinators than more efficient species if they occur in greater abundance. Knowing the effectiveness of all key pollinating species is necessary to compare the relative rate of pollination within and between orchards and regions.

In year 1, a reference collection of flower-visiting species was established by collecting flower visitors across orchards in Central Queensland, South East Queensland and Northern Rivers New South Wales. This formed the basis for identifying key flower visiting species for year 2 surveys.

In year 2, observational surveys of flower-visiting insects were conducted across 23 orchards. Within each orchard, one to three varieties – 741, 344, 842 and/or Daddow – were surveyed. Orchards were located in Central Qld (CQ), South East Qld (SEQ) and Northern Rivers NSW (NR). Twelve trees were surveyed per orchard block and each tree surveyed at three observation periods beginning at 9 am, 12 pm and 3 pm. In total, 180 observation periods were conducted across all orchards.

Preliminary examination of the data indicates 3633 flower-visiting individuals were observed. Honey bees (*Apis mellifera*) were the dominant visitor 77.9% (CQ 86.7%, SEQ 78.4% and NR 55.0%), followed by stingless bees (*Tetragonula carbonaria*) (13.8% SEQ 15.7%, NR 1.6% and CQ n=1). Other flower visiting species were uncommon and numbers varied between regions. The Lycid beetle *Metriorrhynchus rhipidius* was more often observed in SEQ (n=28) and NR (n=15), while the Chrysomelid beetle, *Monolepta australis*, was more commonly observed in NR (n=92).

Numbers of insects observed per orchard were highly variable, with some orchard blocks recording less than five flower visitors across the entire day, whereas in others, more than 100 individuals were recorded.

The preliminary findings indicated that pollination of flowers within racemes was likely to be highly variable and apparently very low in some orchards. This study highlights that for many orchards, few insects are providing a pollination service. There is significant potential to increase the role of insects in pollination through the development of strategies to promote their abundance.

1.1.3 Relationships between avocado flower phenology and temperature

Avocado flowers open first as female, then close and reopen on subsequent days as male. Pollination occurs when female flowers are open and receptive, and when there is pollen available for transfer either from male flowers on the same or other cultivars. Weather conditions affect the timing of flowering opening, and thus the time at which pollination can occur. In previous studies, we established the relationship between overnight minimum temperatures and the timing of the opening of female 'Hass' avocado flowers; colder overnight minimums delay the opening of 'Hass' flowering, shifting the pollination period into the afternoon, evening or even night.

Our aim was to conduct an initial assessment of orchard temperatures in the Tri-State area (Vic, SA, NSW border region near the Murray River) and compare this with the flowering model, to determine whether different pollinators are likely to provide pollination as a response to variable flower opening times. Temperature data loggers were provided to avocado growers in the Tri-State area with data recorded at four orchards: two near Robinvale (Vic), one near Barham (NSW) and one at Merbein South near Mildura (Vic). Using these temperature records, we determined daily maximum and

minimum temperatures and used this in conjunction with our model to determine the likely period of flowering of female 'Hass' flowers for each day.

Temperatures across four orchards in the 24-day period varied between 4.2°C and 40.8°C. More than half of the nights at three sites were warmer than 11.5°C, which was predicted to lead to morning flowering, whereas the fourth site had 62.5% of nights below 11.5°C, predicted to lead to afternoon flowering on these occasions. The same flowering classification was recorded across all sites on 10 out of the 24 days, with 8 of those classified as morning flowering.

Nocturnal flowering was predicted to be probable at all sites apart from at one orchard (Robinvale 1). Nocturnal flowering was confirmed at Merbein South by observation on two nights following low overnight minimums.

These findings indicate high variability in flower opening times in the Tri-State. Therefore, the development of strategies to promote a range of pollinators that will actively forage at these variable times and, subsequently, variable weather conditions, is required to optimise pollination.

1.1.4 The key pollinators of avocados and macadamia

We evaluated pollinator efficiency and effectiveness of honey bees and various wild pollinating species of macadamia (QLD and NSW) and avocado (tristate region). We define:

- efficiency as the rate of pollen transferred to stigmas per minute by an individual of a pollinating species
- effectiveness as the number of stigmas receiving pollen within an orchard by all individuals of a given species per hour, that is, their efficiency multiplied by their abundance.

Macadamia

Honey bees and stingless bees have been considered the most important pollinators of macadamia and previous research has found that honey bees deliver pollen to the stigma of macadamias. To our knowledge, detailed studies of the efficiency of wild pollinating species has not been studied.

Our assessment of five different species ranked stingless bees (*Tetragonula carbonaria*) as the most efficient pollinators, followed by nectar collecting honey bees (*Apis mellifera*) and lycid beetles (*Metriorrhynchus rhipidius*). Soldier beetles (*Campsomeris tasmaniensis*) were the next most efficient followed by nose flies (*Stomorphina discolour*). The scarab beetle, (*Glycyphana stolata*) was also found capable of pollinating. Hover and brown blow flies may also be pollinators as they were found to deliver pollen within 3 mm of stigmas; however, our small sample size did not verify that they place pollen onto the stigma.

We found nectar collecting honey bees to be the most effective pollinating species across all surveys in orchards near Bundaberg (7 blocks, 11 surveys conducted), 29 of 34 surveys conducted near Gympie-Glasshouse (24 blocks) and all but one survey in the Northern Rivers region NSW (14 blocks, 15 surveys). Even so, their contribution across regions and between blocks within regions was highly variable, reflecting variability in their abundance. Despite lower abundance, the greater efficiency of pollen collecting stingless bees as pollinators resulted in them being almost as effective as honey bees in the Gympie – Glasshouse region. Other insects that were found to be capable of pollinating macadamia had negligible effectiveness as pollinators because of very low abundance.

Avocado

This study revealed a surprising finding; that wild non-bee pollinators are the dominant pollinators of avocado in the Tri-State region.

We assessed the pollination efficiency of honey bees and twelve other non bee species. These were a thynnid wasp (Thynnid wasp sp. 1), brown blow flies (*Calliphora stygia*), lesser brown blow flies (*C. augur*), large green blow fly (*Lucila* sp.?), Australian sheep blow fly (*Lucilia cuprina*), dark green calliphorid fly (Calliporidae sp. 1), bristle fly (Tachinidae sp. 3), striped flesh fly (*Oxysarcodexia*? sp. 1), rhiniid fly (Rhiniidae sp. 1), narrow yellow hover fly (*Simosyrphus grandicornis*), black – orange hoverfly (*Melangyna viridiceps*) and the transverse ladybird beetle (*Coccinella transversalis*). Overall, the bristle fly sp. 3 and honey bees were deemed to be the most efficient pollinators, delivering the highest rate of pollen to stigmas per minute. The other species (nine flies, a beetle and a wasp) delivered pollen to stigmas ranging from 0.53 to 0.21 the rate of honey bee pollen deposition per minute.

Despite being generally less efficient pollinators, non-honey bee species were much more effective pollinators overall across Tri-State orchards because of their larger abundances. Our assessment of the pollinator effectiveness of 13 different species/morphospecies (abundance x efficiency) found that, together, these non-honey bee species were more effective pollinators than honey bees in all locations. Honey bees largely appeared to be unreliable pollinators of avocado orchards in the Tri-State region, even though many growers had hives placed within or next to their orchards.

1.1.5 Recommendations for optimising pollination

Macadamia

- For macadamia orchards in QLD and northern NSW, honeybees were either the most effective pollinators or equivalent in effectiveness to stingless bees, mainly because of the larger numbers of honey bees visiting flowers. However, honey bee numbers were often very low in many orchards (frequently less than one individual per tree during each of our survey period) and were unlikely to maximise cross pollination. Better hive placement, monitoring of hive strengths and improved orchard design are the obvious measures growers could take to make the most of potential nut yield increases as indicated by the hand cross-pollination trials. Further research trialling honey bee stocking rates, hive placement and orchard design would help growers to maximise their orchard gate returns.
- In some orchards, feral honey bees were likely playing an important role in pollination. If varroa were to establish, this free service is unlikely to continue, so growers will need to consider how to stock orchards with hives to replace this service.
- Growers themselves can easily assess the level of pollinator activity within their orchards by surveying selected trees for the presence of pollinators (we saw on average 1.7 bees per tree at each survey time). They can also measure whether yields are currently suboptimal by conducting cross-pollination experiments using simple techniques and equipment. This was demonstrated at workshops and is documented in this report. Growers can then evaluate those measures that will most likely improve pollination for their circumstances. We provide several recommendations within our report.
- Stingless bees were estimated to be the most efficient pollinator and, where present, readily foraged on macadamia racemes. If numbers of pollinators are consistently low, the placement of stingless bees within orchards should be considered.
- Further research on the stocking rates of both honey bees and stingless bees is required. Previous reporting has suggested 5–8 honey bee hives/ha for macadamia. Required stocking rates are likely to vary greatly depending on orchard block design and the presence of competing bloom. Encouraging growers to survey their blocks for pollinator activity with or without hives will allow them to evaluate whether their hive numbers are likely adequate within their own specific blocks.

- Many macadamia blocks currently consist of a single variety, reducing the chances of cross pollination. Our findings demonstrate that cross pollination can increase yields. New orchard designs should incorporate more than one variety to improve cross pollen flow. There is a need to design and test orchards containing multiple varieties, including placements of honey bees and stingless bees, to maximise cross pollination. Cross-pollination in existing blocks of one variety may be improved by removing weaker or poor yielding trees and planting different varieties that are most likely to flower simultaneously.
- To encourage honey bees and stingless bees to visit flowers, pruning trees to maximise light exposure to these flowers should increase the likelihood of visitation.
- The timing of pesticide application needs to be considered carefully by growers. For macadamia growers, lace bug (*Ulonemia decoris*) can be extremely problematic and growers have found it necessary to apply insecticides during the flowering period. To avoid the loss of bee pollinators, pesticides that have a short life (less than 12 hours) should be applied during the evening when bees have become inactive. For example, to control lace bug, Trichlorfon should be chosen instead of Diazinon during flowering. The application of shorter lasting pesticides may still cause the loss of non-bee pollinators that remain present within the trees.
- The contribution made by pollinators other than honey bees and stingless bees to macadamia pollination was found to be minor due to their low abundances. Our assessment of other potential pollinating species found lycid beetles, soldier beetles, scarab beetles and nose flies to be capable pollinators. Many other flower visiting insects that we did not assess could also be efficient pollinators. Basic information has previously been published about the lifecycles of some of these currently unmanaged species, but further research on how to rear or maintain them in orchards is required before growers can better utilise them. These species are likely to be present both day and night within orchards and are likely to be adversely affected by pesticide applications, even if instructions are followed to minimise bee losses.

Avocado

- Many growers had placed honey bee hives within or near their orchards. Despite this, honey bee abundances were widely variable between orchards and counts often low. Citrus orchards flowering simultaneously to avocado are known to be more attractive to honey bees and may have contributed to reduced honey bee foraging on avocado. To improve honey bee effectiveness, new avocado orchards should not be sited near to citrus blocks that will provide competing bloom.
- The weather experienced in the Tri-State area during avocado flowering means that the receptive period of 'Hass' flowers will occur at a wide range of times during the day, including in the evening and overnight. Fostering a diversity of pollinators with differing patterns of activity through the day is therefore recommended, to ensure that sufficient pollinators are active when the flowers are open regardless of when that occurs.
- Growers paying for hives should be encouraged to observe honey bee foraging activity at different times and on different days to determine whether honey bees are visiting their orchards. Our data points to honey bees being effective in some orchards but of low effectiveness in others. Further research is required to determine whether current stocking rates and hive placement can be altered to improve their role as pollinators.
- Avocado orchards in the Tri-State area were predominantly pollinated by a diverse range of flies and beetles rather than honey bees. Some of these species have simple lifecycles (blow flies and some hover fly species) with strong potential to manipulate populations to further boost their effectiveness. Growers need to consider the potential impact of rearing some of these species themselves as they can be problematic to other industries (e.g. some blow fly species cause sheep flystrike). Development of strategies to maintain these species is important to ensure current levels of pollination.
- Since most Tri-State avocado orchards are significantly reliant on non-bee pollinators, particularly flies and beetles, it is particularly important to avoid the use of pesticides during

flowering. Non-bee pollinators are more likely to be present in orchards both day and night and strategically applying pesticides to minimise honey bee losses is still likely to cause large losses of non-bee pollinators.

- Changes to land use in the Tri-State, e.g. increased agricultural intensity, may greatly impact non-bee pollinator populations. Further understanding of factors influencing populations of key non-bee pollinators of avocado is needed to inform farmers of management practices that will retain their populations.

1.2 An article published in a macadamia industry magazine

An article on the floral reproductive studies and pollinators has been published in the AMS New Bulletin:

Howlett B, Goodwin M, Cutting B, Cross S, Read S, Wilcox B, Pattermore D 2016. Does pollination between different Macadamia varieties improve yield? AMS News Bulletin, August.

1.3 A reporting seminars conducted (1 macadamia)

During this milestone period we have reported our findings to industry and growers through:

- Howlett BG 2016. , Macadamia Pollination, R&D Speed dating, chaired pollination session. Australian Macadamia Society Conference. Caloundra, 18–20 October 2016.
- Howlett B 2016. Pollination in Macadamias. Bundaberg Pollination Workshop, Macadamia's Australia. QLD, 16 June 2016.
- Howlett B 2016. Pollination in macadamias; update from Plant & Food Research NZ, AMS – Consultants Meeting, Macadamia's Australia. Brisbane, QLD, 8–9 June 2016.

In addition we delivered one more presentation at a Qualicado workshop in Bundaberg (QLD):

Howlett B 2016. A different perspective on pollination of Avocado in Australia's Tri-state region. Qualicado Workshop Bundaberg, QLD, 11 August 2016.

2 Outputs

2.1 Grower magazine articles

Howlett B, Evans L, Wilcox B, Read S 2016. Flies and beetles are key pollinators of Sunraysia's Avocado orchards. *Australian Tree Crop*. April/May: 14–15.

Howlett B, Pattemore D, Rader, Cutting B 2015. Flies can be doing most of the pollination in some Australian avocado orchards. *Talking Avocados* 6(23): 38–39.

Howlett B, Goodwin M, Cutting B, Cross S, Read S, Wilcox B, Pattemore D 2016. Does pollination between different Macadamia varieties improve yield? *AMS News Bulletin*, August.

2.2 Refereed scientific publications

A review of macadamia pollination has now been published in the refereed science journal, *Scientia Horticulturae*.

Howlett BG, Nelson WR, Pattemore DE, Gee M 2016?. Pollination of macadamia: Review and opportunities for improving yield. *Scientia Horticulturae* 197: 411–419.

2.3 Presentations and workshops

Macadamia:

Howlett BG 2016. Macadamia Pollination, R&D Speed dating, chaired pollination session. Australian Macadamia Society Conference. Caloundra, 18–20 October 2016.

Howlett B 2016. Pollination in Macadamias. Bundaberg Pollination Workshop, Macadamia's Australia. QLD, 16 June 2016.

Howlett B 2016. Pollination in macadamias; update from Plant & Food Research NZ, AMS – Consultants Meeting, Macadamia's Australia. Brisbane, QLD, 8–9 June 2016.

Howlett BG 2015. Pollination of Macadamia. Macadamia Consultants Conference. Brisbane, QLD, 10–11 June 2015.

Howlett BG 2014. Macadamia industry and grower workshop: Macadamia pollination and assessing pollination deficit. Lismore, September.

Goodwin R, Cross S 2014. Macadamia industry and grower workshop: Macadamia pollination and assessing pollination deficit. Gympie, September.

Goodwin R, Cross S 2014. Macadamia industry and grower workshop: Macadamia pollination and assessing pollination deficit, Bundaberg, August.

Avocados:

Howlett B 2016. A different perspective on pollination of Avocado in Australia's Tri-state region. Qualicado Workshop Bundaberg. QLD, 11 August 2016.

Howlett B 2016. A different perspective on pollination of Avocado in Australia's Tri-state region. (video presentation) Qualicado Workshop. Pemberton, WA, 23 June 2016.

Howlett B 2015. A different perspective on pollination of Avocado in Australia's Tri-state region. Qualicado Workshop, Robinvale, Vic, 25 November 2015.

Pattemore D 2015. A different perspective on pollination. Avocado Australia's, Tri-state Growers' Qualicado Workshop. Port Macquarie, NSW, 2 December 2015.

Howlett B 2015. A different perspective on pollination of Avocado in Australia's Tri-state region. Qualicado Workshop. Toowoomba, QLD, 9 December 2015.

2.4 Media

A number of interviews and news stories were covered by the media and can be easily found using key word searches on the internet. Key stories include:

Avocado growers urged to embrace flies in orchards as key pollinators.

<http://www.abc.net.au/news/2016-08-30/flies-play-role-in-avocado-pollination/7796976>

Avocado study flies in the face of pollination wisdom, HIA <http://horticulture.com.au/avocado-study-flies-in-the-face-of-pollination-wisdom/>

Fluoro pollen to track bees and insects at work in macadamia. QLD Country Hour orchards <http://www.abc.net.au/news/2015-09-18/fluoro-pollen-tracks-bees/6784808>

Get Farming: Sunraysia flies blow honeybee pollination trend

<http://getfarming.com.au/2016/09/14/sunraysia-flies-blow-honeybee-pollination-trend/>

2.5 Outcomes

Findings from the research have broadened the understanding of growers on the role of wild pollinating species. We have provided delivery of research findings through the following:

Thirty-one macadamia growers that attended workshops held in Bundaberg, Gympie and the Northern Rivers learnt how to assess their orchards to determine whether pollination may be limited. At the meeting we discussed macadamia reproductive biology and pollinators. We provided copies of our macadamia pollination assessment pollination handbook and gave demonstrations on how to assess orchards for yield deficit caused by inadequate pollination. Growers were then asked for their involvements in regional trials to assess pollination deficit. These growers are now capable of assessing their own orchards to determine whether pollination can be improved.

Growers have queried how to improve the role of wild pollinators and at least two growers, one in the Tri-State and one in Western Australia, have already begun trialling methods to increase fly pollinators, demonstrating the interest in the research. Many growers are also very keen for further detail about the pollinator survey results from their orchards. We have provided personal reports to the growers that provided us with access to their avocado farms. The reports provide information on pollinator efficiency, identity and abundances within their own orchards.

The project has also led to some growers and consultants conducting their own field research. Clayton Mattiazi (Hinkler Park, Plantation, Bundaberg) has been conducting additional cross-pollination experiments and has sought our guidance on some aspects. He is willing to share his findings with us. Chris Fuller (Gympie) and an associate also conducted a trial on macadamia cross-pollination to add to research being conducted in the current project. Nut set from the trials has been collected but not yet assessed.

We developed a collaboration with Andrew Robsons 'RnD4Profit Project: Multi-scale monitoring tools for managing Australian tree crops: Industry meets innovation'. In this programme I am supervising PhD student Bryony Wilcox. Bryony is examining tree health relationships with pollination and yield. We have been able to combine some of my key research methods to efficiently collect regional data beyond the initial scope of both projects.

2.5.1 Intellectual property, commercialisation and confidentiality

No IP, commercialisation or confidentiality issues or development to report.

2.5.2 Issues and risks

The research to date and steering group feedback has highlighted the complexity of pollination in both orchard systems. A further focus on delivering comprehensive outputs to growers including workshop discussions (particularly demonstrations), a field guide, and a greater understanding of the key issues, could further encourage growers to uptake strategies focussed on improved pollination outcomes.

Findings from this project and the questions and needs raised by these findings include:

1. Numbers of pollinators are usually low and hugely variable between macadamia orchards – but by the end of this project we will be able to quantify the role of the more common species. To overcome the issue of low pollinator numbers, an ability to test honey bee stocking rates and the development new strategies for boosting other key pollinating species, with the assistance of growers, should be beneficial.
2. All of the macadamia varieties that were tested demonstrated self-pollination to varying degrees. But in all cases, cross-pollination increased yields. Increased cross-pollination can result in increased nut set but this has not been tested this across a range of varieties. Further investigation of this would enable more robust recommendations for different varieties
3. Avocado pollination in the Tri-State is largely being performed by a wide diversity of flies rather than honey bees. We are quantifying the efficiency of these species. It would be particularly useful to link this to actual crop yield. Further work to identify the causes of this diversity and methods to maintain and promote it (within the Tri-State and outside) would greatly assist in improving recommendations.

2.5.3 Other information

Findings from both the macadamia and avocado research will be personally sent through to growers who have allowed research work to be conducted on their orchards, where possible.

The next steps that need to be addressed are:

- Final report received by Horticulture Innovation Australia.
- A seminar communicating research findings and recommendations for the macadamia Industry.
- An article submitted for publication in the macadamia industry magazine.

2.6 Appendix: Optimising pollination of macadamia & avocado in Australia: full report

Overview

In Australia, a significant amount of pollination is thought to be provided as a free service by feral honey bees and possibly by other unidentified unmanaged species. The pollination services from feral honey bees are likely to be significantly diminished or lost should the varroa mite establish in Australia. To optimise pollination currently and to prepare industry for changes necessary if varroa were to become established, it is important to understand current rates of pollination within these crops, the relative contributions of honey bees versus other pollinators, and to identify which key pollinators could be better managed for pollination. The aim of this research was to highlight to growers the importance of pollination and the role of managed and unmanaged pollinators. This was to be achieved by assessing:

- The actual and potential rates of pollination
- The floral biology of macadamia
- The key pollinators of macadamia and avocado
- Recommendations for optimising pollination.

This report focuses on the pollination of macadamia across three regions of Queensland and NSW and avocado in the Tri-State region (orchards within 30 km of the Murray River from Waikerie, SA to Robinvale, Vic). We have found that pollination in these systems is complex, both in terms of floral reproductive biology and the role of insect pollinators. The research within the programme has discovered and delivered a great deal of new information, giving growers new insights into pollination requirements for their orchards. It also provides a platform of new knowledge about the pollination systems of these crops which will enable further research to optimise pollination.

To maximise uptake by growers and ensure our research was relevant and robust, we took a collaborative approach to our research programme. We have:

- Developed industry steering groups for both macadamia and avocado. These consisted of eminent industry and organisation representatives that provided valuable input regarding key priority research requirements.
- Involved growers in our trials, including seeking their assistance to gather data on the effect of cross pollination within macadamia orchards, and deploy temperature data loggers in avocado orchards to assess the potential effect of temperatures on flowering patterns.

- Developed a collaboration with the University of New England through Drs Romina Rader and Andrew Robson to involve PhD student Bryony Wilcox to assess tree health and pollination. Bryony and associated support staff were involved in joint surveys that extended the collection of pollinator survey data for both macadamia and avocado.
- Trained and involved six university students from Agrocampus Ouest, Rennes, France who were studying for their Masters degree in Agricultural Science. The students assisted in all aspects of field survey and data collection on pollinator efficiency and effectiveness.

Key aspects of the research have been communicated through four published industry articles and nine workshops and conferences. The media have also covered stories on both macadamia and avocado research undertaken for this project. We wrote a manuscript reviewing knowledge of macadamia pollination that was published in the international peer reviewed horticultural journal 'Scientia Horticulturae'. Further scientific publications are planned. A list of outputs are provided at the end of this overview.

Macadamia

Macadamias are covered in Section 3 of this report.

Section 3.1 covers macadamia reproductive biology. With the assistance of growers, we demonstrated that cross pollinating single racemes within trees of various varieties increased final nut set within these racemes, but this effect declined in magnitude when we cross-pollinated multiple racemes per tree. We found that self-pollination can occur in some varieties and there appears to be varietal differences as to how many nuts may fully develop. While increasing the number of racemes cross-pollinated per tree was associated with a decline in nut set in two varieties (indicating resource allocation within a tree), an assessment of nut yields in one of these varieties found that hand cross-pollination still led to significantly higher nut yields when we conducted quadrat counts of nut set to cover a higher proportion of racemes on the tree.

Section 3.2 covers the efficiency of different pollinators. We found that a range of insect species were capable of pollinating macadamia including honey bees (*Apis mellifera*), stingless bees (*Tetragonula carbonaria*), Lycid beetles (*Metriorrhynchus rhipidius*), soldier beetles (*Chaliognathus flavipennis*), scarab beetles (*Glycyphana stolata*), and the rhiniid fly (*Stomorphina discolor*). Stingless bees were the most efficient pollinator as individually they deposited pollen between racemes at the greatest rate. However, in most cases they were not the most effective pollinator because of their relatively lower abundance compared to honey bees. Nectar feeding honey bees, lycid beetles and soldier beetles were also efficient. Hover flies and blow flies may also be pollinators, but our samples were too few to confirm this. Pollen collecting honey bees were rarely observed and may be efficient pollinators due to their likely direct contact with the pollen presenters.

Section 3.3 covers pollinator abundance and their overall effectiveness within and between orchards. Pollinator effectiveness is determined by combining a pollinators efficiency at pollinating flowers and their overall abundance. Honey bees were the most abundant species followed by stingless bees across the 60 surveyed orchard blocks located across the Northern Rivers (NSW), near Gympie (QLD) and Bundaberg (QLD). All other insect flower visitors were relatively uncommon. Due to their high abundances, honey bees were the most effective pollinators overall in most orchard blocks. However, stingless bees were found to be the most effective in five orchard blocks near Gympie.

Avocado

Avocados are covered in Section 4 of this report.

Section 4.1 assesses the relationship between avocado flower phenology and temperature in Tri-State orchards. We used knowledge of the relationship between overnight minimum temperatures

and mean first female flower opening time for 'Hass' avocado and data collected from temperature data loggers placed in four orchards located in Robinvale, Vic (2 orchards), Barham, NSW and Merbein South, Vic. Data loggers showed large variation in overnight temperatures at all orchards thereby greatly altering daily female flower opening times. Strategies to encourage pollinator diversity should boost diurnal, crepuscular and nocturnal pollinator activity increasing the opportunity for pollination to occur at widely variable flower opening times.

Section 4.2 assesses the efficiency of key insect flower visitors of avocado. Very little is known about the efficiency of non-honey bee flower visitors, especially in Australian orchards. We believe this is the first study to assess the pollinator efficiency of a range of pollinating species by measuring their ability to deposit pollen onto stigmas and their rates of movements between flowers. As well as honey bees, a range of fly species (blow flies, hover flies, a flesh fly, two bristle flies, a rhiniid fly), two beetle species (a ladybird beetle and the banded pumpkin beetle) and a wasp species were proven pollinators. A bristle fly morphospecies and honey bees were calculated to be the most efficient. However, all of the species examined were potentially very important pollinators when present in high abundance.

Section 4.3 examines the abundance, distribution and effectiveness of avocado insect flower visitors in orchards located near Mildura (Vic), Robinvale (Vic), Renmark (SA) and Waikerie (SA). Flower visitors (more than 69 species/morphospecies) were dominated by flies and beetles. Our assessment of the pollinator effectiveness of 13 different species/morphospecies (abundance x efficiency) found that together, these non-honey bee species were more effective pollinators than honey bees in all locations. Non-bee pollinators included calliphorid flies (including blow flies), hover flies, other muscoid flies (bristle, flesh, rhiniid flies) and ladybird beetles. Although abundant in some orchards, honey bees appeared to be unreliable pollinators across locations. Their low abundances in some orchards could be due to the competing bloom of citrus that is considered more attractive to honey bees.

Recommendations

Macadamia

- For macadamia orchards in QLD and northern NSW, honeybees were either the most effective pollinators or equivalent in effectiveness to stingless bees, largely due to the larger numbers of honey bees visiting flowers. However, honey bee numbers were often very low in many orchards (frequently less than one individual per tree during each of our survey period) and were unlikely to maximise cross pollination. Better hive placement, monitoring of hive strengths and improved orchard design are the obvious measures growers could take to make the most of potential nut yield increases as indicated by the hand cross-pollination trials. Further research trials focusing on honey bee stocking rates, placement and orchard design would help growers to maximise their orchard gate returns.
- In some orchards, feral honey bees were likely playing an important role in pollination. If varroa were to establish, this free service is unlikely to continue, so growers will need to consider how to stock orchards with hives to replace this service.

- Growers themselves can easily assess the level of pollinator activity within their orchards by surveying selected trees for the presence of pollinators (we saw on average 1.7 bees per tree at each survey time), they can also measure whether yields are currently suboptimal by conducting cross-pollination experiments using simple techniques and equipment. This was demonstrated at workshops and is documented in this report. Growers can then evaluate those measures that will most likely improve pollination for their circumstances. We provide several recommendations within our report.
- Stingless bees were estimated to be the most efficient pollinator and, where present, readily foraged on macadamia racemes. If numbers of pollinators are consistently low, the placement of stingless bees within orchards should be considered.
- Further research on the stocking rates of both honey bees and stingless bees is required. Previous reporting has suggested 5–8 honey bee hives/ha for macadamia. Required stocking rates are likely to vary greatly depending on orchard block design and the presence of competing bloom. Encouraging growers to survey their blocks for pollinator activity with or without hives will allow them to evaluate whether their hive numbers are likely adequate within their own specific blocks.
- Many macadamia blocks currently consist of a single variety, reducing the chances of cross pollination. Our findings demonstrate that cross pollination can increase yields. New orchard designs should incorporate more than one variety to improve cross pollen flow. There is a need to design and test orchards containing multiple varieties, including placements of honey bees and stingless bees, to maximise cross pollination. Cross pollination in existing blocks of one variety may be improved by removing weaker or poor yielding trees and planting different varieties that are most likely to flower simultaneously.
- To encourage honey bees and stingless bees to visit flowers, pruning trees to maximise light exposure to these flowers should increase the likelihood of visitation.
- The timing of pesticide application needs to be considered carefully by growers. For macadamia growers, lace bug (*Ulonemia decoris*) can be extremely problematic and growers have found it necessary to apply insecticides during the flowering period. To avoid the loss of bee pollinators, pesticides that have a short life (less than 12 hours) should be applied during the evening when bees have become inactive. For example, to control lace bug Trichlorfon should be chosen instead of Diazinon during flowering. The application of shorter lasting pesticides may still cause the loss of non-bee pollinators that remain present within the trees.
- The contribution made by pollinators other than honey bees and stingless bees to macadamia pollination was found to be minor due to their low abundances. Our assessment of other potential pollinating species found lycid beetles, soldier beetles, scarab beetles and nose flies to be capable pollinators. Many other flower visiting insects that we did not assess could also be efficient pollinators. Basic information has previously been published about the lifecycles of some of these currently unmanaged species, but further research on how to rear or maintain them in orchards is required before growers can better utilise them. These species are likely to be present both day and night within orchards and are likely to be adversely affected by pesticide applications, even if instructions are followed to minimise bee losses.

Avocado

- Many growers had placed honey bee hives within or near their orchards. Despite this, honey bee abundances were widely variable between orchards and counts often low. Citrus orchards flowering simultaneously to avocado are known to be more attractive to honey bees and may have contributed to reduced honey bee foraging on avocado. To improve honey bee effectiveness, new avocado orchards should not be sited near to citrus blocks that will provide competing bloom.
- The weather experienced in the Tri-State area during avocado flowering means that the receptive period of 'Hass' flowers will occur at a wide range of times during the day, including in the evening and overnight. Fostering a diversity of pollinators with differing patterns of activity

through the day is therefore recommended, to ensure that sufficient pollinators are active when the flowers are open regardless of when that occurs.

- Growers paying for hives should be encouraged to observe honey bee foraging activity at different times and on different days to determine whether honey bees are visiting their orchards. Our data points to honey bees being effective in some orchards but of low effectiveness in others. Further research is required to determine whether current stocking rates and hive placement can be altered to improve their role as pollinators.
- Avocado orchards in the Tri-State area were predominantly pollinated by a diverse range of flies and beetles rather than honey bees. Some of these species have simple lifecycles (blow flies and some hover fly species) with strong potential to manipulate populations to further boost their effectiveness. Growers need to consider the potential impact of rearing some of these species themselves as they can be problematic to other industries (e.g. some blow fly species cause sheep flystrike). Development of strategies to maintain these species is important to ensure current levels of pollination.
- Since most Tri-State avocado orchards are significantly reliant on non-bee pollinators, particularly flies and beetles, it is particularly important to avoid the use of pesticides during flowering. Non-bee pollinators are more likely to be present in orchards both day and night and strategically applying pesticides to minimise honey bee losses is still likely to cause large losses of non-bee pollinators.
- Changes to land use in the Tri-State, e.g. increased agricultural intensity may greatly impact non-bee pollinator populations. Further understanding of factors influencing populations of key non-bee pollinators of avocado is needed to inform farmers of management practices that will retain their populations.

3 Macadamias

3.1 Macadamia floral biology and pollination

3.1.1 Introduction

Understanding the floral reproductive biology of macadamia is essential for developing strategies to maximise nut set. Much of the current knowledge of *Macadamia* floral biology and breeding systems has recently been outlined in a review by Trueman (2013); however, current knowledge around many aspects remains restricted to studies assessing relative few varieties. Macadamia trees within orchards can produce over two million flowers in a season, but just 0.3% of flowers may produce a harvestable nut (Ito 1980) with most fruits that initially develop abscising within the first 2 months (Sakai & Nagao 1985; Lavi et al. 1996). Wallace et al. (1996) found that for two varieties, just 3–4% of those fruit present 21 days following pollination were developing into harvestable nuts. To improve yields, it is important to understand the mechanisms behind the high level of fruit drop. Several factors have been implicated in the numbers of harvestable nuts obtained. Among these are donor pollen compatibility (Lavi et al. 1996; Wallace et al. 1996), and cross-pollination of flowers (Trueman & Turnbull 1994).

Macadamia is considered partially self-incompatible and the degree of incompatibility may vary between varieties (Wallace et al. 1996). Multiple pollen grains can germinate on the stigma, including self-pollen, but few reach the full length of the style, with successful pollen tubes taking up to 7 days to reach the ovary (Sedgley 1981, 1983). It is suggested that gametophytic incompatibility may operate to inhibit pollen tube growth and thus reduce the production of self-pollinated fruit (Sedgley 1983). This could explain some of the varying patterns of incompatibility between varieties (Sedgley 1983).

Economic yields are produced within many orchards that contain just a single variety, which suggests that trees of some varieties can produce economically viable nut crops through self-pollination, although the contribution of cross-pollen from trees outside the orchard is not known. The type of self-pollination that may occur (within floret, within raceme, between racemes or between trees) in these circumstances remains unclear. In addition, the lack of cross-pollination and fertilisation is not the sole reason for low yields, as the majority of these small developing fruits, even when cross-pollinated, are dropped early (Sedgley 1981; Sedgley et al. 1990; Trueman & Turnbull 1994).

Initial and final nut set in *M. integrifolia* has been shown to be correlated with increased insect visitation to flowers in variety '508' (Heard 1993); however, flowers are considered only attractive to insects for approximately 3 days and each raceme has open flowers over approximately 1 week (Heard 1993). Flower visiting insects can be attracted by nectar or pollen (Vithanage & Ironside 1986; Kongpitak et al. 2012). Peak anthesis occurs early in the morning on flower opening (McGregor 1999), although Heard (1993) noted that in '508', pollen availability peaks mid-afternoon on the day of anthesis. Pollen quantity and viability varies by variety, with pollen germination in artificial media varying between 45 and 84% (Lavi et al. 1996). Where tested, nectar is reported to peak in flowers early in the morning and again in the early afternoon (Heard 1994; Kongpitak et al. 2012), which possibly influences peak pollinator flower visitation times (Vithanage & Ironside 1986).

Studies conducted on macadamia in this project aimed to further understand floral reproductive biology of commercial macadamia grown in orchard conditions. More specifically our studies assessed:

- the influence of cross-pollination on final nut yield within racemes for various combinations of macadamia varieties with the help of growers located in the Northern Rivers region of NSW, SE Queensland (Gympie) and Central Queensland (Bundaberg)
- the influence of cross-pollination intensity (varying number of hand cross-pollination within trees) on '741' (pollen donor '816') and '842' (pollen donor '344')
- whether self-pollination (within raceme, between raceme, between tree) influences final nut yield compared with cross-pollination in varieties '741', '816' and 'A203'.
- the timing and volume of nectar production, flower opening and pollen availability in varieties '741' and '842'.

3.1.2 Materials and methods

Hand cross-pollination versus open pollinated on single racemes

To understand the degree to which cross-pollination influences nut yield, trials were conducted between varieties (Table 3.1.1), involving the transfer of pollen by hand from one variety to the stigmas of another variety. Macadamia growers were trained by scientists to conduct the majority of varietal crosses through field day workshops that were held near Bundaberg, Gympie and Lismore. The growers were then invited to repeat the experiment at their own orchards.

Trials were conducted on between four and twenty trees for each variety. For each tree receiving the pollination treatment, two racemes of similar floral development, located in similar positions (height, aspect, branch location) but on separate branches, were marked. One was randomly assigned to be cross-pollinated. This raceme was hand pollinated using a glass test tube covered with pollen of the other variety. To collect pollen, the test tube was placed over a raceme of the donor variety so that the pollen presenters of flowers (containing dehiscent pollen) within the raceme had good contact with the sides of the test tube (Figure 3.1.1). Twisting the test tube was adequate for transferring pollen onto the test tube wall. The amount and distribution of pollen within the tubes could then be quickly assessed through visual examination. Tubes were considered to have adequate pollen for transfer if pollen was observed to be evenly distributed across the inner length of the test tube. To transfer pollen, the test tube containing the donor pollen was then placed over the marked raceme and twisted, allowing the cross-pollen to be picked up by the pollen presenters (containing the stigmas) on the treatment raceme. The second marked raceme was not hand pollinated and represented the open-pollinated control.

Table 3.1.1. Hand cross-pollinations conducted between different macadamia varieties (Fruiting tree variety x Pollen donor variety). Location of orchards are in parentheses, BD is Bundaberg (within 100 km); Gympie (within 100 km) and NR is the Northern Rivers, New South Wales (within 100 km of Lismore).

Varietal Crosses (Orchard Location)		
741x246 (BD)	Ddwx741 (BD)	A16xA4 (NR)
741x246 (NR)	Ddwx842 (BD)	A203xA203 (BD)
741x344 (BD)	Ddwx849 (BD)	A203x246 (BD)
741x344 (BD2)	DdwxA16 (BD)	A203x344 (BD)
741x788 (BD)	DdwxA203 (BD)	A203x741 (BD)
741x816 (BD)	DdwxA268 (BD)	A203x788 (BD)
741x816 (BD)	DdwxA38 (BD)	A203x816 (BD)
741x842 (BD)	DdwxA4 (BD)	A203x842 (BD)
741x842 (BD2)	A268x246 (BD)	A203x849 (BD)
741x849 (BD)	A268x344 (BD)	A203xA16 (BD)
741xA16 (BD)	A268x741 (BD)	A203xA268 (BD)
741xA203 (BD)	A268x788 (BD)	A203xA38 (BD)
741xA203 (BD2)	A268x816 (BD)	A203xA4 (BD)
741xA268 (BD)	A268x842 (BD)	A203xDdw (BD)
741xA38 (BD)	A268x849 (BD)	842x246 (Gympie)
741xA4 (BD)	A268xA16 (BD)	842x344 (Gympie)
741xDdw (BD)	A268xA203 (BD)	A16x344 (NR)
Ddwx246 (BD)	A268xA4 (BD)	842x741 (BD)
Ddwx344 (BD)	A268xDdw (BD)	842x816 (BD)
Ddwx816 (BD)	816x741 (BD)	344x816/Ddw (NR)
Ddwx788 (BD)		



Figure 3.1.1: Hand pollinating a raceme with a glass test tubes.

A second trial was conducted on '842' using pollen from '816'. Four different treatments were conducted to assess the effectiveness of pollination methods. The treatments were:

- Hand pollinated with paintbrush
- Open pollinated control
- Hand pollinated with tube then bagged
- Hand pollinated with tube then left open.

The treatments were randomised across racemes at similar flowering stages and similar positioning in each tree. Each treatment set was repeated across 20 trees.

In addition, the lengths of flowering racemes were measured for cultivars '816' and '741', to test whether nut set was dependant on flower number (with raceme length as a proxy for flower number), or whether nut set could be considered regardless of flower number or raceme length.

With the exception of orchards where growers conducted their own cross-pollination experiments, all first year floral biology studies were conducted on a 200-ha macadamia orchard 24.95°S, 152.36°E near Bundaberg, Qld. The orchard had macadamia varieties planted in rows of 10, with 5 m between rows and 2 m spacing between trees. The grower had reported problems with nut set.

Initial nut set was examined for trials on 13 October 2014 and final nut set on 15 December 2014. Data on initial set were also collected by several of the cooperating growers. The data between cross-pollination and control treatments, along with treatment type, were compared to determine their influence on nut yield and whether a pollination deficit is currently limiting potential yield. Data were analysed using paired t-tests.

Capacity for self-pollination

To examine the degree that self-pollination may occur in *Macadamia*, the mode of self-pollination within floret (autogamy), or between florets but within variety (geitonogamy) was examined by conducting hand pollination experiments on single racemes. For all varieties, treatments were also compared with open pollinated control racemes and with a cross-pollinated raceme. For '741' the donor pollen was obtained from '816' while for '816' and 'A268', donor pollen was from '741'. Experiments were conducted across three varieties near Bundaberg – '741', '816' and 'A268' (18 September 2015) 24.77°S, 153.01°E.

Eight hand pollination treatments were conducted for each variety:

1. open pollinated (no pollination)
2. caged autogamy
3. bagged autogamy
4. within-raceme pollination and bagged
5. between racemes within-tree and bagged
6. between-tree racemes within-variety and bagged
7. between racemes of different varieties and bagged
8. between racemes of different varieties and caged.

Bags to prevent visitation by flower visiting insects were constructed from fine mesh material and sealed along their length and base using polyethylene tape that was stapled every 3–4 cm. Their size (30 x 15 cm) allowed them to completely envelop flowering macadamia racemes when tied around the upper petiole. Cages were also prepared and used in two treatments due to the concern that bags alone might potentially rub pollen between florets within a raceme, increasing the chance of non-autogamous pollination. These cages were constructed from wire mesh (gap diameter 2.5 cm) to create cylinders, 10 cm in diameter and 20 cm in length. Wire mesh platforms were secured within the upper and lower sections of the cage (at approximately 10 cm above the cage base) through which

the treatment raceme could be threaded through. The cages were then encased in fine mesh bags (40 x 25 cm). The cages were placed over inflorescences to minimise pollen movement between florets that could occur if bags rubbed against the raceme.

The experimental procedure for each macadamia variety utilised a random block design. The eight treatments were replicated across 10 blocks for varieties 741 and 816 and eight blocks for A268. Each individual treatment was assigned to one tree and a single, late budding raceme was selected and marked. Racemes of similar length, position on branch and orientation on the tree were chosen and marked as selected treatment racemes on adjacent trees within rows. For varieties 741 and 816, three blocks were selected per row for three rows and one block in a fourth row. For A268 three sets of two blocks were selected per row across four rows. Blocks within rows were separated by a gap of two or three trees.

Racemes used within experiments were selected at late budding stage and were marked and depending on treatment, bagged, caged or left exposed. They were monitored every 1–2 days and when in full flower, treated based on the requirements of the treatment. To conduct the hand pollinations, individual disposable plastic acetone sheets (A4 size) were rolled into cylinders with an aperture of diameter 5 cm and sealed using polypropylene sealing tape. For treatments where hand pollinations were required, pollen was collected inside a cylinder by placement over a fully flowering raceme that met the requirements of the treatment. That is, a raceme within the tree for within-tree pollination, a raceme from a different tree of the same variety for between-tree intra-varietal pollination and a raceme from a tree from a different variety for cross-pollination treatments. To collect pollen, a cylinder was placed directly around the raceme ensuring that flower pollen presenters were in full contact with the sides of the cylinder. For intra-raceme pollination, a cylinder was twisted on the treatment raceme allowing for pollen collected from the pollen presenters to be transferred onto the inner cylinder walls and transferred onto the pollen presenters of other florets within the raceme. Following pollen transfer within all hand pollination treatments, the cylinder would be observed to ensure pollen had been evenly collected on the cylinder wall. If this was not the case then a second placement and twist would be administered (this was not required in any cases). Each cylinder was used for just one treatment to avoid unintentional pollen transfer between treatments.

Following the application of each treatment, bags were re-applied and the racemes left for two weeks to ensure all florets within the racemes had completed flowering. Bags were then removed and marked racemes left to develop nuts. Nut counts were then conducted for all treatments on January 25 (varieties '741' and '816') and January 26 ('A268') 2016.

Intensity of cross-pollination

Cross-pollination experiments have demonstrated that in the majority of cases cross-pollination of racemes by another variety increase final nut set within single racemes compared to open pollinated racemes. However, the effect of cross-pollination on nut set of multiple racemes within a tree has not been examined. Trials on '741' in an orchard near Bundaberg 24.77°S, 153.01°E. and 842 in an orchard near Gympie 26.18° S, 152.67° E were conducted to test whether the number of hand cross-pollinated racemes within trees results in higher nut set across multiple pollinated racemes and for the Bundaberg orchard, whether this influenced nut set within racemes that were not hand cross-pollinated but remained exposed to open pollination. Five hand pollination treatments were conducted, each on an individual tree: no hand pollination (control trees) (0x), for the other four treatments hand pollination were conducted on 1) one raceme (1x) 2) three racemes (3x) 3) fifteen racemes (15x) and 4) one hundred racemes (100x)(in the Bundaberg orchard only).

A random block experimental design was applied. All treatments were replicated 10 times (10 trees) with exception of the 100x treatment that was replicated six times (six trees). For each treatment tree, all hand pollinated racemes with the exception of the 100 hand pollinated racemes, were marked with tape. In addition, a further six fully flowering racemes that were not hand pollinated were marked also

on each tree to assess fruit set. Hand pollinations and marked racemes for all but the 100x treatment were located on a single tree branch with basal diameter between 15 and 30 cm of similar height and orientation on each tree. For the 100x treatment, cross-pollinations were randomly conducted on flowering racemes across the entire tree to a height of 2 m due to the limited number of flowering racemes available in a single branch. Following the hand cross treatment, 15 cross-pollinated and six not cross-pollinated flowering racemes were marked on a single branch to assess final nut counts.

Hand cross-pollinations were conducted using individual disposable cellulose acetone tubes (as described in self-pollination experiments). Pollen from flowering racemes from '816' was used as the donor pollen. Collection and application of pollen was the same as described in the self-pollination experiments. Tubes were used once for all treatment with exception of 100x where each tube was used to pollinate two racemes. Racemes were then left to set nuts, from which a subset was then counted on January 26 2016.

In addition, nut counts were conducted on four sides (opposite and perpendicular) of each tree (two within row on opposite sides and two perpendicular to the tree row on opposite sides). At each side, a fold-out quadrat forming a 0.75 m³ cube was held at a height of 1.5 m, and all nuts contained within were counted. In addition, at each quadrat point the number of nuts on 10 racemes were counted within an area of 0.75 m³ (including racemes with no nuts) by selecting those nearest to the outer midpoint of each quadrat and at a height of 1.5 m to determine whether location of racemes within the tree influenced nut set. Nut counts were also conducted on all four tree sides at 50 cm within the tree canopy and at the midpoint between the tree trunk and outer canopy, again by selecting the 10 racemes nearest to the height point of 1.5 m as measured by a tape measure. Nut counts and surveys were conducted on 26–27 January 2016.

Nectar production and diurnal pollen availability

Nectar was sampled using micropipettes at hourly intervals from open pollinated flowers of '842' between 0750 h and 1530 h on 27 August 2014; between 0855 h and 1700 h on 28 August; and between 0900 h and 1500 h on 29 August. Flowers were sampled from a different raceme on a different tree during each hour. In total, 63 racemes were sampled on 27 August and 62 each on 28 and 29 August. Nectar extraction was also attempted from open-pollinated flowers for '741'; however, no nectar could be extracted.

To assess nectar flow, nectar was collected from racemes that had been enclosed in plastic or mesh bags that inhibited visitation by insects as well as open flowers. Plastic and mesh bags were both employed as they may potentially alter the volume of nectar through evaporation. Flowers within mesh bags were sampled for nectar on three consecutive days (27–29 August 2014) at 1200 h and 1700 h, while flowers enclosed in plastic bags were sampled over the same days at 1400 h and 1600 h. The sugar concentration was measured using a refractometer designed to measure small quantities. Where there was less than about 0.3 µL extracted from the flower nectaries, the nectar from more than one flower from the same raceme was collected together and tested.

The timing of flower opening was established using 10 marked racemes, each on a different tree, for two varieties, '842' and '741'. These were marked at c. 1500 h for '741' and at c. 1530 h for '842' on 27 August 2014. For each raceme, the number of flowers that had opened was counted every hour for 2 full days, beginning on 28 August 2014 between the hours of 0715 and 1730. The stigmas from any open flowers were then removed. The timing of anthesis was assessed by determining the relative amount of collectable pollen. This was measured by placing a test tube over a raceme and collecting pollen, using the same method as the cross-pollination experiments (described above). For each variety, a test tube was twisted over 20 racemes of similar flowering stage each hour. The pollen within the test tube was then washed from the test tube by adding 50 mL of 70% ethanol that was shaken for 1 min to dislodge pollen. Pollen grains from a standardised volume (200 µL) of the solution were then counted on a haemocytometer.

Pollen present on pollen presenters and styles versus other floral parts following pollen dehiscence was also examined. Eight flowers from 842 that had dehisced were harvested each hour between 0900 h and 1300 h on 28 August 2014. The flowers and style/pollen presenter were separated immediately and placed in separate vials, washed in ethanol, and the pollen grains counted using a haemocytometer.

Data analysis

Data throughout this report were mainly examined using descriptive statistics as patterns, and trends could be easily determined using these types of analyses. We conservatively assumed that missing or lost marked racemes indicated pollination failure, rather than assuming other sources of damage (herbivory, wind, etc.). To assess whether nut set was best predicted by raceme length or pollination treatment, we used a generalized linear mixed model using package 'lme4' in the programme R (Bates et al. 2015; R Core Team 2016). Region and site were random effects, and the fixed effects modelled were raceme length, treatment, pollen source, nut tree and interactions between these factors. Terms were dropped stepwise to minimise the Akaike information criterion (AIC), and the resulting best fit model was reported.

3.1.3 Results

Hand cross-pollination versus open pollinated on single racemes

Nut set, raceme length and pollination treatment

For the two fruiting varieties assessed ('816' and '741'), raceme length was not found to be a significant predictor of nut set, but its inclusion improved the fit of the model. The factors that were significant predictors of nut set in the model were the identity of the fruiting tree ('816' fruiting tree had lower nut set) and the source of the pollen (cross-pollination by all varietal sources increased set compared to open racemes) (Table 3.1.2). This indicates that pollination experiments in macadamia can be conducted at the raceme level without taking floret number into account.

Table 3.1.2. The results of a generalized linear model (GLMM) for predicting nut set on individual racemes of macadamia trees variety '816' and '741' following hand cross-pollination versus open pollination. The explanatory variables included raceme length, fruiting tree and pollen source trees. Significant variables ($P < 0.05$) are shown in bold.

Explanatory variable	Estimate	Std. Error	z value	Pr(> z)
Intercept	0.21814	0.48566	0.449	0.653
Raceme Length	0.02964	0.01967	1.507	0.131
Fruiting Tree '816'	-1.29925	0.30424	-4.270	<0.001
Fruiting Tree '741'	-0.08382	0.22481	-0.373	0.709
Pollen Source Tree 'A203'	1.80907	0.23132	7.821	<0.001
Pollen Source Tree '816'	1.28878	0.19271	6.688	<0.001
Pollen Source Tree '741'	1.71469	0.18971	9.039	<0.001
Pollen Source Tree '344'	0.90959	0.19877	4.576	<0.001
Pollen Source Tree '246'	0.79534	0.29328	2.712	0.007

Initial and final nut set comparison:

The initial nut set was higher compared to final nut set for all crosses where this comparison was made (Figure 3.1.2). In all but one of the trials, final nut set was lower than initial nut set but higher than the final set compared with open pollinated racemes. An exception was '741' crossed with the

pollen of '842' that initially had significantly higher nut development but final nut set was low and not significantly different to open pollinated racemes (Figure 3.1.2).

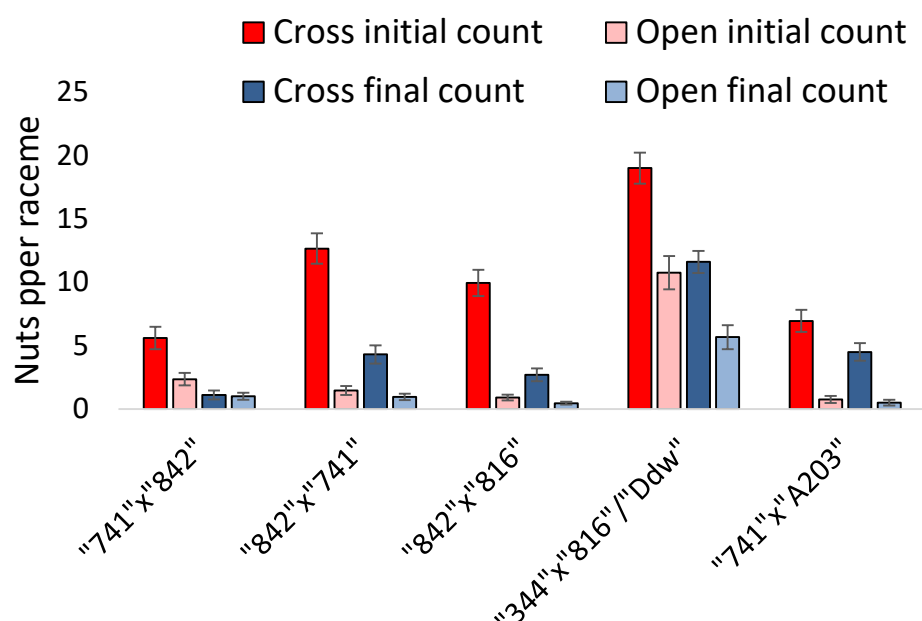


Figure 3.1.2. Initial and final nut set of hand cross pollinated (cross) and open pollinated (open) racemes in five different macadamia variety crosses. The vertical lines are standard error bars.

Final nut set comparisons across orchards and regions:

Hand cross-pollinations resulted in higher final nut sets than in hand self-pollinated controls in the vast majority of cases, (Figure 3.1.3a–e) and many of these comparisons were significant.

We were unable to glean any obvious patterns regarding regional variation in final nut yield patterns (nut set from cross- or open-pollinated racemes). By far the most extensive dataset was obtained from Bundaberg (57 trials), followed by those from Northern Rivers (four trials) and Gympie (one trial). In Bundaberg and the Northern Rivers, data were widely variable and this may have reflected the type of crosses made, in addition to other factors that may affect nut yield, including weather variability and orchard management. However, in the majority of individual trials irrespective of region, cross-pollination increased raceme yields.

Although the pollen source has a significant influence on nut set for most cultivars, differences in performance between varieties are still important (Figure 3.1.3a–e). 'Daddow' is a poor tree for nut set. Variety '816' appears to also be poor for nut set but provides a good pollen source for most other varieties. More cross-pollination trials are needed to assess nut set for varieties such as '816', '344' and 'A16'.

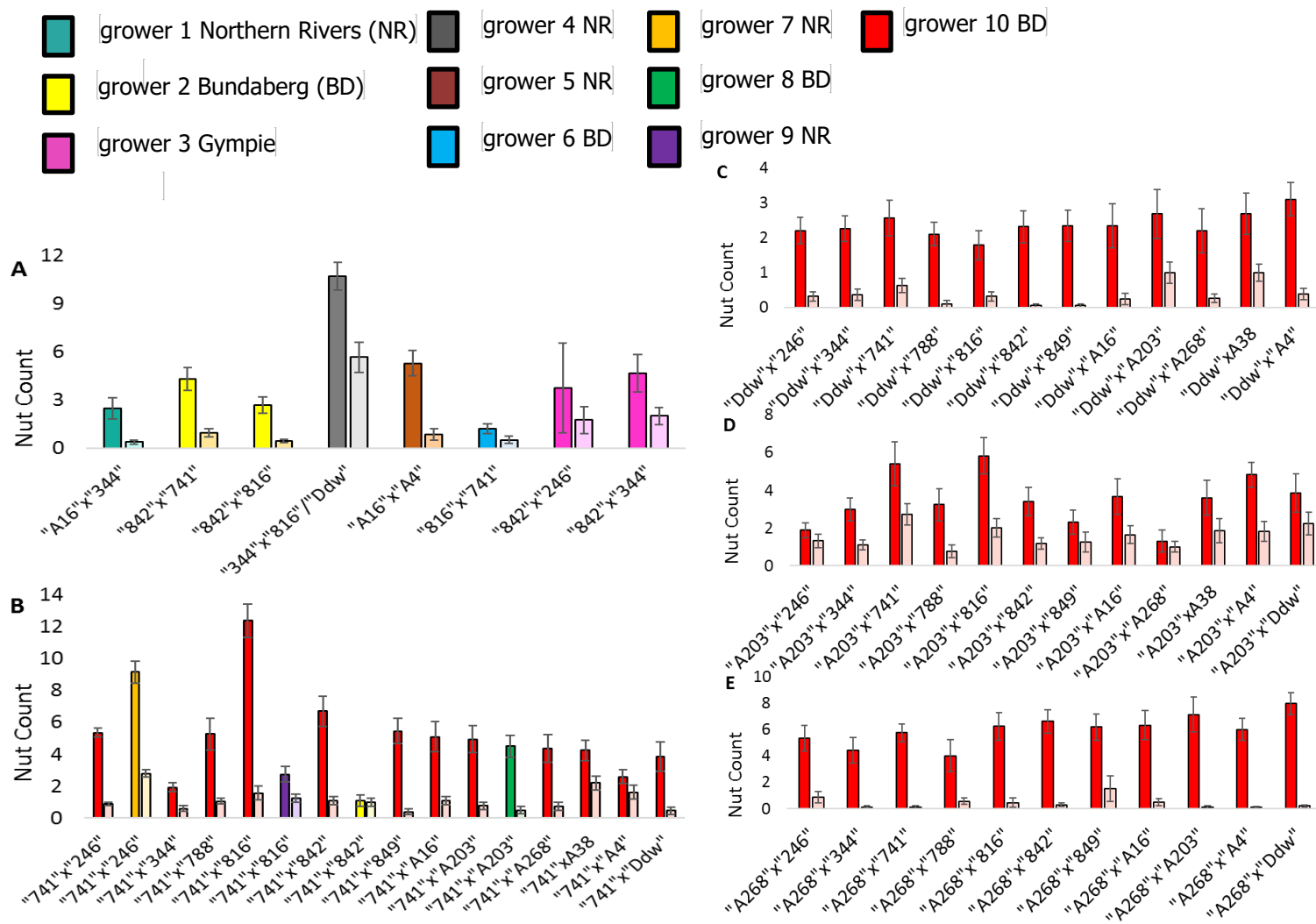


Figure 3.1.3. Mean final nut set (\pm SE) from macadamia racemes that were hand cross-pollinated (fruit tree x pollinizer tree) compared with open pollinated racemes within the same trees. Bold colours are cross-pollinated, pale colours open pollinated. Different colours represent different grower orchards. 3.1.2A are results from a range of crossings conducted between polliniser and fruit tree varieties and conducted across several orchards, 3.1.2B are '741', 3.1.2C variety 'Daddow' (Ddw), 3.1.2D variety 'A203' and 3.1.2E variety 'A268' crossed with a range of different pollinisers.

Cross-pollen limitation variation between nut cultivars

An assessment of nut set of different macadamia varieties irrespective of donor cross-pollen source indicated cross-pollination significantly increased nut set on single racemes for all varieties. However, the total nut set and the scale of the difference between hand cross-pollinated and open racemes varied between cultivars (Figure 3.1.4). Nut set on open racemes for '344' is equal to or greater than cross-pollination on all other cultivars. Cross-pollination nut set on '816' is not significantly different from nut set on open pollinated racemes of most other cultivars. However, the replication for '816' is lower than other varieties.

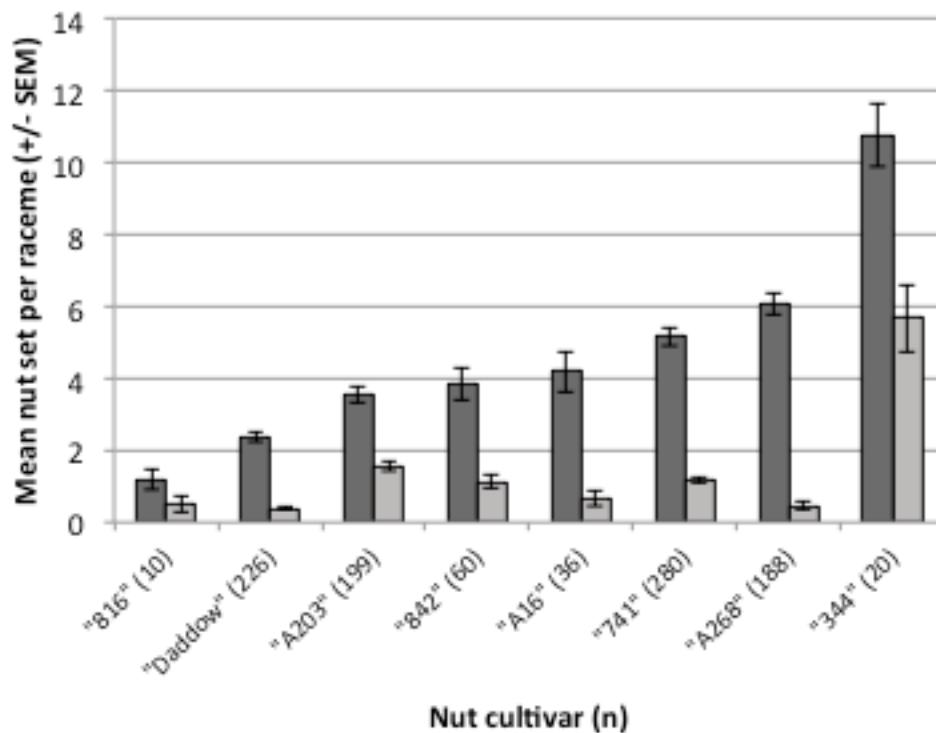


Figure 3.1.4. Nut set from cross-pollination (from various pollen sources, dark grey bars) and open pollination (light grey bars) on single racemes across eight nut cultivars. Sample size is number of trees.

Capacity for self-pollination

Selfing capability for '741', '816' and 'A268' varied (Figure 3.1.5), but all self-pollination treatments produced low nut set compared to open and cross-pollination treatments. '741' self-pollination treatments produced higher final nut counts while 'A268' self-pollination treatments produced just one nut. The various selfing treatments to assess autogamous versus geitonogamous pollination did not show any difference in final nut counts. This data suggests that at least some varieties can produce fully developed nuts from pollination within the flower. Even though self-pollination can result in some nut development to varying degrees in at least some varieties, in all varieties assessed, self-pollinated raceme treatments yielded lower final nut counts than cross-pollinated and open-pollinated treatments.

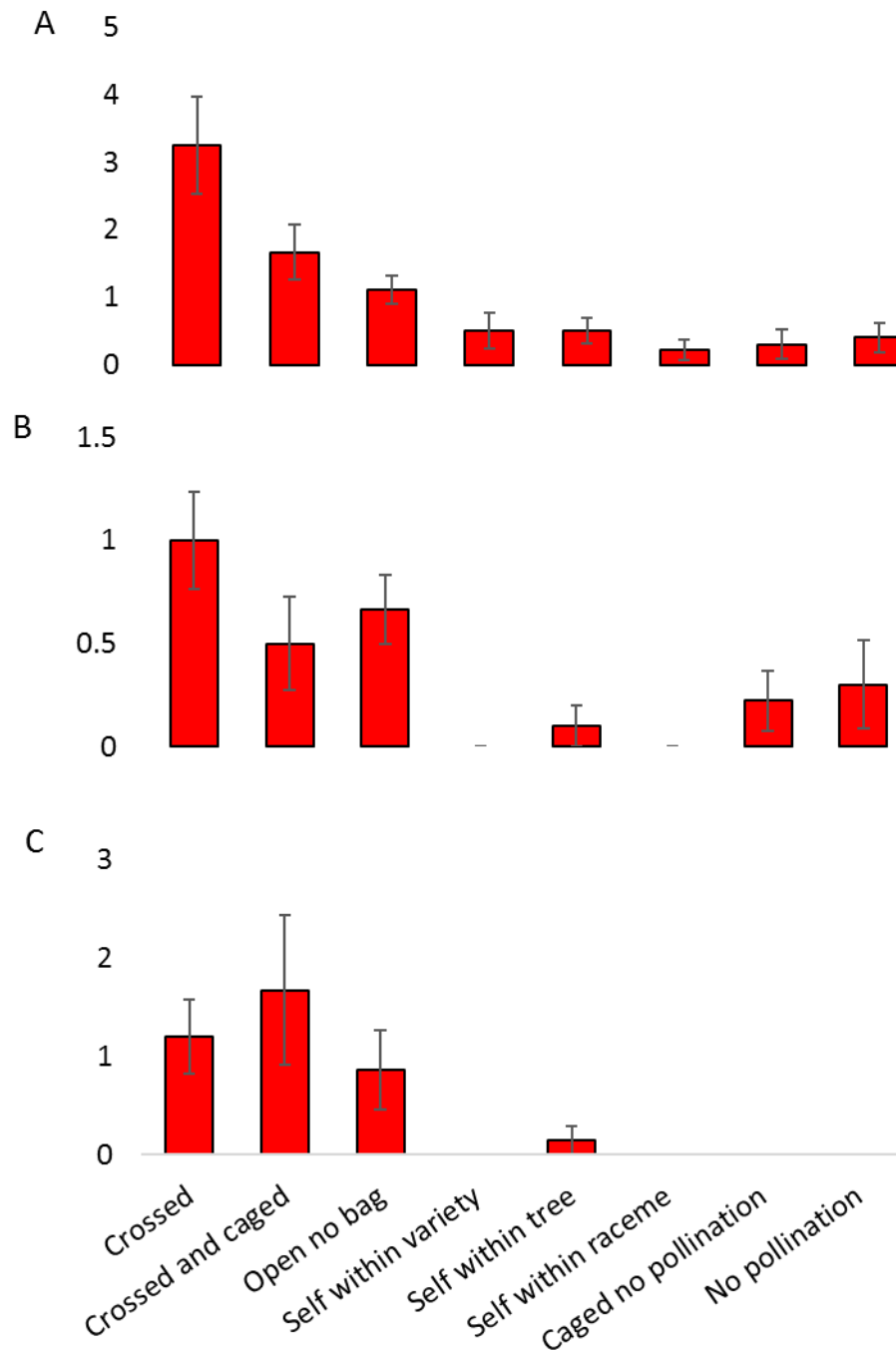


Figure 3.1.5. Final nut counts per raceme for three macadamia varieties A. '741', B. '816' and C. 'A268' following selfing and crossing treatments. Selfing treatments were applied to assess autogamous pollination (racemes bagged or caged with no self-pollination applied), and geitonogamous pollination (pollen movement to florets: 1 within a raceme, 2 between racemes within a tree, 3 between trees of the same variety). Florets of variety '741' were crossed with pollen of '816' ('741' x '816'). The other crossings were '816' x '741' and 'A268' x 'A203' with either bags or cages applied following hand pollination. An additional treatment was included to assess open pollination (flower left exposed to pollinators until bagging alongside other treatments).

Intensity of cross-pollination

As with the single raceme cross experiments, hand cross-pollination of a single raceme within trees of varieties '741' and '842' resulted in a significant final nut set count compared with open pollinated racemes in control trees (both varieties) and open pollinated racemes (assessed in '741' only). However, hand cross-pollinating more racemes within a tree resulted in lower final nut counts on these treated racemes (Figure 3.1.6 and 3.1.7). This pattern was consistent for the two macadamia varieties trialled. Interesting for '741', although there was a decrease in final nut counts/raceme, there was an increase in final nut counts in the control racemes.

Overall, nut counts per raceme within cubic quadrats (all racemes counted whether treated or not) placed in the 100 cross-pollinated raceme trees were higher than the control trees for '741' ('842' not assessed) (Figure 3.1.8). This suggests such a treatment, even when conducted on a single day, can still benefit tree yield overall, despite declining nut set in the cross-pollinated racemes alone.

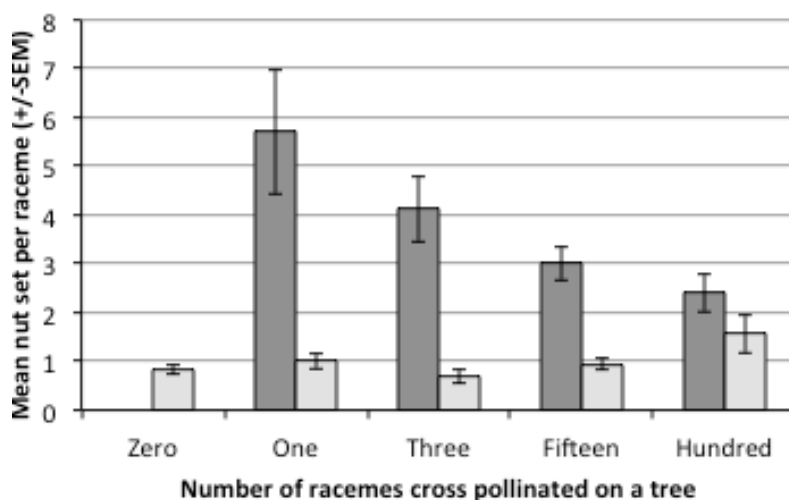


Figure 3.1.6. Mean nut set of hand-crossed (dark grey) and open (light grey) racemes of '741', with varying numbers of racemes hand crossed per tree. Errors bars show standard errors of the mean.

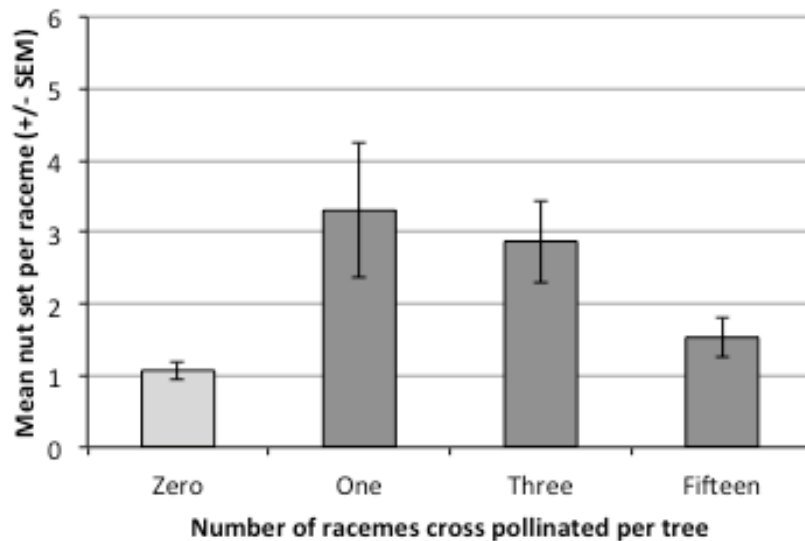


Figure 3.1.7. Mean nut set of hand crossed (dark grey) and open (light grey) racemes of '842', with varying numbers of racemes hand-crossed per tree. Errors bars show standard errors of the mean.

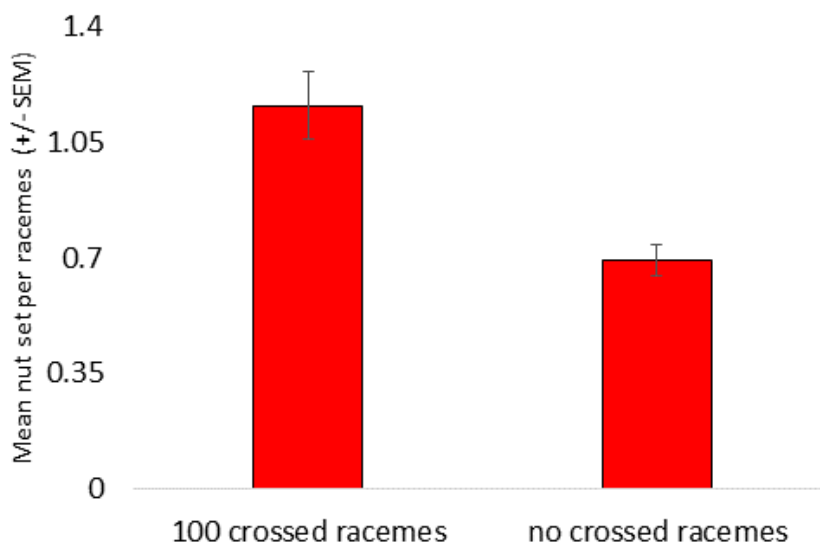


Figure 3.1.8. Mean nut set of racemes within 0.75 m³ quadrats position within trees North, South East and West at a base height above ground of 1.5 m. Comparisons are between trees with hand cross-pollination and those not hand pollinated.

Nectar volume and diurnal pollen availability

Nectar volume in '842' decreased throughout the day, while sugar concentration increased (Figure 3.1.9). Nectar volume was higher when racemes were bagged.

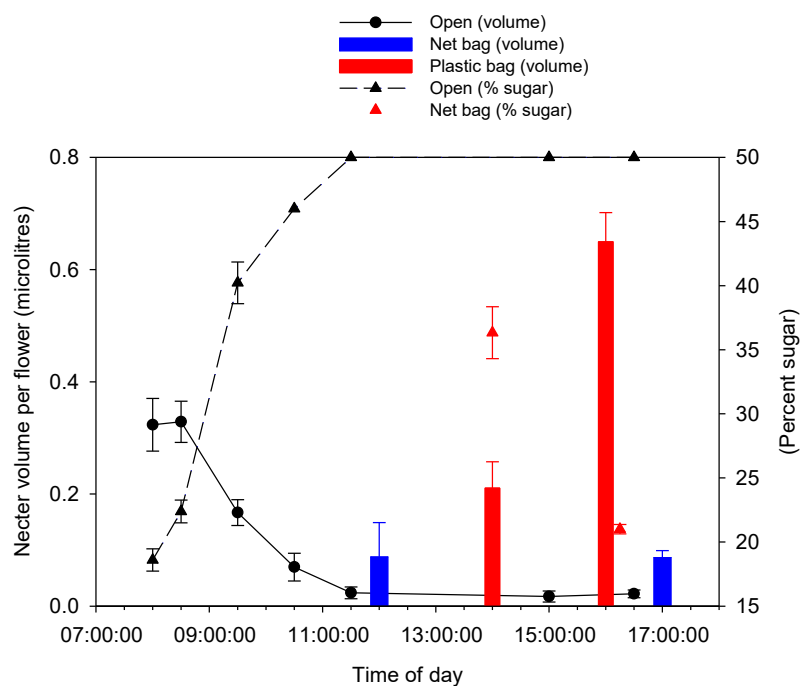


Figure 3.1.9. Nectar volume and percentage sugar concentration recorded from macadamia flowers exposed and not exposed to insects throughout the day.

In both varieties, most flowers ('741' = 95.8%, 842 = 97.3%) opened after 1200 h (Figures 3.1.10 and 3.1.11). Flowers opened earlier on 29 August than on the previous day for both varieties, showing diurnal variability in flower opening time.

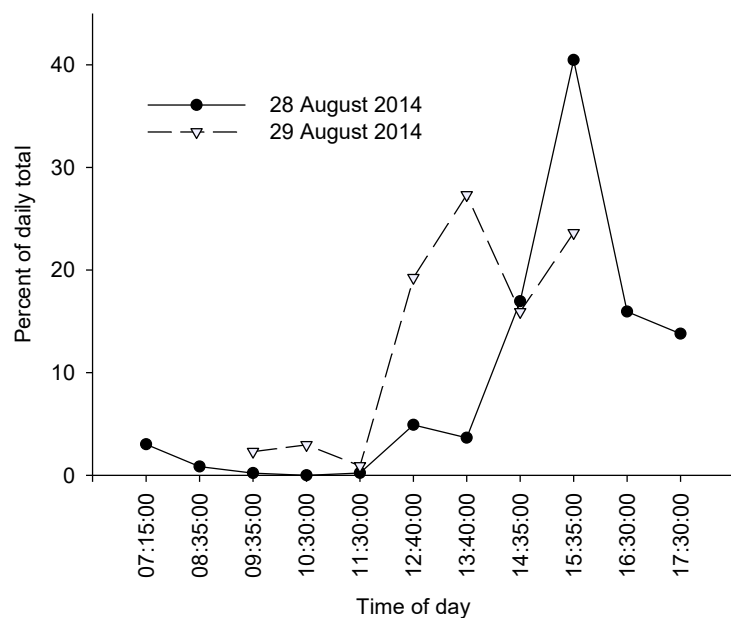


Figure 3.1.10. The number of macadamia flowers that opened each hour expressed as a percentage of the total number opened on each day (of variety '741').

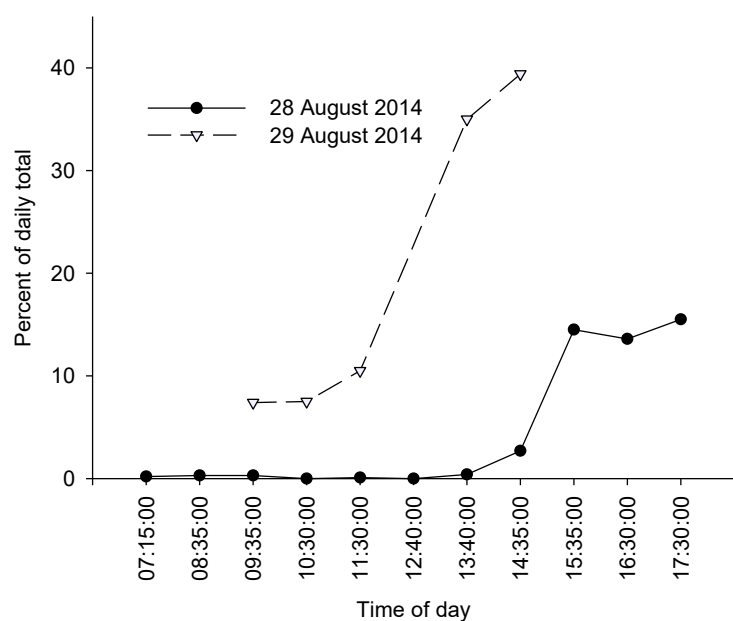


Figure 3.1.11. The number of macadamia flowers that opened each hour expressed as a percentage of the total number opened on each day (of variety '842').

Following pollen dehiscence, between 9.3 and 20% of the total amount of pollen (flowers plus pollen presenters/styles) was present on pollen presenters/styles (Figure 3.1.12).

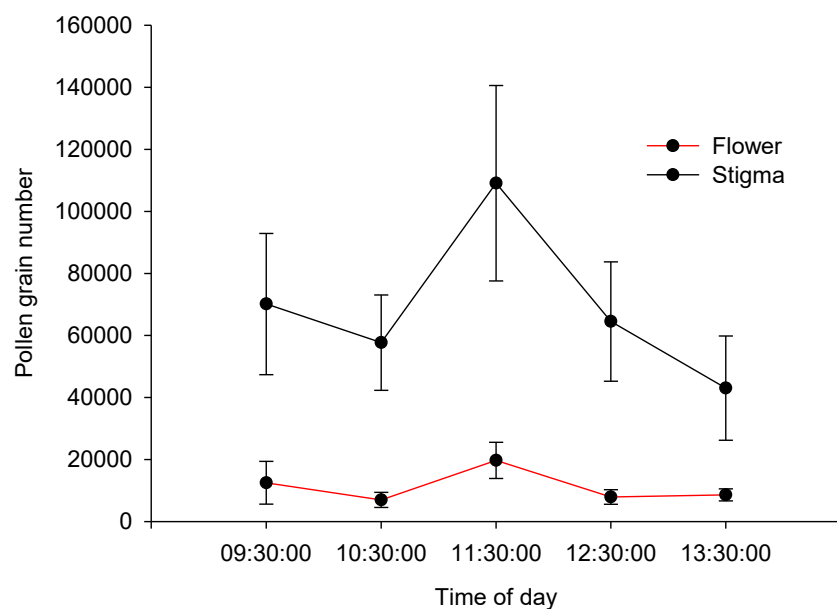


Figure 3.1.12. Pollen on variety '842' macadamia flowers (excluding stigmas) and pollen presenters/styles (labelled stigma) at different times of the day.

3.1.4 Discussion

Hand cross-pollination versus open pollination on single racemes

Our findings extend knowledge on the effect of cross-pollination on final nut set within single racemes providing further insight into the potential benefits of cross-pollinating a range of macadamia varieties with various pollen donor varieties. Many previous studies have assessed the role of cross-pollination between different varieties and its potential influence on nut development; however, these have tended to focus on initial nut set as a measure of cross-pollination effectiveness. Wallace et al. (1996) demonstrated that initial nut set may not be an accurate measure of final nut set. The authors found that in some cases, initial nut set in cross-pollinated treatments (at 14 and 21 days) but not final nut set could be significantly greater than self-pollination and no pollination treatments. Although our studies focussed on final nut set we did assess initial nut set in a small number of trials. For these, our results support Wallace et al. (1996) finding that initial nut set did not always correlated with final nut set. For the varieties we assessed, final nut set was lower than initial nut set but remained higher than the final set compared with open pollinated racemes. An exception was '842' crossed with the pollen of '271' that initially had significantly higher initial nut set but final set was low and not significantly different to open pollinated racemes.

There are a large and growing number of macadamia varieties currently available and being grown by orchardists in Australia and the impact of cross-pollination on tree yields between them has not been assessed on a large scale. This information is key to understanding the nut yielding capacity of these varieties. We hand-crossed a broad range of varietal fruit trees with pollen donors by conducting 62 separate trials (57 at orchards near Bundaberg). In nearly all cases, cross-pollination resulted in higher numbers of final nuts set compared to open pollinated racemes. It is important to note that open pollinated racemes were fully exposed to visit by pollinating insects and therefore cross-pollination may have occurred in these racemes. However, in most cases this appeared insufficient to maximise nut set.

In the few cases where cross-pollination did not significantly increase nut set, nut yields were low. The reason for this was not assessed. One factor may have been poor compatibility between the fruiting tree and pollen donor variety. The relatedness between varieties has not been considered, but many varieties are considered very closely related. For example, Hawaiian varieties are derived from a small number of *M. integrifolia* (Pearce et al. 2008) and this may have impacted on compatibility between pollen donor and recipient varieties (Storey 1965). Our data suggest that some varieties (e.g. '816') may be better pollen donors for a range of varieties resulting in high raceme nut yields. Moreover, other unexplored factors such as tree health, orchard management, pests and diseases and weather could also have impacted on final nut set within each trial.

Further work to assess the role of the various factors that influence final nut set needs to be conducted to determine the best combinations of fruiting tree and pollen donor varieties that can optimise nut yields. However, our assessments in these trials point to the potential for increased crop yields by incorporating multiple varieties within orchard blocks. This is a recommendation that has been suggested by previous authors (Sedgley et al. 1990; Rhodes 2001).

Capability for self-pollination

Although macadamia has been considered to be partially self-incompatible, we found that self-pollination can result in fully developed nuts. Variety '741' produced more self-pollinated nuts than '816' while 'A268' produced just one self-pollinated nut. These results may explain why nut yields are produced in single variety blocks that are relatively isolated from blocks of other varieties. However, self-pollination racemes, irrespective of mode of self-pollination applied [autogamy (no pollination treatments of racemes enclosed in either bags or cages within bags) or geitonogamy (self-pollination

treatments by pollinating flowers within racemes, between racemes and between trees of the same variety) were lower for all three varieties when compared with cross-pollination or open pollinated racemes. This supports our findings (and the findings of other studies) that cross-pollination of macadamia has the potential to increase yields within racemes. This is despite the capability of at least some varieties to produce fully developed nuts through self-pollination.

We did not find any significant differences in the type of self-pollination. Our treatments to test autogamy produced a small number fully developed nuts in '741' and '816'. This occurred even within cages that were designed to minimise the chance of pollen movement between florets. If these treatments truly reflect autogamous pollination then it indicates that self-pollen on the pollen presenter is capable of pollinating the stigma, even though flowers are protandrous (exposing pollen 1-2 days prior to the stigma becoming receptive (Sedgley et al. 1985). We cannot entirely rule out the possibility that developed nuts from these treatments were not produced through autogamous pollination. There was a chance that between flower pollen movement may have occurred via movement of small vectors (e.g. tiny arthropods such as thrips) whose presence could have been missed during bagging or alternatively through possible air borne pollen within the cages and bags.

Intensity of cross-pollination

To our knowledge, this study is the first to assess nut set on racemes following hand cross-pollination of varying numbers of raceme within trees. For '741' and '842', there was a drop in mean number of nuts set per raceme as the number of hand crossed racemes increased within a tree. Therefore, resulting nut set from a single crossed raceme is likely to overestimate the potential nut set within the tree, if the result alone is used to calculate tree yield potential.

However, for racemes not hand cross-pollinated ('741' only assessed), we saw an increase in the number of nuts set per raceme the more we cross-pollinated racemes within a tree. A possible reason for this is that pollinators were more readily transferring cross-pollen from hand cross-pollinated racemes to the open racemes as they moved between the treated and untreated racemes. Moreover, we observed an overall increase in final nut counts per 0.75m³ quadrat in trees where we cross-pollinated 100 racemes compared to control trees (no cross-pollination) strongly suggesting that cross-pollination can boost overall yields.

The maximum number of racemes hand pollinated per tree in this trial – 100 racemes over a single day – was just a small fraction of the numbers of racemes that would have flowered within the tree during its flowering period. We do not know what effect further increasing cross-pollination within trees might have on a tree's overall yield capacity and its potential consequences on tree health and future bearing capability.

Using tubes to collect and transfer pollen

The use of test tubes (or similar) to hand cross-pollinate racemes by collecting pollen and transferring to a tree of another variety is very frequently used as a method by researchers. This technique is convenient as rapid cross-pollination of many racemes can be conducted. Using this method consistently should provide data that is comparable between different treatments. However, the technique does not guarantee that the stigmas of all florets come into contact with cross-pollen. Whether hand cross-pollination of individual florets (more time consuming) would have resulted in higher (or different nut set) would be a useful method to determine whether raceme nut counts using this technique, may impact the capacity of final nut set within racemes.

Diurnal patterns of nectar production, pollen dehiscence and flower opening

These results suggest nectar flow within flowers occurs in the evening, at night or very early morning (before 0800 h). The decrease in volume throughout the day indicated that flowers in this variety do not continually release significant amounts of nectar. Other studies have also reported nectar peaking in volume in the morning but unlike the findings of this study, they also report a second peak is reported to peak in the early afternoon (Heard 1994; Kongpitak et al. 2012). Nectar volume has been suggested as a potential influence on peak pollinator flower visitation times (Vithanage & Ironside 1986).

Corresponding increases in sugar concentrations with decreasing nectar volume within flowers throughout the day may be due to evaporation. Evaporation is known to increase sugar concentrations in the nectar of plants (Corbet 2003). The volumes of nectar and sugar concentrations from flowers sampled from the plastic bags during the afternoon suggest the bags inhibited nectar evaporation, while some evaporation is likely to have occurred from the flowers contained in the mesh bags.

In varieties, '741' and '842', the majority of flowers opened just before (first day of monitoring) or just after midday (second day) demonstrating that diurnal variation occurs between days. The amount of pollen on pollen presenters also corresponded closely with pollen on the flowers themselves (not on the presenters). The presence of pollen on flowers, but not on the pollen presenter/style, may have been due to rapid redistribution by a range of flower-visiting species or possibly by the wind. However, wind pollination, is considered to play an insignificant role in pollination because of the tendency for pollen to be released in viscous clumps as well as the small size of the receptive stigmatic surface (Schroeder 1959; Scholefield 1982; Wallace 1999). The diurnal pattern between the number of pollen grains on the pollen presenter/style versus elsewhere on the flower showed similar relative count patterns, with the largest counts being at 1130 h (Figure 1.22). This may reflect increased pollen dehiscence by flowers at this time. These finding appears to fall in the middle of two previous studies. McGregor (1999) found that peak anthesis occurred early in the morning on flower opening while Heard (1993) noted that pollen availability peaks mid-afternoon on the day of anthesis. However, flower opening appears to vary somewhat between days, even for the same variety (Figures 1.9 and 1.10).

Key Points

- Cross-pollination of single racemes within trees of various varieties demonstrated that in the majority of cases, final nut yield is increased. This suggest a high degree of flexibility in mixing macadamia varieties within blocks to increase yields.
- A degree of self-pollination occurs in at least some varieties; however, this may vary with variety. Self-pollination is likely to explain why isolated blocks of a single variety still produce nuts. However, cross-pollination of racemes still resulted in increased nut set pointing to potential yield increases in single variety orchards.
- Hand cross pollinating larger numbers of racemes within trees of variety '741' and '842' resulted in a lower number of nuts set in these racemes compared to tree where just one raceme is cross-pollinated. Despite this, our quadrat counts still showed a mean increase in nut set compared with trees that were not hand cross-pollinated (only '741' assessed). As cross pollination still results in increased nut set, we recommend orchard designs incorporate more than one variety to promote cross-pollination.

3.1.5 References

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3.2 Efficiency of macadamia pollinators

3.2.1 Introduction

An understanding of the contribution and efficiency of pollinators to cultivated macadamia is necessary to develop strategies that growers can implement that target key species. Here we define efficiency as the rate of pollen deposited onto stigmas by an individual of a species. Findings from this study and others suggest that cross-pollination can increase yields in macadamia across a number of varieties (de Lange 1974; Sedgley 1983; Masis & Lezama 1991; Trueman 2013). This requires the removal of self-pollen from the pollen presenter of the flower and replacing it with pollen from a different variety (Wallace 1999). Insects are considered key pollinators of *Macadamia* (Howlett et al. 2015 and references within) with initial and final nut set in *M. integrifolia* having been shown to be correlated with increased insect visitation to flowers (Heard 1993). Honey bees and stingless bees are considered the most important pollinating species in Australia and are currently the only readily available managed pollinators that are used by macadamia growers for pollination purposes (Rhodes 2001; Halcroft et al. 2013).

A number of studies have assessed aspects of the efficiency of honey bees as pollinators of macadamia. These have included foraging patterns such as movements between flowers and their ability to deposit pollen onto stigmas. Nectar-collecting individuals have been reported to interact infrequently with stigmas as flower nectaries located at the base of the style are too distant for the body of the bee to directly contact the stigma when foraging (Heard 1994). Vithanage and Douglas (1987) found honey bees to be capable of depositing pollen onto stigmas with the amounts deposited correlated with the amounts being carried on the body of honey bees at specific times. Heard (1993) estimated that a macadamia flower required 150 honey bee visits for adequate pollination; however, other studies have not found a correlation between honey bee visits and nut set (Rhodes 1986; Blanche et al. 2006).

In contrast to honey bees, individuals of some stingless bee species, (including *T. carbonaria* in Australia), have been observed collecting pollen rather than nectar, resulting in frequent contact with the stigma (Heard & Exley 1994). This has led to some studies concluding that they are more efficient pollinators than honey bees (Heard 1987, 1994; Kongpitak et al. 2012). The majority of foraging stingless bee individuals from orchard based hives have also been found collecting macadamia pollen (Heard 1987). This is in contrast to orchard-based honey bee hives from which the majority of individuals may collect pollen from other plant species (Heard 1987, 1994). To date, information quantifying the amount of cross-pollen they are capable of transferring to stigmas or their rate of movement between macadamia varieties (or even trees) is not apparently available. Moreover, some individuals have been observed robbing pollen from opening flowers where the stigma has not been fully exposed (approximately 18% of foraging bees between 14:00 and 15:30 from data collected across 10 orchards) (Heard 1994). Temperatures below 18°C are also not considered favourable for *T. carbonaria* foraging (Heard & Exley 1994) potentially limiting their usefulness on cooler days.

Other insects and vertebrates have also been considered to be potential pollinators of macadamia; however, published information on their efficiency is relatively limited. Other bee species have been noted as flower visitors of macadamia of which some may be pollinators (Vithanage & Ironside 1986; Heard & Exley 1994; Kongpitak et al. 2012). The beetle species *Metriorrhyncus rhipidius* (Macleay) and *Campsomeris tasmaniensis* (Sassure 1885)(Hymenoptera: Scolidae) have been considered to be potentially important pollinating species within some orchards as they carried pollen and were noted moving between racemes (Vithanage & Ironside 1986). Other flower visitors recorded include various species of Diptera (flies), Lepidoptera (both butterflies and moths) and Hymenoptera (wasps) (Masis & Lezama 1991; Heard & Exley 1994; Kongpitak et al. 2012). Birds have also been observed

feeding on the nectar of flowers and could potentially deposit pollen over distances of at least several hundred metres (Heard & Exley 1994; Howlett et al. 2015).

The aim of the current study is to measure the key pollinator efficiency parameters of common flower visiting species within macadamia orchards in Australia. These are pollen deposition on stigmas, movement between flowers, movement within and between racemes both within and between trees, and rate of contact with stigmas. Together, these parameters are necessary to determine and rank different pollinating species. We also examine the potential and consistency of likely pollen flow throughout the day within five orchard blocks, two located near Gympie (South East Queensland) and three near Bundaberg (Central Queensland) by placing fluorescent powder onto the pollen presenters of selected racemes at different times and tracing its movement using a UV light source.

3.2.2 Materials and methods

Pollen deposition onto stigmas

Data was collected in 2014 from within two orchards near Bundaberg (24.7753° S, 152.2744° E; varieties '741' and '344'), and three orchards near Gympie (26.2609°S, 152.5864°S; varieties '842' and '344'). Additional data was collected in 2015 from '842' from an orchard near Gympie. For each insect visit, we recorded the insect taxa, time of visit, insect behaviour (pollen or nectar collecting), weather variables at the time of visit (temperature, humidity, light intensity and wind speed).

We particularly focused on collecting data on more common flower visiting species: **honey bees**, *Apis mellifera* L. (Apidae); **stingless bees**, *Tetragonula carbonaria* Smith, 1854 (Apidae); **lycid or net-winged beetles**, *Metriornychus rhipidus* Macleay (Lycidae); **soldier beetles**, *Chaliognathus flavipennis* W.J. Macleay, 1872 (Cantharidae); **nose flies**, *Stomorhina discolor* Fabricius, 1794 (Rhiniidae); and **Australian brown blowflies**, *Calliphora stygia* Fabricius, 1781 (Calliphoridae). Where additional flower visiting insect species were measured, we make note of our findings.

To assess pollen deposition on flowers, we selected racemes containing a combination of fully open flowers (pollen presenters fully exposed), opening flowers (pollen presenter still contained within the bud) and buds. Flowers deemed recently opened (neighbouring opening flowers) with styles fully expanded but with no evident disturbance to the self-pollen on the pollen presenters were selected as test flowers.

The flowers were carefully abscised approximately 10 mm below the receptacle using forceps. A cube ($10 \pm 2 \text{ mm}^3$) of gelatine fuschin (gf)(Dafni 1992) (stained a deep wine red colour) was melted at approximately $30 \pm 5^\circ\text{C}$ on a glass slide. The style and stigma of the test flower was then completely coated by the melted gf using forceps to manipulate the flower. The flower was then removed and the gf left to set for 1–2 min outside of direct sunlight and at air temperature ($18\text{--}23^\circ\text{C}$). Once set the gf was carefully peeled off using forceps removing the bulk of the self-pollen. Any remaining pollen was stained and therefore identifiable from new pollen that may arrive on the surface of the style or stigma.

Following the removal of self-pollen, the test flower was then exposed to a targeted pollinating species to determine their ability to transfer pollen to the style and stigma. This was achieved by carefully orienting the treated flower using forceps amongst a flowering raceme that already had a target insect foraging upon it. Care was taken to orient the treated flower into a similar position to those flowering within the raceme, without contacting the flowers within the raceme. Following an insect visit to the treated flower, the style and stigma were abscised from the ovary, the style was then abscised from the stigma at 3 mm below the stigma, and each floral part was placed onto a small cube of gelatine-aniline blue. The cube was then melted at low heat (approximately 30°C) and a cover slip gently pressed over the melted gelatine-aniline blue and style or stigma and allowed to set.

Flowers visited by insects were then compared with control flowers. Control flowers were collected following the same procedure as insect-visited flowers; however, an insect was not observed to make contact with the flower. Control flowers were exposed within a raceme for between 15 and 20 s.

Slides were later examined in the laboratory and counts conducted of clear macadamia pollen grains in contact with the stigma, within 3 mm of the stigma, and on the styles conducted using a compound microscope. Pollen grains that had been stained red by the gf treatment were not counted.

Movement of insects between flowers, racemes and trees

To compare how frequently different pollinators are likely to move pollen, we tracked pollinators and recording rates of movement among flowers, inflorescences and trees. Data were collected by speaking movement codes into audio recorders. These were conducted within the same orchards as those used to assess pollen deposition on stigma/styles by insects. Flower-visiting species were targeted based on their abundances to ensure adequate replication of the most common taxa. Data were collected opportunistically throughout the day when pollinators were active. Movements between racemes within trees and between trees were recorded, as well as weather variables (temperature, humidity, light intensity, wind speed). These data are assessed to compare rates of movements between species and where possible, whether movement patterns are influenced by the macadamia variety.

Additional data were also collected in year 2 to increase the replication of those insect species evaluated in year 1 and also to gather additional data on *Chaliognathus flavipennis* W.J. Macleay, 1872 (Cantharidae) and *Metriorrynchus rhipidus* Macleay (Lycidae) so that they may be included in pollinator efficiency assessments.

Behaviour of pollen and nectar collecting honey bees

To further understand the effectiveness of honey bees as pollinators, close-up videos of honey bees visiting flowers of '842' were taken. The behaviour of foraging bees was assessed, along with the number of visits to racemes and the length of time of their visits. Chosen racemes were located within 30 m of a group of c. 50 beehives. Two racemes of '842' were filmed for five minutes during every hour for 5 days, using two cameras. On 26 August 2014, recordings were taken between 0830 h and 1630 h; on 27 August between 0830 h and 1330 h (no recording at 1230 h because of rain); on 28 August between 0900 h and 1700 h; on 29 August between 0900 h and 1400 h; and on 31 August between 0820 h and 1720 h. At some periods during the day, video recording was halted because of rain.

On 30 August 2014, two racemes from '741' were similarly recorded every hour between 0930 h and 1515 h. Because of a lack of honey bee activity on this variety (just one bee was observed), further recordings were not conducted.

Honey bees foraging for nectar and pollen were captured from racemes of '842' and the numbers of loose pollen grains were counted. We also aimed to capture honey bees from '741'; however, there were too few bees foraging to do this. It is common for there to be a correlation between the number of pollen grains on the body of a pollinating insect and the amount of pollen deposited onto stigmas (Vithanage & Ironside 1986; Howlett et al. 2013). This was assessed for macadamia by comparing these data with data assessing the ability of honey bees to deposit pollen onto macadamia stigmas. A total of 41 bees were collected between 27 and 30 August 2014 and between the hours of 0940 and 1525.

Assessing pollen flow in three macadamia orchards

To assess whether low flower visitor abundance inhibited pollen flow between and within racemes and whether time of day influenced pollen flow, experiments were conducted in two orchards near Bundaberg (Gooburrum Road 24°45'36.01"S, 152°16'34.59"E; Moore Park Road 24°46'35.02"S, 152°16'45.68"E and a single orchard near Gympie (26.26089°S, 152.58635°E). Two different macadamia varieties contained in separate blocks were assessed at each orchard. At Gympie these were '741' and '842', at Moore Park Road they were '741' and '816' while at Gooburrum Road they were 'A264' and 'A209'. Separate experiments were conducted in each orchard. At Gooburrum Road two varieties – '741' and '816' – were assessed in separate experiments.

At the two Bundaberg orchards fluorescent powders were applied between 6 and 7 am; 9 and 10 am; 12 and 1 pm; 3 and 4 pm; and 6 and 7 pm, while at the Gympie orchard they were applied at all of these times except 6–7 am. For each time, raceme applications were conducted on two trees in three locations within each block. The locations were at opposite ends and in the centre of each block. Treatments at the end of each block were applied to trees located within three trees of the end of the block and at each time, the two treatment racemes were selected on trees at least four rows apart and treatments and just one raceme treatment was applied on a tree. A different-coloured fluorescent powder colour was applied to each treatment at each different time.

A different experimental design was applied at the Gympie blocks to assess whether the colour of the fluorescent powder might influence flower visitation and powder movement. Two trees were selected at each block location (either end of the orchard and in the centre), and all time treatments were conducted within the tree. Four racemes were chosen per tree for powder application, each raceme receiving one powder application at a separate time. A different-coloured powder was applied to a raceme for a given time; however, the colour of the powder application to a raceme differed to the other racemes marked at different times. Trees selected for the treatments were located at least four rows apart.

To apply the fluorescent powder, individual disposable plastic acetone sheets (A4 size) were rolled into cylinders with an aperture of diameter 7 cm and sealed using polypropylene sealing tape. The base of the tube was also sealed by applying a double layer of the sealing tape to the base to minimise fluorescent powder sticking the tape and to prevent the loss of the powder. Separate cylinders were constructed for each powder colour. Approximately two tablespoons of powder were placed in each tube and evenly distributed across the inner wall of each tube by shaking, rubbing and knocking the tube. To apply the powder to a flowering raceme, the cylinder was placed directly around the raceme, ensuring the pollen presenters of the flowers contacted with the sides of the cylinder (Figure 3.2.1). The cylinder was then twisted on the treatment raceme, allowing for powder to be transferred from the cylinder wall to the pollen presenters of the raceme. Following powder transfer, the raceme would be examined for even distribution of powder to the pollen presenters. If this was not the case then the tube containing the powder was shaken and re-applied until even distribution was obtained.



Figure 3.2.1. Application of fluorescent powder to the pollen presenters of a flowering raceme.

In all blocks, powder-treated racemes were exposed for three hours and then detached and disposed of to prevent further powder being removed from the raceme. A UV lamp was then used to view fluorescent powder at night (Figure 3.2.2), thereby visualising the movement of powder within and between trees. Neighbouring racemes and trees up to 20 m away were examined for powder. Where powder was detected, distance was measured along with the location of the powder (e.g. petal, style, stigma, leaf, petiole, branch).

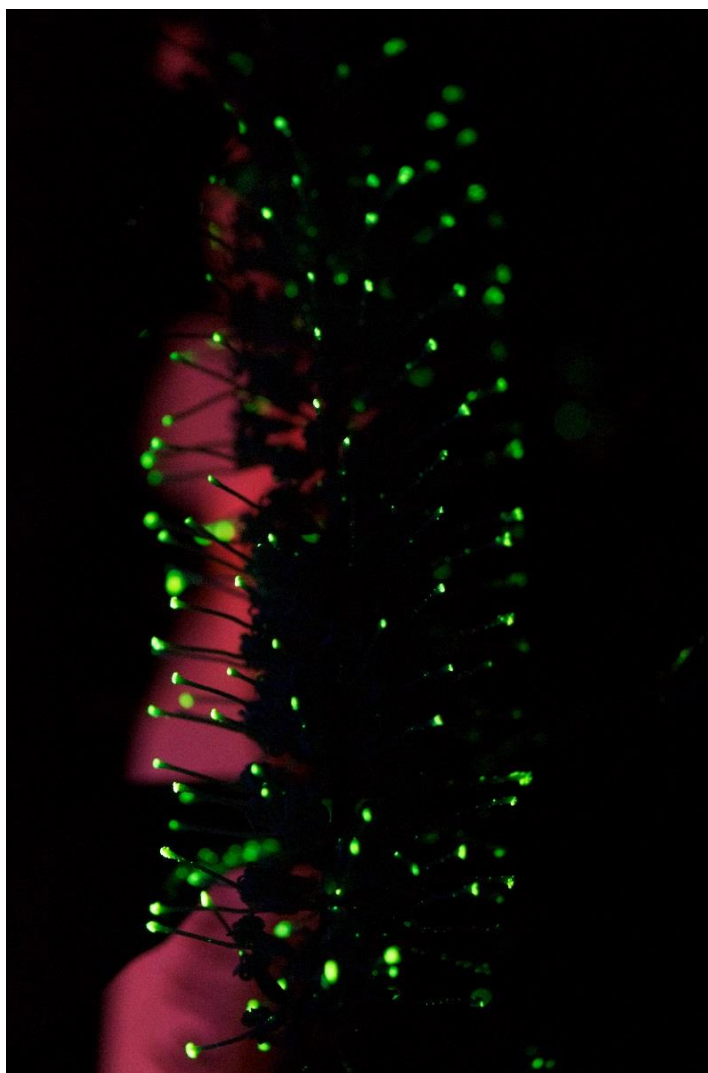


Figure 3.2.2. A flowering raceme showing the distribution of fluorescent powder on flower pollen presenters following treatment and under UV light (photograph by Brian Cutting, Plant & Food Research).

3.2.3 Results

Pollen deposition onto stigmas

A total of 185 individual insect visits to test flowers were obtained, ranging from 104 visits by the honey bee (*A. mellifera*) to three visits by the scarab beetle *Glycyphana stolata* (Table 3.2.1).

In all cases the majority of visits deposited either none or very few pollen grains (<3) with the exception of the lycid beetle (*Metriorrhyncus rhipidius*), soldier beetle (*Chauliognathus flavipennis*) (Figure 3.2.3) and *G. stolata* (min 4, max 45).

The mean (\pm SE), pollen grain deposition directly onto stigmas implies lycid beetles (0.62 ± 0.33), stingless bees (0.58 ± 0.10), honey bees (0.40 ± 0.1), nose flies (*Stomorphina discolor*) (0.40 ± 0.22) and soldier beetles (0.36 ± 0.15) all capable of pollination as these data were higher than for controls (0.12 ± 0.06) (Figure 3.2.3). No pollen grains were deposited on stigmas for scarab beetles, hover flies (*Melangyna viridiceps*) or blow flies (*Calliphora stygia*), although numbers of individuals tested of these insects were lower than the other species.

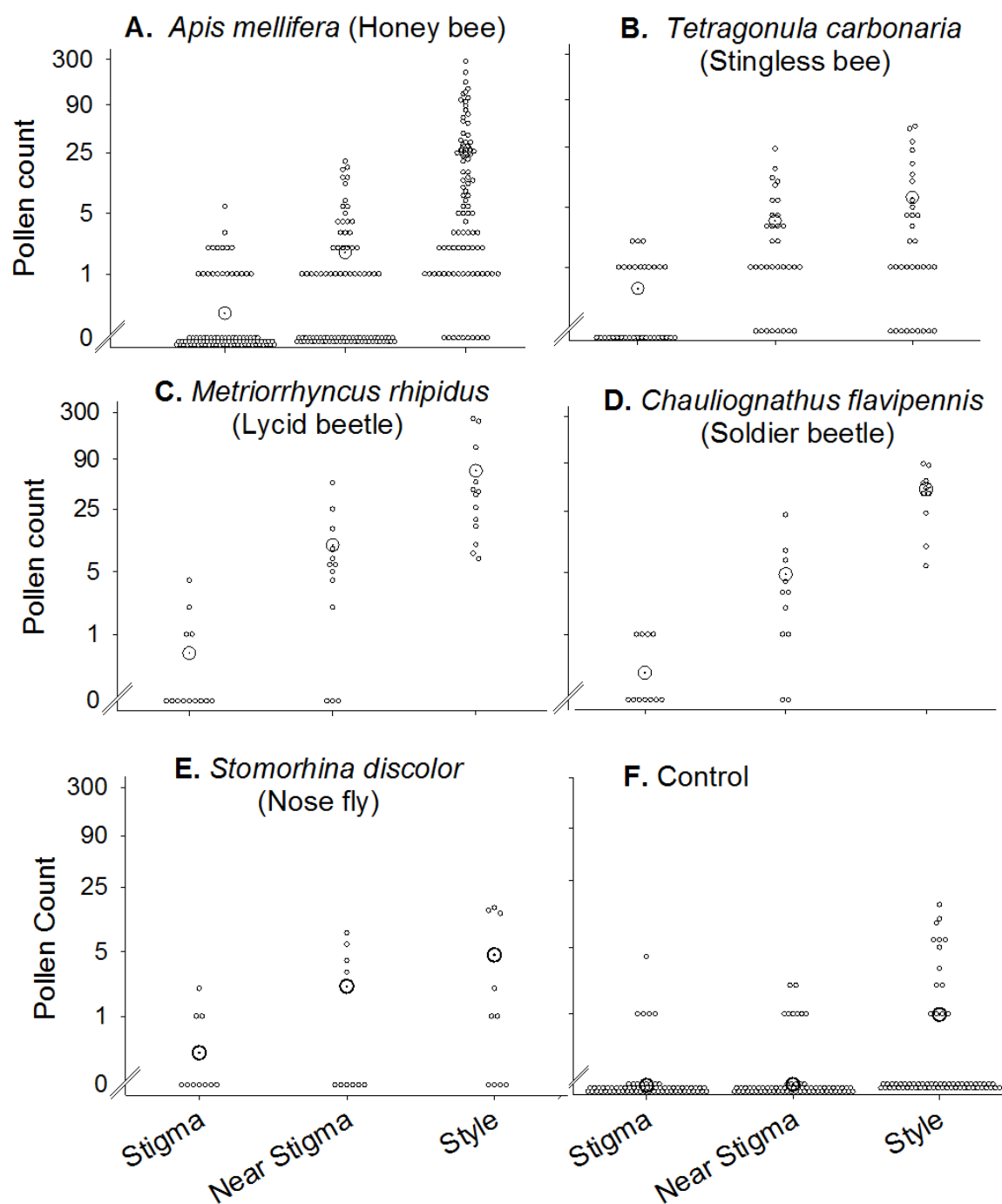


Figure 3.2.3. Pollen deposition by individuals of five different flower visiting insects in or near the stigma and on the style of macadamia flowers (black dots). Means are larger clear circles. Vertical scale is presented as natural log.

The mean pollen deposition on pollen presenters (within 3 mm of the stigma) by the various pollinators was higher than on stigmas. Lycid beetles recorded a mean \pm SE of 10.7 ± 3.78 , soldier beetles 5.30 ± 2.01 , stingless bees 4.43 ± 0.89 , honey bees 2.13 ± 0.46 , nose flies 1.83 ± 0.95 , scarab beetles 1.67 ± 1.20 , brown blow flies 1.25 ± 0.90 and hover flies 0.57 ± 0.42 . All values were higher than the controls (0.15 ± 0.05).

Mean numbers of pollen grains deposited on styles by these insects were higher than on or near stigmas (Figure 3.2.3). Lycid beetles recorded a mean \pm SE of 71.50 ± 25.24 , soldier beetles 48.60 ± 7.85 , scarab beetles 27.00 ± 12.10 , honey bees 25.84 ± 4.60 , stingless bees 6.42 ± 1.32 , hover flies 5.63 ± 4.78 and nose flies 4.60 ± 2.10 . Only data for brown blow flies 1.00 ± 0.71 was similar to controls 0.99 ± 0.32 .

Stingless bees deposited pollen the most frequently, followed by soldier and lycid beetles, nose flies and then honey bees (Table 3.2.1). For pollen deposition near stigmas, the lycid and soldier beetle were as equally efficient as stingless bees while scarab beetles also delivered pollen on two of three occasions tested. Honey bees, brown blow flies delivered pollen at close to 50% of the time while all other species also exceeded controls (Table 3.2.1). All individuals of soldier beetles and lycid beetles successfully delivered pollen to styles while honey bees also deposited more than stingless bees. Percentage pollen delivery to styles by all other tested insects exceeded controls (Table 3.2.1).

Table 3.2.1. Insects visiting test flowers and the proportion that delivered pollen.

Insect Honey bee	Family Apidae	Visits 104	Percent of visits delivering pollen		
			On stigma 20.2	Near stigma 42.1	On style 87.3
Stingless bee	Apidae	33	45.5	75.8	72.7
Lycid beetle	Lycidae	13	30.8	76.9	100.0
Soldier beetle	Cantharidae	11	36.4	81.8	100.0
Scarab beetle	Scarabidae	3	0.0	66.7	66.7
Nose fly	Rhiniidae	10	30.0	40.0	60.0
Hover fly	Syrphidae	7	0.0	28.6	42.9
Brown blow flies	Calliphoridae	4	0.0	50.0	50.0
Control		68	7.4	11.8	20.6

There were no obvious differences between the three macadamia varieties regarding the amount of pollen deposition across stigmas and styles for honey bees (the most commonly sampled insect across varieties) (Figure 3.2.4). However, the style controls sampled from '842' contained more pollen than the other varieties. Pollen grains across all stigma-style control slides were comparatively low compared to those visited by insects.

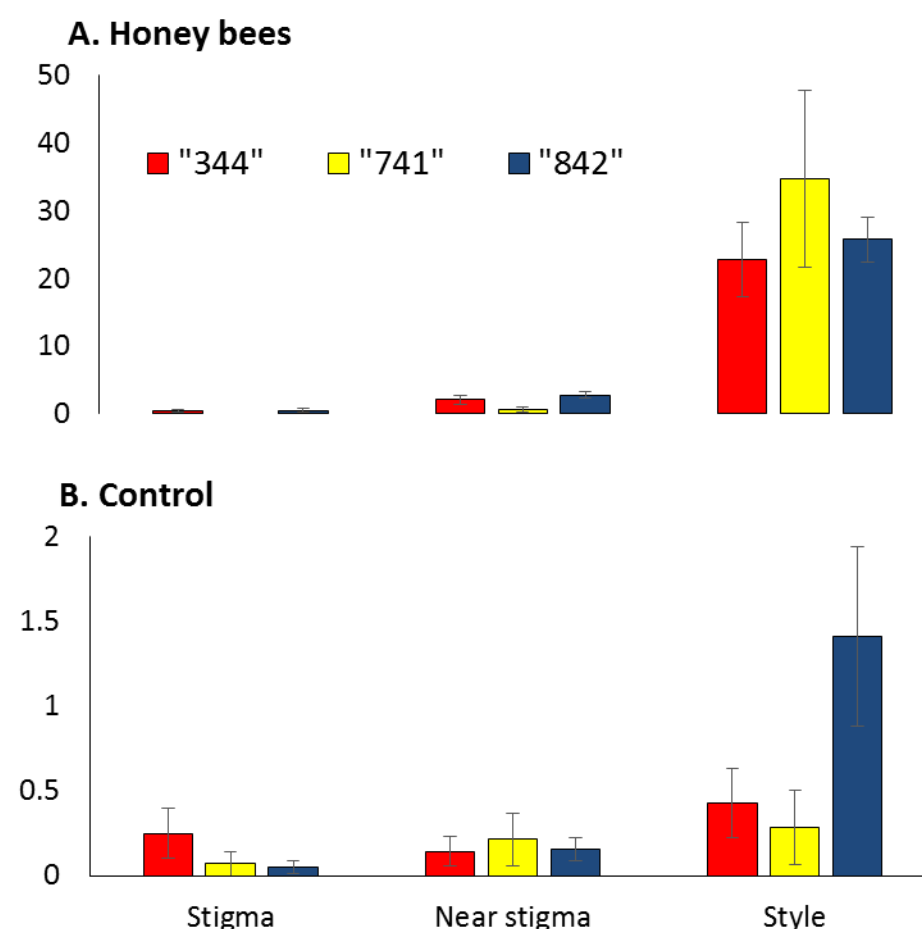


Figure 3.2.4. Count of pollen grains and their location on stigmas and styles following a single flower visit to three macadamia varieties by (A.) honey bees compared with pollen grains on stigmas and styles of unvisited flowers.

Movement of insects between flowers racemes and trees

Audio data was collected for six flower visiting species across 11 separate days with honey bees being the most frequently recorded (Table 3.2.2).

Table 3.2.2. Number of separate audio recordings and total number of raceme visits for each insect species.

Insect	No. recorded	No. raceme visits
Honey bee (<i>Apis mellifera</i>)	69	951
Stingless bee (<i>Tetragonula carbonaria</i>)	34	298
Lycid beetle (<i>Metriorrhyncus rhipidius</i>)	11	117
Nose Fly (<i>Stomorphina discolor</i>)	13	77
Soldier beetle (<i>Chauliognathus flavipennis</i>)	10	54
Brown blow fly (<i>Brown blow flies</i>)	7	44

Interactions with the stigma

All honey bees observed in video, survey and audio recordings were primarily nectar collecting. Where honey bees were observed pollen collecting they interacted with the stigmatic region of the flower on all visits. Nectar collecting honey bees did not appear to purposely interact with the stigmatic region, but occasional contact was observed. Assessment of video recordings of 11 nectar foraging honeybees and assessment of 79 flower interactions revealed honey bees contacted the stigmatic region of a flower on 24.3% of occasions. This contact was made by various body parts of the insect including abdomen, head, side and underside of thorax, wings. For the 48 flower interactions observed by stingless bees ($n = 13$), individuals interacted with the stigmatic region on 58.3% of flowers visited.

In total, 50 5-min video recordings were taken between 26 and 31 August 2014 on '842'. A total of 22 honey bees visited the flowers during this time, with just one collecting pollen. The nectar foragers spent an average (\pm SE) of 1.88 ± 0.22 seconds visiting each flower. As only one pollen forager was viewed during the initial set of recordings, a further 35 honey bees (28 nectar collectors and seven pollen foragers) were video recorded while they were visiting racemes of the same macadamia variety. The nectar foragers from the additional recordings took an average of 2.4 ± 1.7 seconds to visit each flower, whereas the pollen foragers took an average of 2.0 ± 0.37 seconds per flower (not significantly different: t -test $P = 0.23$). However, pollen foragers visited fewer flowers per raceme (6.2 ± 0.91), than nectar foragers (11.6 ± 0.99 ; t -test $P = 0.008$). All honey bee pollen foragers were observed to touch the pollen presenter in the region of the stigma.

Rate of flower and stigma visitation between different pollinators

Although stingless bees on average contacted more flowers and more stigmas per minute when compared with the other flower visitors, there was a high degree of variation between individuals for all insect species (Figure 3.2.7). All flower visitors had at least some individuals that were observed to move frequently between flowers and contact the stigmatic region. However, an exception was stingless bees that collected pollen from the anthers of opening flowers [may be described as robbing (Heard 1994)] with the stigma not yet released. Ten stingless bees were observed robbing and they either did not contact stigmas or rarely did. These ten individuals represented the ten lowest values for stingless bees in Figure 3.2.7 (B) showing frequency of stigma contacts.

In contrast, the three pollen collecting honey bees observed had much higher rates of stigmatic region contact than other honey bees, and were similar to the top stigma contact rates of stingless bees (Figure 3.2.7 B).



Figure 3.2.5. Lycid beetle are comparatively large insects that often contact the stigmatic region of macadamia flowers as they walk across a raceme (photograph by Brian Cutting, Plant & Food Research).



Figure 3.2.6. Stingless bees are small insects but contact the stigmatic region of macadamia flowers as they collect pollen (photograph by Brian Cutting, Plant & Food Research).

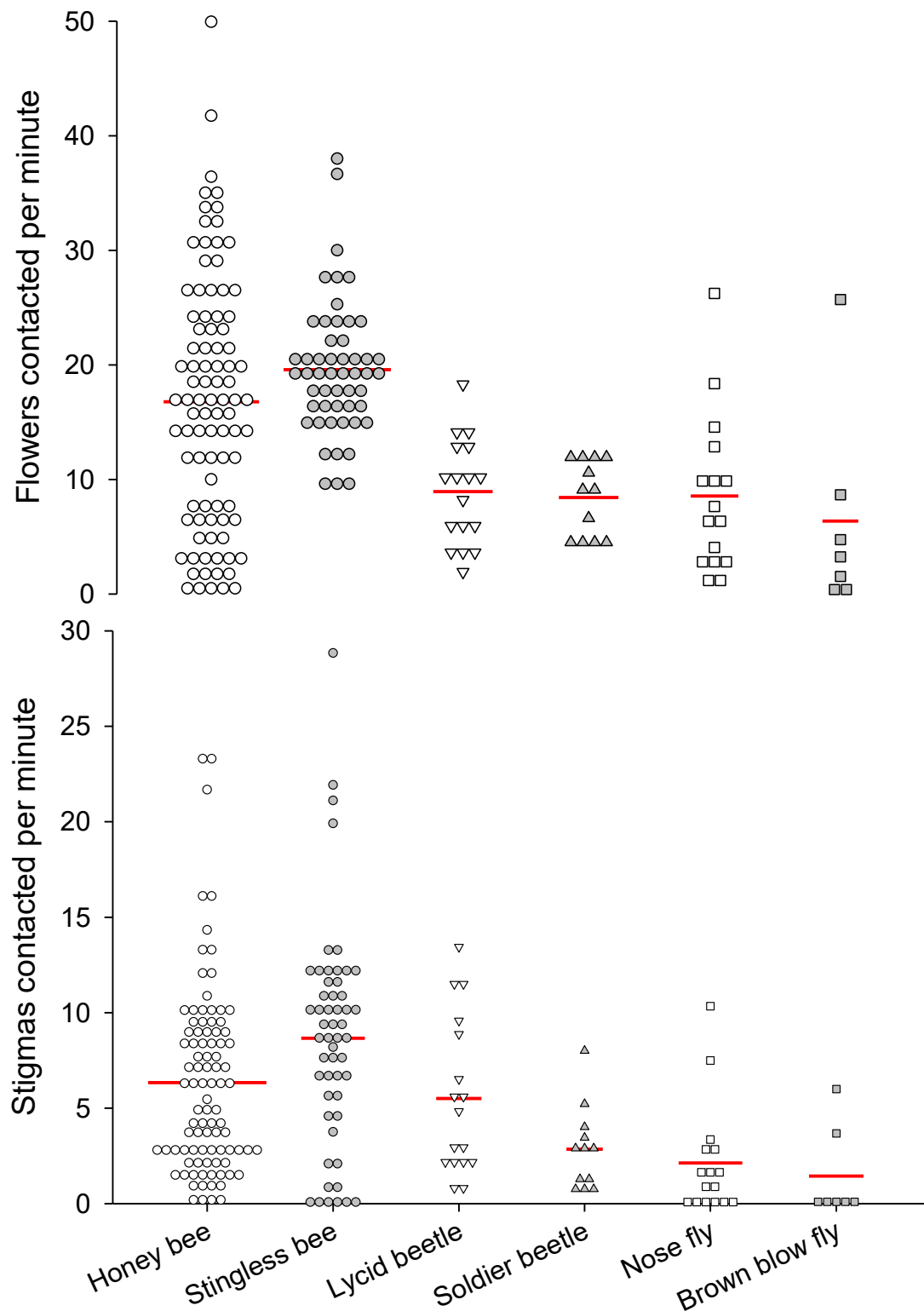


Figure 3.2.7. Number of flowers (A) and stigmas (B) contacted per minute for two bee, two beetle and two fly species. Shapes are mean number of flowers or stigmas contacted for each individual insect recorded. The mean for each species is represented by a red horizontal line.

On average, stingless bees visited the highest number of racemes per minute (Figure 3.2.8), meaning they are likely to transfer pollen between inflorescences at the fastest rate. However, there is a wide

variation between individuals of all assessed species. The beetles were on average, the slowest movers on this measure.

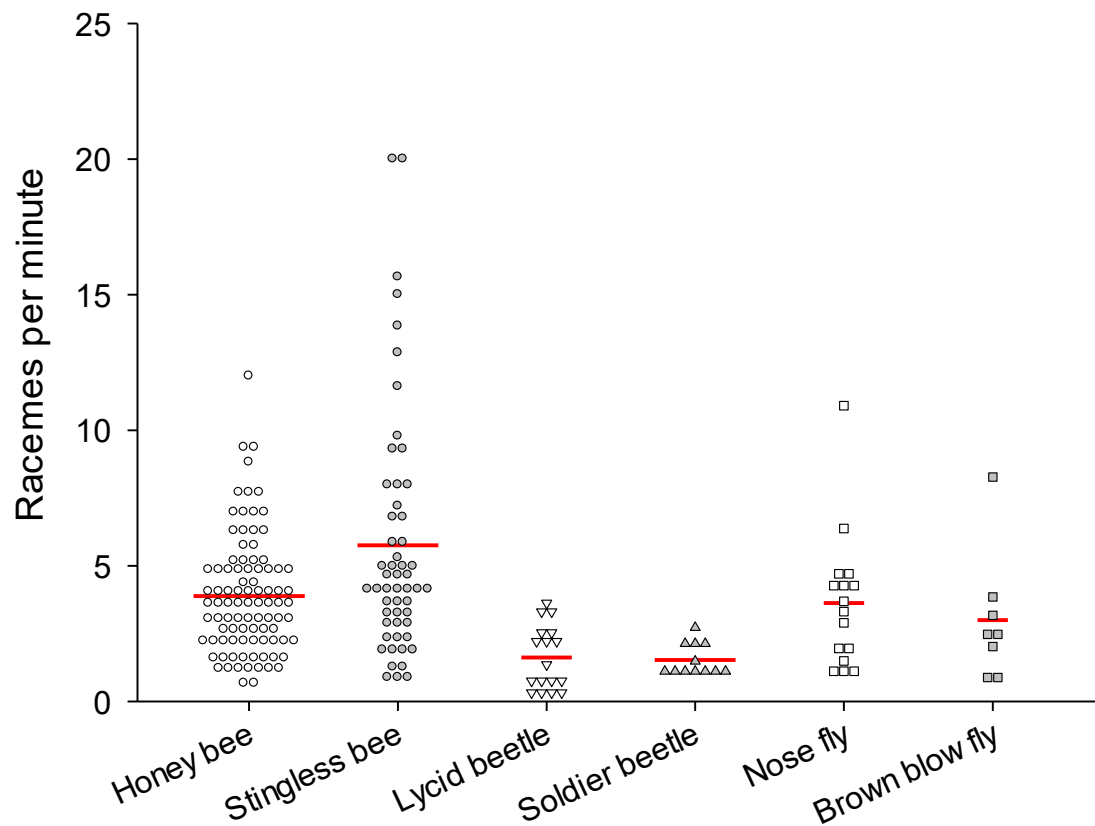


Figure 3.2.8. Number of racemes visited per minute for two bee, two beetle and two fly species. Shapes are mean number of racemes contacted for each individual insect recorded. The mean for each species is represented by a red horizontal line.

Between-tree movement is necessary for cross-pollination to occur. Of the insects observed, inter-tree movement was not commonly observed. Of the 69 honey bees, 15 between-tree movements were observed. Also observed moving between trees were lycid beetles (seven out of 11 individuals) and soldier beetles (three out of 10 individuals) (Figure 3.2.9).



Figure 3.2.9. Estimated number of trees visited per hour for honey bees, lycid and soldier beetles.

Assessing pollen flow in three macadamia orchards

Insects were observed carrying the fluorescent powder (Figure 3.2.10) from treated racemes. Subsequent observation found powder movement in all treatment blocks with some movement occurring between trees. A summary of numbers of fluorescent powder grains recovered on adjacent trees to the treatment tree in each cultivar block is provided in Table 3.2.3 and demonstrates high variability (more than one magnitude difference).

Table 3.2.3. Total number of fluorescent powder grains moved from marked racemes for different macadamia varieties in different blocks.

Region	Variety	Total grains moved
Bundaberg	'741'	38
Bundaberg	'816'	99
Bundaberg	'A203'	34
Bundaberg	'A264'	16
Gympie	'842'	426
Gympie	'344'	215



Figure 3.2.10. Honey bee (*Apis mellifera*) with fluorescent powder grains following an interaction with a treated raceme.

Of the powder that had moved from the marked raceme and that we traced was found on other racemes. Most was found on pollen presenters (near the stigmatic region of the flower), styles or petals. A relatively small proportion of powder was found on buds (Figure 3.2.11)

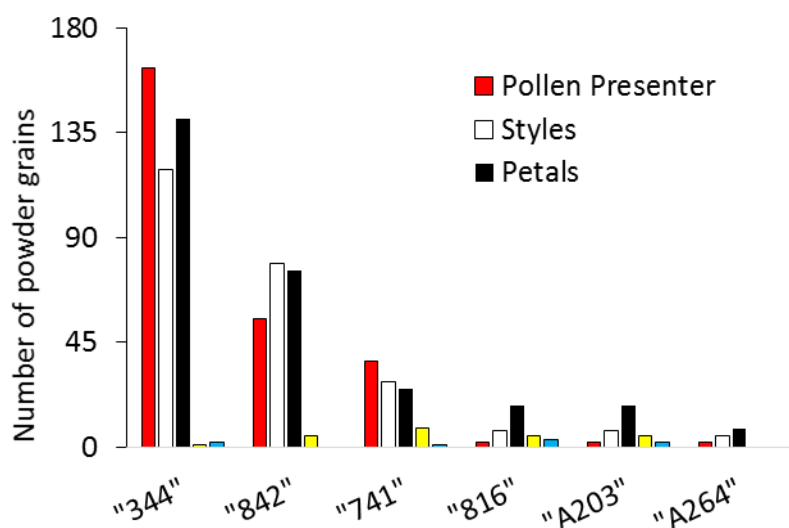


Figure 3.2.11. Occurrence of fluorescent powder that had moved from the marked racemes onto different plant parts.

There was no consistent pattern regarding powder grain flow throughout the day between the orchards, except that in all cultivars, the least amount of pollen flow was recorded after 6 pm (Figure 3.2.12).

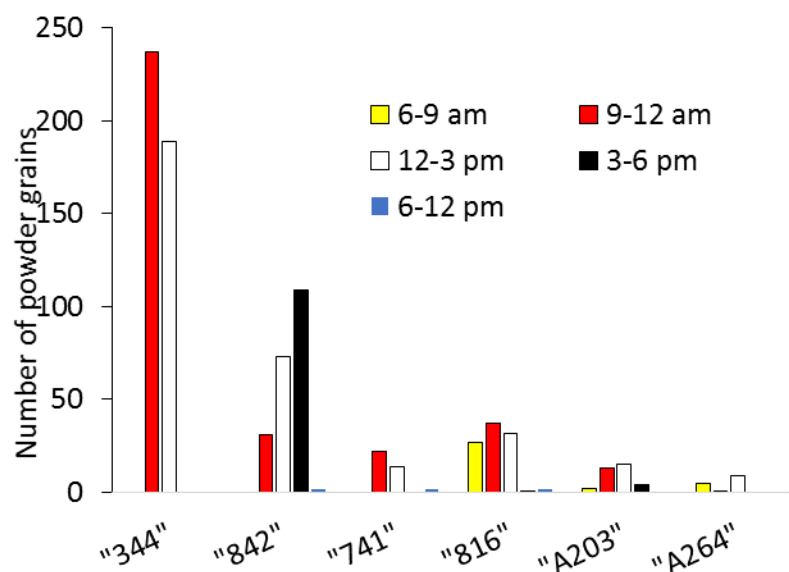


Figure 3.2.12. Number of fluorescent powder grains moved from the marked racemes at different times during the day.

In all orchard blocks, most of the powder grains detected were moved to racemes close to where the marked raceme was located (data for two orchards are presented in Figure 3.2.13 A and B). Between tree movement was detected in four of the six blocks and the maximum distance of powder moved from a treated raceme was 6.25 m.

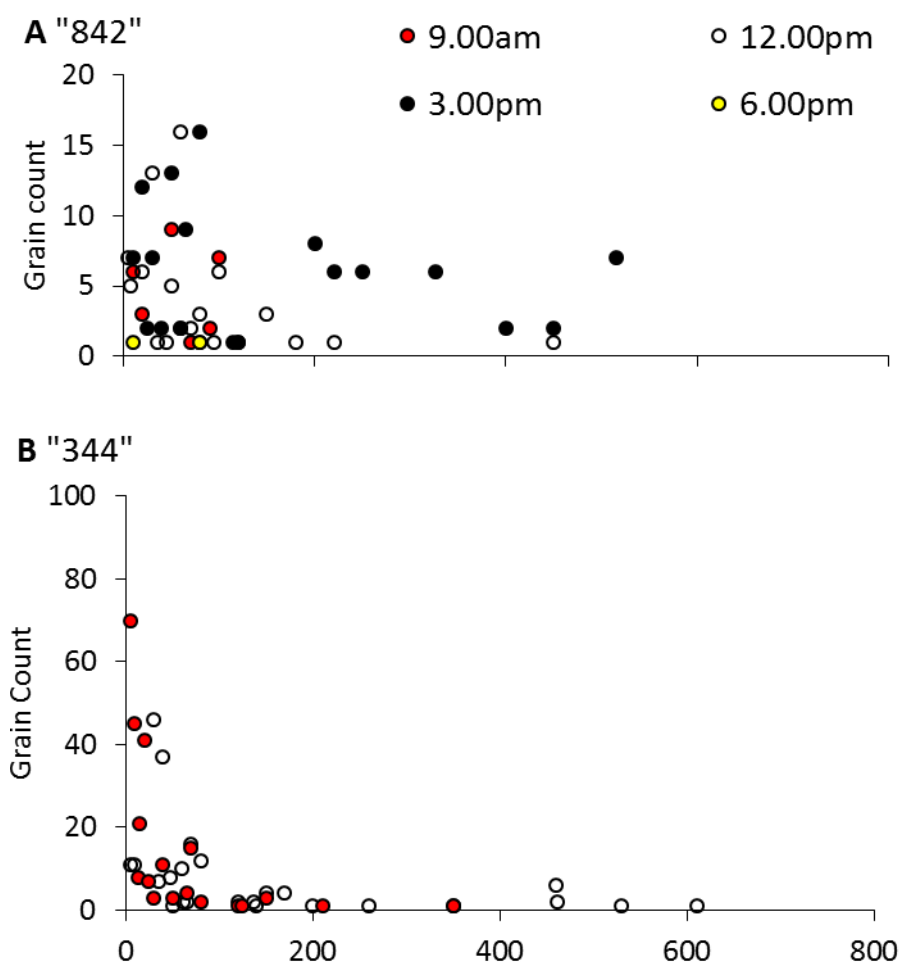


Figure 3.2.13. Distance moved by fluorescent powder grains applied at different times of the day to the stigmatic region of flowers within fully flowering racemes. Figures A and B are for different macadamia varieties within separate blocks located near Gympie. Racemes were left exposed to flower visiting insects for 3-h periods.

3.2.4 Discussion

Single pollen deposition by different flower visiting species

As with the study by Vithanage and Douglas (1987), we found honey bees capable of placing pollen onto the stigmas of macadamia. This was deposited at a rate of approximately one in five flowers visited and fits within the range of observed macadamia stigmatic contact by honey bees reported by these authors. The mean number of pollen grains delivered to macadamia stigmas on each flower visit was low (0.4 grains per visits).

To our knowledge, this is the first study that has examined the ability of other flower visiting species to deposit pollen onto stigmas. Stingless bees deposited pollen to stigmas more frequently than honey bees (one in every two visits) and the average number of pollen grains deposited per flower was the highest of all insects visited. Other insects that were found capable of delivering pollen to stigmas were lycid beetles, soldier beetles and nose flies. In these cases approximately one in every three visits resulted in pollen deposition. However, the data for these species have small sample sizes (between 10 and 13 individuals). Interestingly, when compared with stingless bees, lycid beetles, soldier beetles and honey bees deposited more pollen on styles, and on a higher proportion of flowers visited. This may reflect their differences in behaviour. Stingless bees were more likely to directly visit the stigma region for possible pollen collection. However, most honey bees observed were not collecting pollen and therefore were less likely to interact with stigmatic region. Other insects walked across flowers and therefore did not appear to seek deliberate contact with the stigmatic region of the flower. The hover fly *Melangyna viridiceps* was also found to deliver pollen to the style and near to the stigma but we did not record it delivering pollen onto the stigma.

Although this study found that a number of species were capable of delivering pollen to stigmas and styles, the technique used was different to that used by Vithanage and Douglas (1987). Similar to their study, we removed the self-pollen from our test flowers. In addition, we assessed non-visited control flowers [unlike Vithanage and Douglas (1987)]. Our method also differed from theirs as we used a stain (gelatin-fuschin) to clean the style and stigma of self-pollen. This also stained any remaining self-pollen, which allowed us to assess pollen delivered by insects (non-stained). However, the removal of pollen from the stigma may result in differences in behaviour of stingless bees compared with their interaction with flowers containing self-pollen. The delivery of pollen to stigmas is likely to more closely resemble their visits to flowers where self-pollen had previously been removed by other flower visitors. We did not assess the ability of these insects to deposit pollen onto stigmas in the presence of self-pollen.

The removal of the flower and its placement by hand within racemes (positioned in a similar manner to attached flowers) is a technique that has been used in other studies on plant-pollinator interactions (Thomson & Goodell 2001; Adler & Irwin 2006). A potential drawback of this method is that it may unintentionally have influenced insect behaviour on the raceme or its contact with the flower. However, the data for this study did detect differences between the species that may reflect their visitation pattern. That is, stingless bees delivered more pollen to stigmas through their movement directly onto the pollen presenter, whereas the other species interacted more with the styles as they walked through the raceme or attempted to obtain nectar.

There is a possibility that some of the large numbers of pollen grains deposited onto styles by the various flower visiting species may eventually arrive at stigmas via additional insect transportation.

This scenario has been postulated by Heard (1994) following observation that stingless bee movement on styles and stigmas may result in the relocation of pollen deposited by honey bees on the style to the stigma. Further study on how pollen grains may be vectored in addition to understanding the longevity of pollen grain viability may provide a more complete picture of how pollen flow and pollination may occur over time and distance within and between orchards.

These data were collected across three different macadamia varieties ('344', '741' and '842'), but for honey bees, we did not see any altered pattern in the number and distribution of pollen grains onto the styles, near to the stigma and on stigmas when comparing between these varieties. The numbers and distribution of other pollinating species did not make it possible to compare their patterns of pollen deposition among the varieties.

Rate of flower and stigma visitation between different pollinators

Pollen collecting honey bees were rarely observed visiting macadamia flowers as opposed to nectar collecting individuals. The efficiency of pollen collecting honeybees may be quite different to nectar foragers. Heard (1994) notes that pollen collectors contact the stigmatic region during their visits to collect pollen, while in contrast, nectar collectors may only incidentally contact stigmas. Regarding nectar collectors, our findings support this, with the stigmatic region of approximately one in every five flowers being contacted by various parts of the honey bee's body (Figure 3.2.14). Therefore, nectar foraging honey bees can be considered effective pollinators, particularly if cross pollen movement is transferred between racemes of different macadamia varieties.



Figure 3.2.14. Nectar foraging honey bee having collected fluorescent powder across various parts of its body. Note the wing contacting the stigmatic region of a flower (photograph by Brian Cutting, Plant & Food Research).

Stingless bees contacted the stigmatic regions of flowers they visited on almost 60% of occasions. On average, they visited slightly more flowers per minute than honey bees and also contacted more stigmas per minute. A proportion of individuals was observed collecting pollen from anthers of flowers that has not fully opened (30% of individuals) and either did not interact with the stigmatic region or

rarely did so. Even so, inclusion of these insects in our overall assessment resulted in a higher rate of contact with the stigmatic region compared with honey bees.

The lycid and soldier beetles and nose and brown blow flies were also observed readily moving between flowers. Lycid beetles made contact with the stigmatic region almost as frequently as honey bees and at twice or more the rate of the soldier beetles and the fly species. As with nectar foraging honey bees, this contact appeared to be incidental. The relatively large size of lycid beetles relative to the other species may explain the higher rate of contact. The movement of the beetles between racemes was lower than the other species; however, these beetles tended to spend more time flying over larger distances. We observed relatively frequent movement of these beetles between trees at a comparable or higher rate than honey bees. Inter-tree movement (particularly inter-varietal) is a key requirement to maximise cross pollination. Our records of cross-tree movements was limited due to their relative infrequency, and therefore ranking species on this measure is tentative. In addition, we did not obtain data on inter-tree movements for stingless bees or fly species where their small size or fast flight made observing their movement over distance extremely difficult. For a range of other crops, hover flies have been observed to move more frequently between varieties; however, whether this is the case for macadamia is unknown.

Efficiency of different insect pollinators

The data collected in this study demonstrates that various insects contribute to the pollination of macadamia grown in orchards. It is important to note that our study looked at stigmas where self-pollen had been removed. We are thus unable to assess the efficiency of pollinators when there is self-pollen present. However, given that self-pollen is presented approximately 2 days prior to the stigma becoming receptive, self-pollen may largely have been removed by visiting insects prior to stigma receptivity.

Assuming all flowers, racemes and trees are equally attractive to each pollinating species (i.e. they do not discriminate between flowering racemes), stingless bees were found to be the most efficient pollinating species. They contacted more stigmas and visited more racemes per minute than other insects. Nectar foraging honey bees and the lycid beetle appear to have similar pollination efficiency. Honey bees contacted more stigmas and racemes per minute but lycid beetles deposited pollen more frequently on the stigmas of visited flowers. Of the remaining species where data for both pollen deposition on stigmas and flower visitation data are available, soldier beetles were the next most efficient and nose flies the least.

Pollen flow within orchards

Using fluorescent powder applied to pollen presenters as a surrogate for pollen, we measured potential pollen movement by insects within four different orchards, each of a different variety. We found that nearly all grains were moved to the floral parts of other racemes; mainly the style, pollen presenter and petals. This movement strongly suggests that flower visiting animals were responsible for vectoring the powder, as movement via wind would have resulted in a much larger proportion of grains being present on leaves and branches.

There was very large variation in the number of moved powder grains detected between orchards, with almost 30 times more grains between highest and lowest. This could potentially reflect the abundance of pollinators. Of the two orchards where insect surveys were conducted, both the numbers of pollinators counted and the number of powder grains transported varied by similar amounts. Further surveys coupled with powder movement experiments are required to confirm this possible link.

The potential variation in pollen flow throughout the day showed variation between orchards. The data suggest that large amounts of pollen flow can occur throughout the day and this pattern does not

appear consistent between regions or orchards. Vithanage and Ironside (1987) assessed pollen flow in a single orchard of variety of 'Keauhou' across 8 days using a method assessing pollen delivery to the stigmatic region of flowers. They found that most pollen flowed in the morning, consistent with the availability of pollen on pollen presenters. We did not assess whether this might explain the variable patterns across the varieties we examined. Other factors that affect insect activity such as weather and competing bloom are likely to play a significant role in pollen flow caused by insects.

Key points

- Pollination of macadamia can be conducted by various insect species including bees, beetles and flies.
- Our assessment of five different species (assuming all flowers, racemes and trees are equally attractive to all five species) ranks stingless bees as the most efficient pollinators, followed by nectar collecting honey bees and lycid beetles. Soldier beetles were the next most efficient followed by nose flies and brown blow flies. The scarab beetle, *Glycyphana stolata*, was also found capable of pollinating. Hover and brown blow flies may also be pollinators as they were found to deliver pollen within 3 mm of stigmas; however, our small sample size did not verify that they place pollen onto the stigma.
- We were unable to assess the efficiency of pollen collecting honey bees as these were very rarely observed within our study orchards.
- Pollen flow appears highly variable between orchards and may reflect different pollinator abundances.
- There is potential to encourage the presence of multiple insect species, especially stingless bees, to contribute to pollination.

3.2.5 References

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3.3 Abundance, distribution and effectiveness of insect visitors to macadamia flowers across three regions in Australia

3.3.1 Introduction

The presence of wild pollinators often benefits crop production. Recent studies have demonstrated that for 20 insect-pollinated crops, the presence of wild pollinating species alongside honey bees increases crop yield compared to crops in which honey bees are foraging alone (Garibaldi et al. 2013). Moreover, increased pollinator density and diversity can also assist in increased crop yields (Garibaldi et al. 2016).

The improved crop yields associated with the presence of diverse wild pollinating species may be due to number of factors. These include complementarity of foraging patterns of different pollinator species under variable weather conditions (Howlett et al. 2013), and improved pollinator efficiency through the interaction of pollinating species (Brittain et al. 2013).

Assessing the efficiency of both wild and managed pollinators allows them to be ranked according to their potential contribution, but their overall effectiveness within orchards can only be determined by measuring the relative abundance of each species (Rader et al. 2009, 2012). The efficiency of a variety of insect pollinators of macadamia is assessed (Section 2) in this report. However, knowledge of the distribution and abundance of flower visiting species within and between macadamia orchards grown across different regions is needed to determine where shortfalls of pollinating species and subsequent pollination is likely to occur.

In Australia, two published studies, Vithanage and Ironside (1986) and Heard and Exley (1994) have previously shown that a number of insect species (and in the latter study - birds), visit the flowers of *Macadamia* spp. grown in orchards. The study by Vithanage and Ironside (1986) surveyed four orchards over two seasons while Heard and Exley surveyed 15 orchards over three seasons but compared seasonality over two seasons for six orchards. Vithanage and Ironside (1986) recorded at least 28 flower visiting species while Heard and Exley recorded at least 55. The most common visitors were bee species (Apoidea), other Hymenopteran (wasp), Coleopteran (beetle) species [particularly Lycids (Lycidae)], Dipteran (fly) species and Lepidopteran (moths and butterflies) species.

Honey bees were recorded as the most abundant species in both the Australian surveys followed by stingless bees [*Tetragonula* spp. (all specimens caught by Heard and Exley (1994) were *T. carbonaria*) with these species representing 60.5% and 35.8% of all flower visits respectively. Overall, other individual flower visiting species represented less than 1% of flower visits across all orchards in the Heard and Exley (1994) study, however, other species may be relatively abundant in individual orchards (Vithanage & Ironside 1986). Heard and Exley (1994) found that the visitation rate to racemes of both honey bees and stingless bees varied greatly between orchards. For honey bees this ranged from 0.8 visits per hour in one orchard to 10.5 in another orchard (overall mean \pm SE of 5.0 ± 0.7 visits per hour) while for stingless bees this ranged from 0.0 (five orchards)—10.7 raceme visits per hour in another orchard (mean \pm SE of 2.9 ± 0.8) (Heard & Exley 1994).

Honey bees were also found to significantly prefer exposed racemes (less than 20 cm from the edge of the canopy) with an average 6.5 visits/hour compared to hidden racemes (more than 1 m within the canopy), with an average 4.4 visits/hour (Heard & Exley 1994). A similar preference was also noted for stingless bees with an average of 9.6 visits/hour to exposed racemes compared to 3.6 visits/hour to hidden racemes (Heard & Exley 1994). Moreover, the number of foraging individuals for both bee species followed a bimodal pattern throughout the day with numbers highest at mid-morning (10–11 am) and early afternoon (1–2pm) (Vithanage & Ironside 1986). This trend was not apparent in the study by Heard and Exley (1994).

It is possible that the variety of macadamia may also influence the visitation rate of pollinating species. For example, it was noted that the number of honey bees visiting variety “246” per day was more than 2.8 times the number visiting variety “508”. Preferential visitation by honey bees to particular varieties within the same plant species has been noted across other crops (Erickson et al, 1979; Evans et al. 2011) and may be the case for other bee species (Howlett et al. 2015), potentially reducing the effectiveness of these flower visitors as cross pollinators.

Since the last published survey by Heard and Exley (1994) there has been the establishment of new orchards, particularly near Bundaberg, QLD. Our goals in this study were to:

- Determine what flower visitors currently visit macadamia orchards, by collecting flower-visiting species from orchards located in Bundaberg (coastal central Queensland), Gympie - Glasshouse (South East Queensland) and the Northern Rivers near Lismore (New South Wales).
- Assess the abundance and distribution of flower-visiting species within orchards across these regions through surveys. On each survey day, flowering intensity was assessed as well as the distribution of flower visitors on flowering racemes located on the outer and inner canopy.
- Determine whether there are noticeable differences in flower visitor abundances between different macadamia varieties located on the same farm by conducting simultaneous daily surveys on selected farms
- Compare pollen removal from florets in relation to flower visitor abundance.
- Estimate pollinator effectiveness by evaluating pollinator efficiency and abundances across regions and orchards.

We then discuss suggestions to improve pollinator effectiveness, with a focus on orchard design and hive placement.

3.3.2 Materials and methods

Preparation of a reference collection of flower visiting insects

Flower-visiting insects were opportunistically collected from macadamia orchards in both 2014 and 2015. These were collected by hand from flowering racemes either directly into specimen containers containing tissue soaked with ethyl acetate or by using a sweep net. Specimens collected by sweep net were then transferred to a container with ethyl acetate. Ethyl acetate is an effective killing agent for insects, resulting in quick immobilisation. Following collection, specimens were stored in the shade and transferred to a refrigerator set at 4°C. Specimens were then pinned and boxed within four days of capture. The most common flower visiting species have been identified to species level, while many of the less common species will be identified as part of an ongoing PhD project conducted by Bryony Wilcox, University of New England, Armidale, NSW, where the collection is currently lodged.

Insect flower visitor surveys

Selection of orchards:

Surveys were conducted from 31 August to 19 September 2015, across three different regions in Australia (Figure 3.3.1). From north to south: Seven orchards near Bundaberg (within 100 km of Bundaberg, 24.8S 152.3E), ten orchards in Gympie - Glasshouse (within 200 km of Gympie – Glasshouse Mountains, 26.2S 152.6E - 26.9S 152.9E), and six orchards in the Northern Rivers of New South Wales (within 100 km of Lismore 28.8S 153.4E) (Figure 3.3.1).

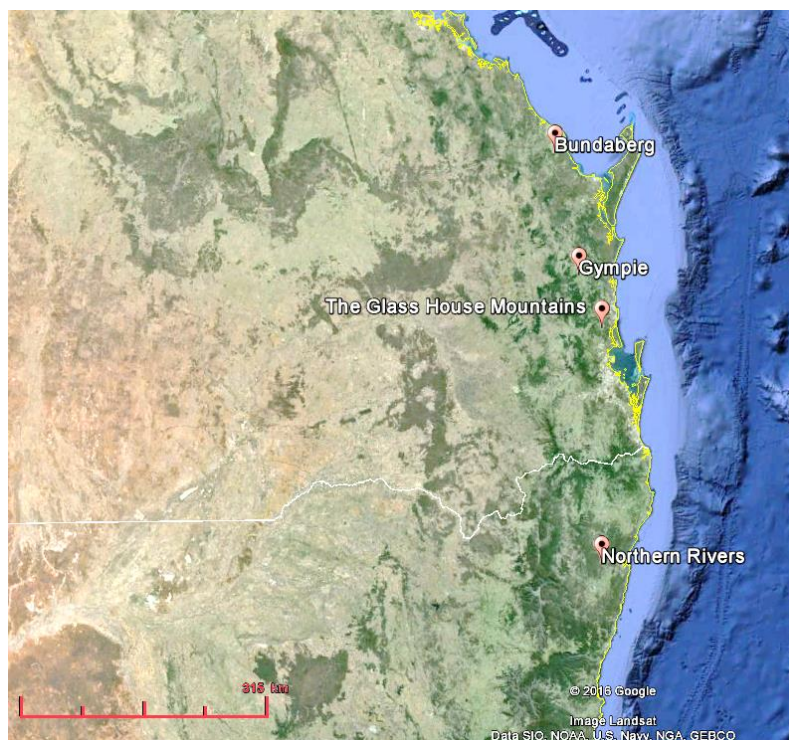


Figure 3.3.1. Location of the different macadamia orchards (Google Earth imagery).

Four different varieties of macadamia : “344”, “741”, “842” and “daddow” were selected for surveying with a key focus on orchards containing “741” across regions. A selection of orchards were chosen to survey on two separate days to assess between day variation of flower visiting species.

For each survey, trees were observed three times during the day beginning at 8:30 am, 12pm, and 3pm. During each survey, a 1.5 m long measuring pole was held with the base at a vertical height of 0.5 m above the ground (determined by an attached piece of string). The pole was also equipped with a perpendicular horizontal measure of length 0.75 m. This was used to restrict insect counts on racemes to the area between the outer canopy and 0.75 m within the canopy. In some cases where the base of the foliage had been pruned approximately 1 m above the ground, it was necessary to hold the pole base at a higher point in the foliage to ensure the area containing flowering racemes was equivalent to other orchards. The observer then counted flower-visiting insects around the entire circumference of each tree. Care was taken to minimise brushing the foliage with the pole. Where a flower-visiting species was particularly abundant, a hand-held counter was used to record numbers. Insects were recorded on a spreadsheet to species level where possible. If the species were unfamiliar to the observer, an attempt was made to capture the specimen for identification purposes. Based on the flower-visiting insect species collected across several orchards in 2014, as well as the survey data collected by Vithanage and Ironside (1986) and Heard and Exley (1994), it was predicted that the most abundant species observed would be the honey bee (*A. mellifera*) followed by the stingless bee (*T. carbonaria*). There was also the expectation that particular species of Coleoptera,

Diptera, Hymenoptera and Lepidoptera could be observed, and these were listed on the observers' spreadsheets. Only insects observed on flowering racemes were counted.

The survey design contained 12 observation trees per orchard. Each was observed across the three survey times. Three observation trees were marked consecutively along a separate row within the orchard block. The first set of three trees were located in a corner of the block, while another set of three in the opposite corner. The other two sets of trees were located within the orchard to form a diagonal survey of three grouped trees across the orchard (Figure 3.3.2).

Visitation of Inner Canopy versus outer canopy racemes

In a selection of orchards, separate surveys of flower-visiting insects visiting within canopy racemes versus outer canopy racemes were conducted. Counts were conducted on two sets of racemes (inner and outer) on either side of the tree facing directly onto each inter-row. Twenty-five fully flowering racemes at each canopy location (inner and outer canopy) were counted by placing a tape measure on the outer most point of the tree at a height of 1.5 m and counting the nearest racemes located no further than 10 cm within the foliage of the canopy. Inner raceme counts were conducted by measuring along the same line as the outer canopy racemes but at a point 0.5 m inside the canopy (height of 1.5 m) and counting flower visitors on the nearest racemes to the point (at 0.5 m or deeper inside the canopy).

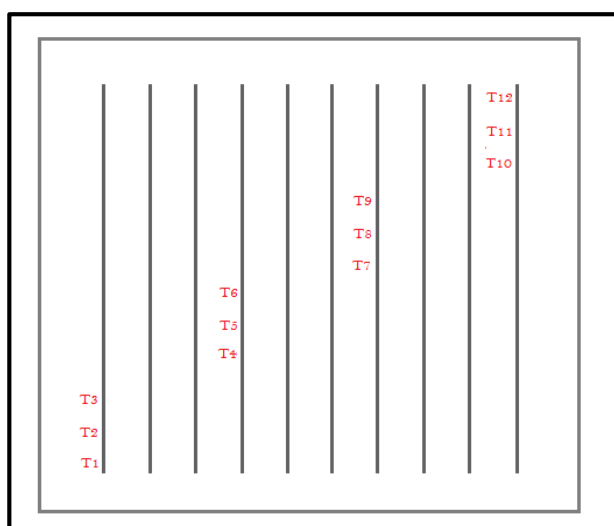


Figure 3.3.2. Survey design within an orchard block with observation trees arranged in sets of three that run diagonally from one corner of the orchard to the other.

Weather variables were recorded at the beginning and at the end of every survey. Measurements taken were for air temperature (°C), relative humidity (%), wind speed minimum and maximum (km/h), light intensity (north, south and towards the sun) (W/m²). The GPS coordinates were also recorded for each orchard.

Flower density

Counts of flowering racemes on each survey tree were also conducted during the day of survey to estimate flowering density. Separate counts were conducted on four sides (opposite and perpendicular) of each tree (two within the row on opposite sides and two perpendicular to the tree row on opposite sides). At each side, a fold-out quadrat forming an 0.75m³ cube was held at a height of 1.5 m, and separate counts of budding racemes, racemes with less than 10 open flowers, fully flowering racemes and racemes that had completed flowering were conducted (Figure 3.3.3).

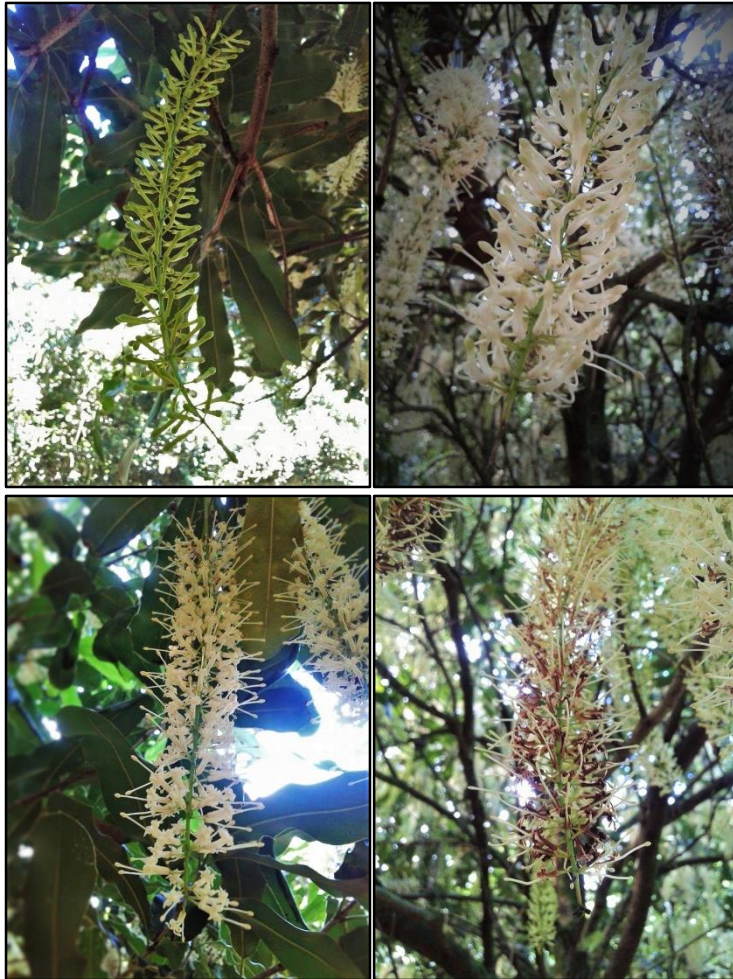


Figure 3.3.3. Racemes at four stages of blooming were counted within each tree (from left to right : budding, less than 10 open flowers, fully flowering and completed flowering). (Photographs by Thomas Besnier).

Pollen removal and flower visitor abundance

To assess whether there might be a relationship between pollen removal from fully flowering racemes within orchards and insect flower visitor abundance, assessments of racemes was conducted immediately following the completion of an insect flower visitation survey between 4 and 6 pm. The stigmas/pollen presenter of the 10 uppermost fully open florets, 10 bottom most and ten most central were examined for the presence or absence of self-pollen. Fully intact pollen maintained a 'match-stick head' appearance with all pollen tightly clumped and visible on the pollen presenter and surrounding the stigma. On removal of pollen from the pollen presenter, the surface was left smooth. Florets where pollen had not been completely removed (i.e. still clumps of pollen present on the pollen presenter) were grouped with florets containing self-pollen.

Six of the twelve trees (every second tree) that were surveyed in the insect surveys were chosen to assess removed pollen. We assessed racemes within two 0.75 m³ quadrats that were positioned on either side of each tree. Therefore, twelve racemes per tree were assessed overall. Placement of the quadrat was conducted using the same method as for the flower density surveys. Within each quadrat, two fully flowering racemes were first selected closest to the nearest left hand corner of the quadrat and florets within each assessed. This was followed by two fully flowering racemes located closest to the centre of the quadrat. Finally, another two fully open racemes were then assessed nearest to the furthest right hand point of the quadrat were assessed.

Calculating pollinator effectiveness within orchards

Pollinator effectiveness considers both the pollinator efficiency of an insect and the abundance of the insect species. To calculate pollinator efficiency we used data from section 2 of this report, on the rate of pollen movement for a given species. This included time taken for the pollinator species to move between racemes, the number of stigmas they contacted per minute and the amount of pollen they deposited on stigmas. We then considered the abundance of each pollinator (this section) to calculate a measure of pollinator effectiveness. This is a calculation of the frequency at which stigmas within a raceme potentially receive pollen per hour by a given pollinating species.

3.3.3 Results

Reference Collection

The reference collection of flower-visiting insect species, excluding the European honey bee *Apis mellifera*, has been developed to assist in the identification of key pollinating species across macadamia orchards located near Bundaberg, Gympie-Glasshouse and the Northern Rivers. Specimens were collected opportunistically when observed and were collected from orchards that were visited during observation surveys. The collection has also been used to train staff and transfer knowledge to growers of the key flower-visiting species within macadamia orchards.

Insects captured visiting macadamia flowers were dominated by hymenopteran and dipteran species. These will be identified to species/morphospecies level. Currently we have identified:

Bees

Colletidae

- *Leioproctus (Leioproctus)* sp. Rous Rd., Alstonville (Northern Rivers, NSW) visiting variety “A4”, on 10 September 2014 female, no pollen
- *Hylaeus (Prosopistemon)*, Rekow (Bundaberg) visiting variety 34, on 7 September 2014 female. These bees carry pollen internally

Halictidae

- *Lasioglossum (Chilalictus) polygoni* Rous Rd visiting variety A1, on 10 September 2014 carrying pollen. female, three specimens
- *Homalictus* sp. Sontag, Welcome Ck. (Bundaberg) visiting “741” on 7 September 2014

Apidae

- *Tetragonula carbonaria* (Meliponini) Karana Farm, Anderleigh road, Gympie visiting “344” on 9 and 10 September 2014, 17 specimens, with seven carrying pollen

Other Hymenoptera

Ichneumonidae

- Ichneumonid wasp Sontag, Welcome Ck. (Bundaberg) visiting “741” on 7 September 2014

Eumenidae

- *Odynerus* sp. or *Paralastor* sp. solitary wasp (Vespoidea) Sontag Welcome Ck. Visiting “741”, on 7 September 2014

Vespidae

- *Polistes* sp. (paper wasp) Rous Rd, Alstonville visiting variety “A16”, on 10 September 2014

Formicidae

- Three morphospecies, three specimens

Diptera

- Calliphoridae: four morphospecies, six specimens
- Rhiniidae: *Stomorphina discolor*, six specimens
- Tabinidae: two morphospecies, two specimens
- Anthomyiidae: *Anthomyia punctipennis*
- Muscidae: one morphospecies, one specimen
- Tachinidae: two morphospecies, two specimens
- Syrphidae: *Melangyna viridiceps* (Macquart, 1847) At least one specimen
- Syrphidae: *Simosyrphus grandicornis* (Macquart, 1842) At least one specimen
- Syrphidae at least three other morphospecies, at least three specimens
- Other Diptera: three morphospecies, three specimens

Coleoptera

- *Monolepta australis* (Chrysomelidae): seven specimens (six from Alstonville, one from Bundaberg).
- Coccinellidae: one morphospecies, one specimen
- Lycidae: *Metriorrhyncus rhipidius* (Macleay) At least one specimen
- Cantharidae: *Chauliognathus flavipennis* (W. J. Macleay, 1872) at least one specimen
- Scarabeidae: *Glycyphana stolata* (F.) at least one specimen
- Other Coleoptera: two morphospecies, two specimens

Hemiptera

- Pentatomidae: three morphospecies, three specimens
- Other Heteroptera: one morphospecies, one specimen

Neuroptera

- One morphospecies, one specimen
- Collections from years 1 and 2 are currently residing at the University of New England for further identification to species level where possible.

Insect flower visitor surveys

A total of 45 macadamia blocks were surveyed across 23 different macadamia farms. Varieties assessed are listed in Table 3.3.1. All blocks were surveyed across a single day (once) with exception of six blocks of “741” (surveyed twice) one block of “344” (three times), another two blocks of “344” (twice), one block of “842” (three times and another two blocks of “842” (twice).

Table 3.3.1. Number of orchards surveyed for each macadamia variety.

Variety	Bundaberg	Gympie - Glasshouse	Northern Rivers
"741"	7	6	4
"344"	0	7	5
"842"	0	4	4
"816"	0	1	0
"daddow"	0	4	1
Mixed variety	0	2	0

In total, 3633 flower-visiting insects were observed across all surveys. The majority of these were in Gympie - Glasshouse orchards; 3169 individuals compared to 291 individuals in Northern Rivers and just 173 in Bundaberg. Of these, 77.9% were honey bees and 13.8% stingless bees. All other species combined represented 9.2%.

For each region, honey bees represented the highest proportion of flower visitors near Bundaberg 86.7%, followed by the Gympie - Glasshouse 78.4% and Northern Rivers 55.0%. Just one stingless bee was observed across all surveys in orchards in the Bundaberg region and represented just 1.6% of visitors in the Northern Rivers, but represented 15.7% of visitors in Gympie – Glasshouse.

Although few in number, the most common of the other flower-visiting species observed in Bundaberg orchards were the Dipteran species *Stomorphina discolor* (n=4), the lycid beetle *Metriorrhynchus rhipidius* (n=3) and ladybird beetle species (Coccinellidae) (n=3). In Gympie - Glasshouse these were *M. rhipidius* (n=28), *S. discolor* (n=27) and *Calliphora* sp. 1 (n=15). In the Northern Rivers region these were the Chrysomelid beetle *Monolepta australis* (n=92), *M. rhipidius* (n=15) and *Calliphora* sp. 1 (n=5).

At the orchard block level, honey bees were observed visiting flowering macadamia racemes in 39 of 45 orchard blocks, however, their abundance was highly variable (Figure 3.3.4). Stingless bees were also seen across 21 orchard blocks and, like honey bees, were highly variable in abundance (Figure 3.3.4). All other insect taxa were observed in relatively few orchards and in relatively low numbers, in all but a few cases, counts were below ten (Figure 3.3.4).

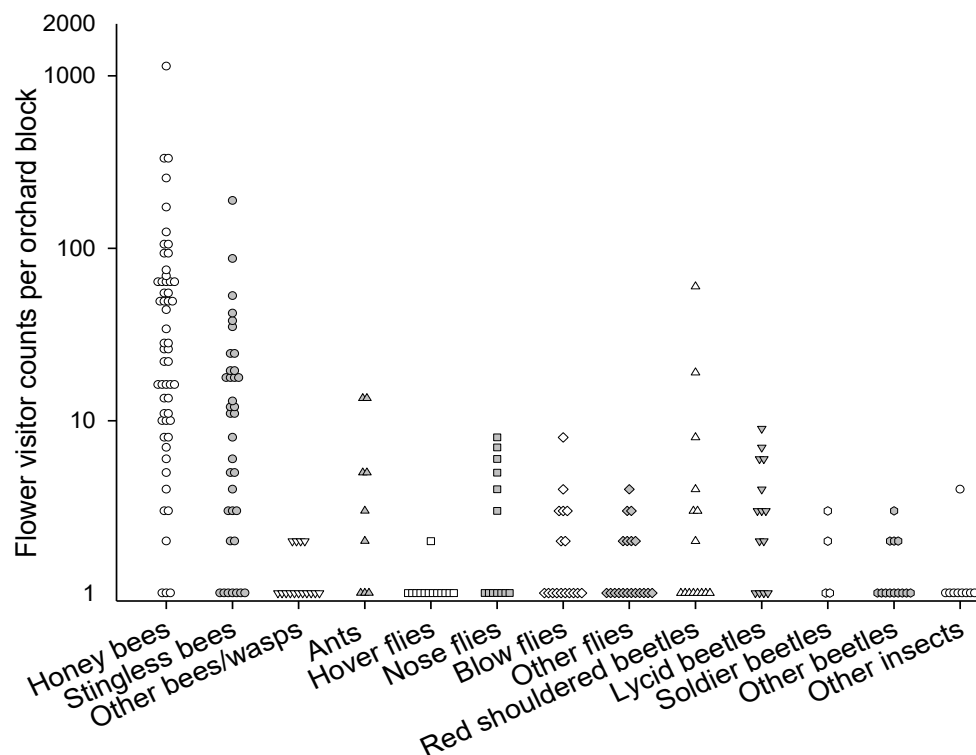


Figure 3.3.4. Counts of insect taxa observed visiting flowering macadamia racemes within orchard blocks. Only data for blocks where the insect was observed (i.e. $n \geq 1$) are included. Each symbol represents total counts within an orchard block.

Flowering intensity and insect counts

Flowering intensity varied between orchard blocks across survey days. For Bundaberg blocks, the average number of fully flowering racemes across the twelve surveyed trees within each block ranged from 1.7 to 9.9 per m^3 across all survey days. For Gympie – Glasshouse it was 0.2 to 4.7 per m^3 while for Northern Rivers it was 0.6 to 6.1 m^3 . Overall, flowering intensity was highest for orchard blocks in Bundaberg and lowest in Gympie – Glasshouse (Figure 3.3.5)

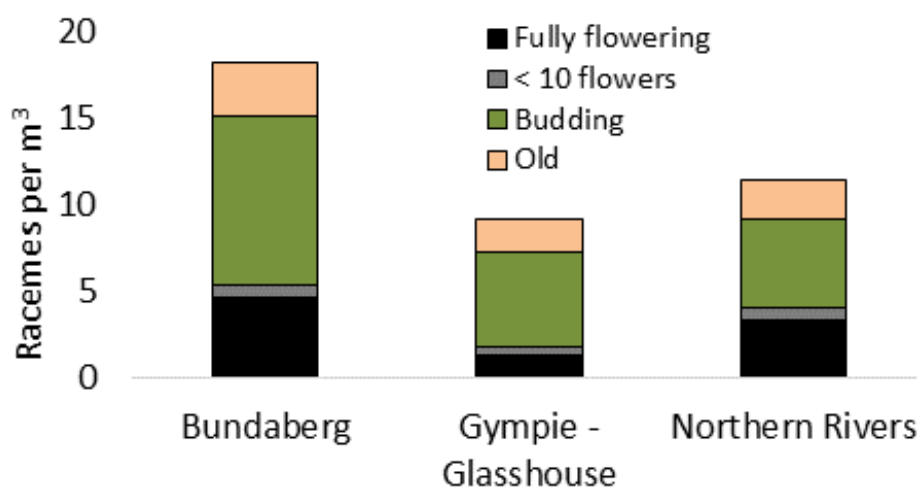


Figure 3.3.5. Average number of racemes per cubic metre and their development stage for trees within surveyed blocks located across three regions.

A comparison of flowering intensity between macadamia varieties found more variation between blocks in the Northern Rivers with varieties “741” and “daddow” having more flowers per cubic metre on survey days compared to varieties “344” and “842” (Figure 3.3.6). In the Gympie – Glasshouse region the variation between varieties was less, with “daddow” having more fully flowering racemes compared to the other varieties (Figure 3.3.6).

Conversion of observed insect counts into an estimate of numbers of insects observed per 100 fully flowering racemes (to account for variation of flowering intensity between orchard blocks) further emphasised differences in the patterns of flower visitor abundances between regions. For each survey period (three per day), the number of honey bees observed for Gympie – Glasshouse orchards averaged (\pm SE) 8.5 ± 0.6 , compared to Northern Rivers 0.5 ± 0.1 and Bundaberg with just 0.3 ± 0.1 . This is emphasised by the counts of honey bees visiting fully flowering racemes within orchard blocks (Figure 3.3.7). Therefore, factors associated with regional differences influenced pollinator abundance more than flowering intensity.

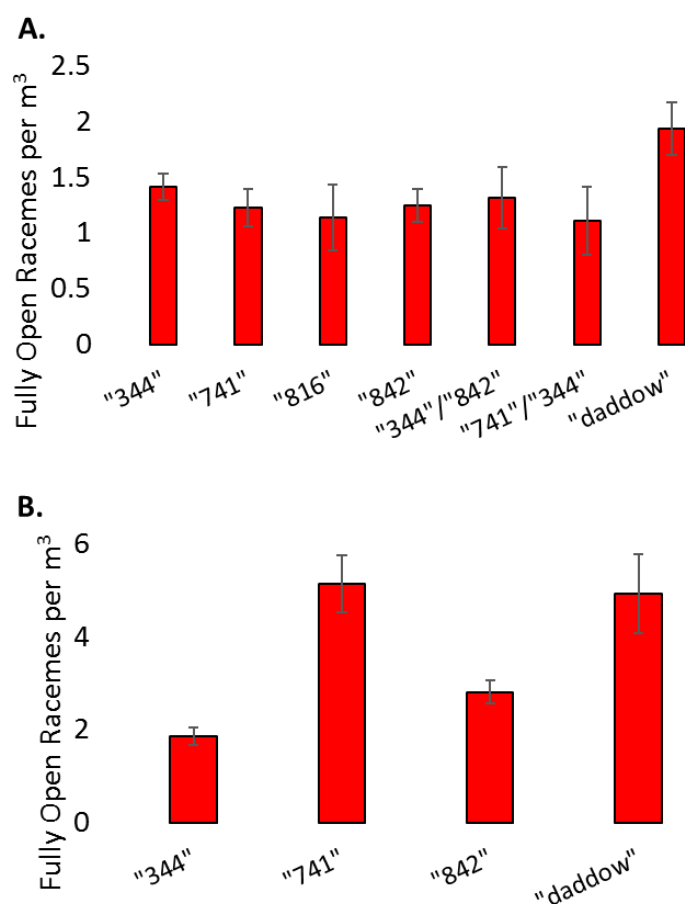


Figure 3.3.6. Average number of fully flowering racemes per cubic metre within surveyed blocks of each macadamia variety, A. are orchard blocks in the Gympie – Glasshouse region, B. are blocks in the Northern Rivers.

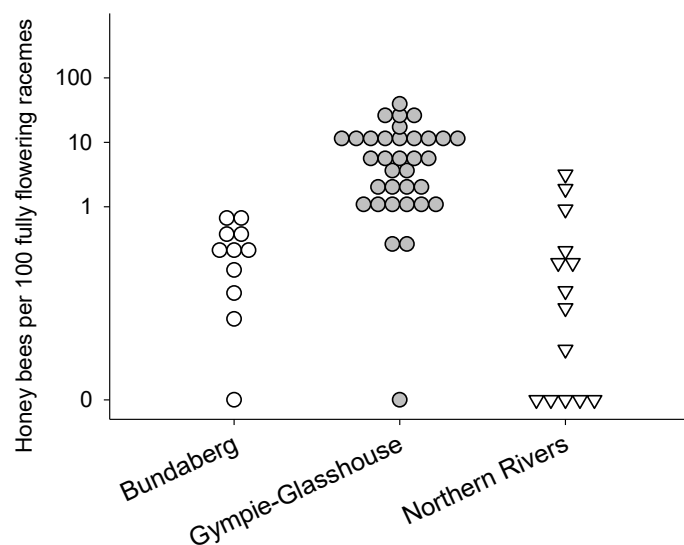


Figure 3.3.7. Counts of honey bees per 100 fully flowering macadamia racemes within orchard blocks across three regions. Each symbol represents total counts within an orchard block.

Insect counts and survey time

Although there was high variability in the number of flower visitors between orchards, there was a trend of increasing numbers of flower visitors from the morning to afternoon surveys. This trend was more noticeable in orchard blocks in Bundaberg (Figure 3.3.8)

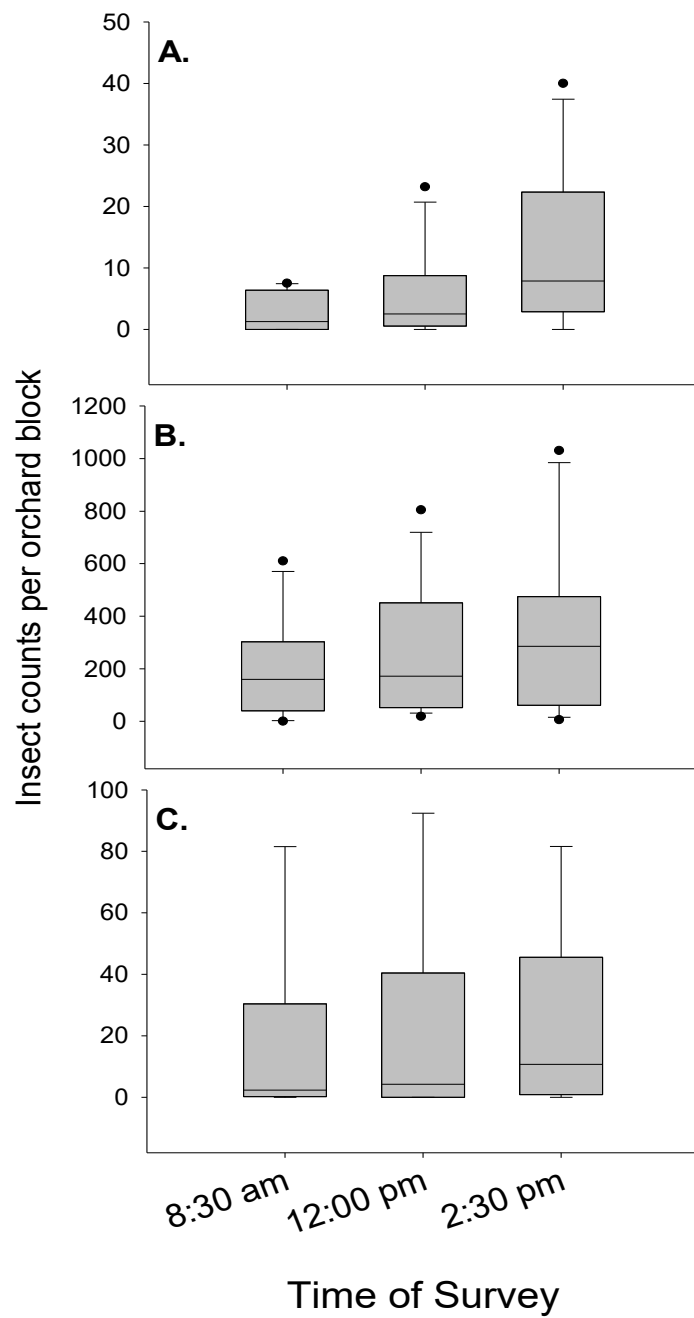


Figure 3.3.8. Counts of flower visitors to macadamia trees across three survey times within orchard blocks. Blocks were located across three regions A. Bundaberg, B. Gympie – Glasshouse and C. Northern Rivers.

Flower visitor counts between different macadamia varieties located in different blocks on the same farms.

Flower visitor counts between different varietal blocks located on the same farms (estimated numbers per 100 fully flowering racemes) were often variable, but not always (Figure 3.3.9). On one farm, variety “344” maintained marginally higher counts of flower visitors than variety “842” across three different survey days, despite the change in flowering intensity between the two varieties across survey days (“344” having higher flowering intensity in the first two surveys, “842” in the third). Overall, there was no pattern evident between flowering intensity the number of insects visiting flowers of different varieties. This may indicate difference in the attractiveness of different varieties, but could also be due to factors such as block location, different management practices between blocks and difference in tree age or shading of surveyed racemes.

Comparison of visitation of racemes positioned near the outside versus within the canopy.

Honey bees and stingless bees were the only flower visiting insects in sufficient abundance across multiple orchards to compare visitation to fully flowering racemes near the outside of the canopy versus the inner canopy. Both species were in higher abundance near the outside of tree canopies although we also observed both species visiting racemes located in the inner canopy (Figure 3.3.10).

The relationship between pollen removal and flower visitor abundance

An assessment of eleven orchards also found higher pollen removal from individual florets within orchards with higher abundance of insects per 100 fully flowering racemes as recorded in the observation surveys. (Figure 3.3.11).

Weather and flower visitor occurrence.

Temperatures at survey times varied from 17.6 - 33.1°C (mean 23.6°C), Light intensity from 79 -1312 W.m² (mean 842 W.m²), humidity from 25 – 87% (Mean 49.1%) and average wind speed from 0.0 – 20.1 km/h (mean 3.3 km/h) . Table 3.3.2 shows the range of weather variability at which various insect taxa were observed actively visiting flowering racemes. These may not fully reflect the range of weather variability that these insects may be active under as the variability reflects the frequency of weather measures recorded across all surveys and the abundance of the specific taxa.

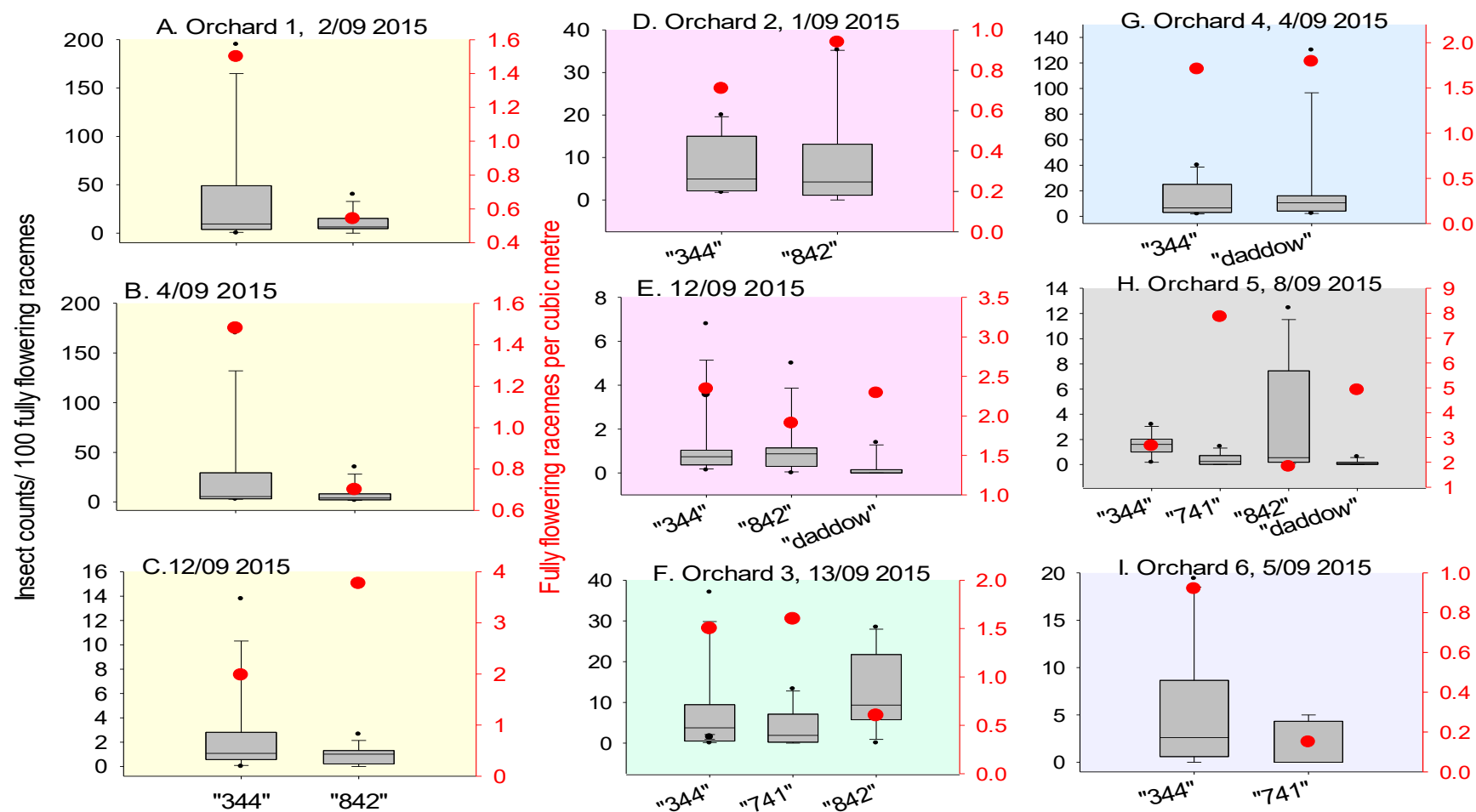


Figure 3.3.9. Counts of flower visitors per 100 fully flowering racemes for blocks of different varieties within the same farm. Data are presented as box-plots with median (y-axis in black). Average number of fully flowering racemes per cubic metre are present as red circles (y-axis in red). Differently colour graphs are different farms.

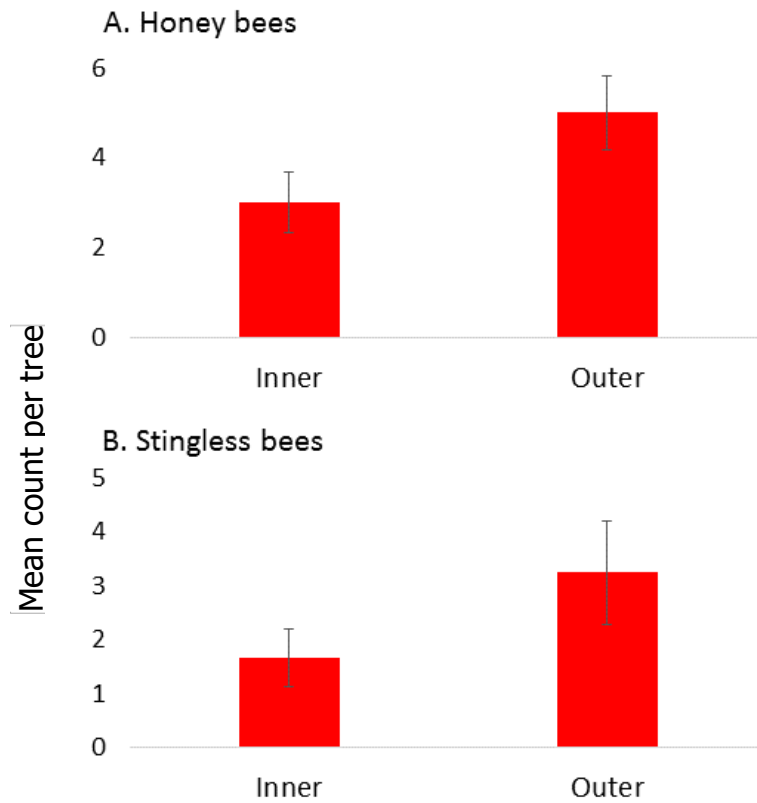


Figure 3.3.10. Average counts (\pm S.E.) of A. honey bees and B. stingless bees on fully flowering racemes located near the outside of the canopy versus the inner canopy

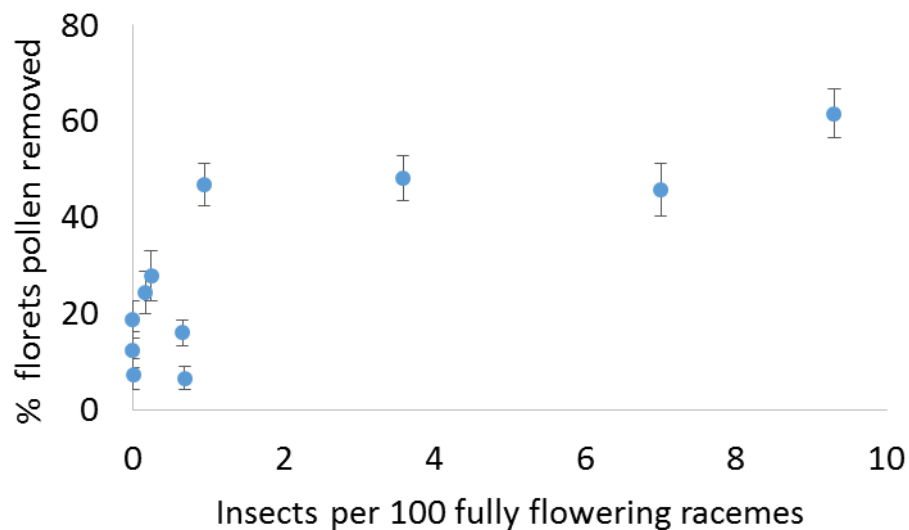


Figure 3.3.11. Relationship between the proportion of florets with pollen removed from pollen presenters (average \pm S.E.) and the average number of insect flower visitors per 100 fully flowering racemes.

Table 3.3.2. Range of weather variability that flower visitors were observed visiting flowering macadamia racemes.

Taxa	Observed	Temperature (°C)	Light Intensity (W/m ²)	Humidity (%)	Wind speed (km/h)
Honey bees	2796	17.8 - 33.1	108 - 1300	25 - 80	0.0 - 20.1
Stingless bees	503	17.6 - 31.6	257 - 1235	25 - 63	0.0 - 10.5
Hoverflies	13	19.9 - 27.1	500 - 1075	31 - 54	0.0 - 6.5
Blow flies	37	17.8 - 33.1	79 - 1135	29 - 63	0.0 - 10.5
Nose flies	32	19.9 - 31.2	79 - 1030	29 - 59	0.0 - 9.1
Ants	26	21.2 - 26.5	216 - 1300	36 - 60	0.0 - 10.9
Lycid beetles	43	21.6 - 33.1	79 - 1235	26 - 69	0.0 - 10.5
Other beetles	118	17.8 - 27.0	188 - 1235	32 - 70	0.0 - 20.1

Pollinator effectiveness and pollen flow between orchards

Calculations of pollinator effectiveness (pollinator efficiency x abundance) found large variability between orchard blocks within and between regions (Figure 3.3.12). Regarding the potential for stigmas to receive pollen per hour, median values for blocks in the Bundaberg and Northern Rivers region were very low (Bundaberg 0.15 and Northern Rivers 0.12) with Northern Rivers blocks being more variable (Figure 3.3.12). In contrast blocks within the Gympie – Glasshouse regions had a much higher median (6.79).

Of the pollinating species contributing to the pollen delivery to stigmas, honey bees were by far the most effective pollinator across all orchard blocks in Bundaberg where pollen flow was recorded. The very low abundance of other insect pollinating species in Bundaberg orchard blocks suggests their contribution in this region is negligible. In the Northern Rivers orchard blocks, honey bees were also determined to be the most effective pollinator, however in two orchards where honey bees were very low in abundance, lycid beetles were more effective in one and stingless bees in the other (Figure 3.3.12). In contrast, for orchard blocks within the Gympie – Glasshouse region, stingless bees were almost as effective as honey bees contributing on average 5.6 ± 1.5 pollen grains to each raceme per hour compared to honey bees 5.96 ± 1.1 . In this region they were more effective than honey bees within daily surveys of nine of the 34 daily orchard block surveys (Figure 3.3.12). All other pollinating species assessed for pollination efficiency contributed very little to pollen flow to raceme stigmas in this region.

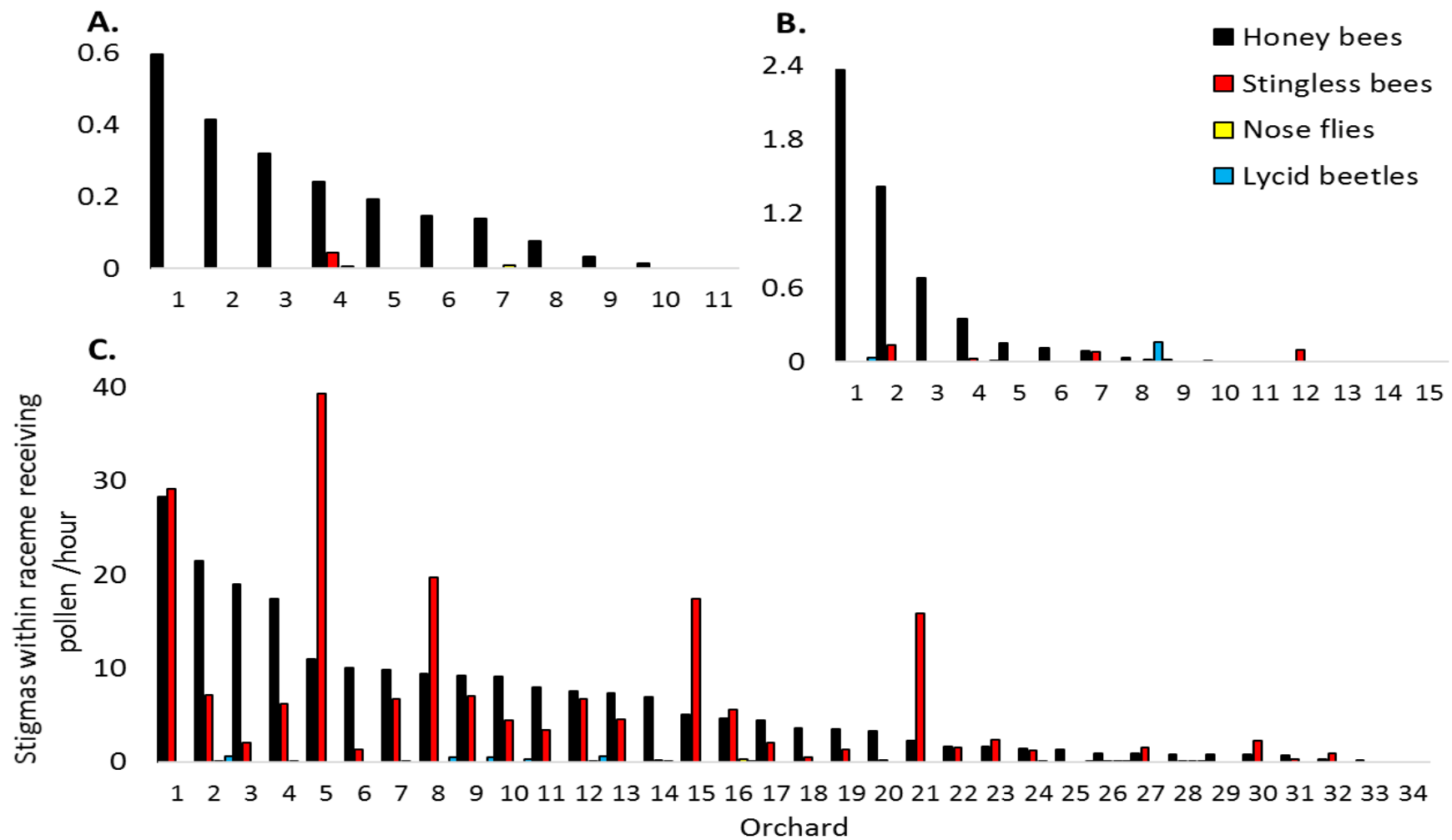


Figure 3.3.12. Estimated pollinator effectiveness of four different insect species across daily surveys of macadamia blocks located in three regions A. Bundaberg (Central Coast Queensland) , B. Northern Rivers (North New South Wales) and C. Gympie – Glasshouse (South East Queensland).

3.3.4 Discussion

Pollinators and flower visitors of macadamia in the Northern Rivers, Gympie-Glasshouse and Bundaberg regions.

A wide range of insect species were collected visiting flowering macadamia racemes and many of the species were the same as those recorded by Heard (1994). Although many may be capable of pollinating macadamia, here we provide some basic information, where available, on the more common flower visitors and those taxa that are, or have been suggested as, pollinators.

Many growers already allow or request for the provision of managed honey bees and, to a lesser extent, stingless bees within their orchard blocks and these are the species that are considered as key pollinators of macadamia (Howlett et al. 2015). Apart from honey bees and stingless bees (Apidae), other bee species have been observed as macadamia flower visitors and potential pollinators, particularly species from families Colletidae and Halictidae (Vithanage and Ironside, 1986; Heard and Exley, 1994). We also collected bees from these families, specimens of which are contained within our reference collection of macadamia flower visitors. These species differ from the Apidae species as they are mainly ground nesting (*Leioproctus*, *Lasioglossum* and *Homalictus*) (Danforth & Ji 2001; Donovan 2007) or nest within the cavities of plant stems or twigs (*Hylaeus*) (Donovan 2007) and are not managed species. Methods have been trialled and used to successfully transfer *Leioproctus* between one site to another in New Zealand (Donovan et al 2010), and ongoing research is further exploring methods to better manage these pollinators in cropping systems.

Flies from a range of different families have also been reported visiting macadamia flowers (Heard and Exley, 1994). We also collected a wide range of Diptera from macadamia flowers. The more common species were nose flies (Rhiniidae), blow flies (Calliphoridae), house flies (Muscidae), bristle flies (Tachinidae) and hoverflies (Syrphidae). Many of these flies are known pollinators of a range of crops (Rader et al. 2016; Howlett et al. 2012, Rader et al 2009) and our findings demonstrate that at least one species, the nose fly *Stomorhina discolor* is capable of pollinating macadamia (transferring pollen directly onto stigmas), while hoverflies and blow flies can at least contribute to pollen flow between flowers (pollen delivered to styles and to within 3 mm of stigmas). Nose fly larvae have been reported predated other dipteran larvae such as house flies (Kurahashi & Fauran 1980). The larvae of blow flies (*Calliphora stygia* and *Lucilia cuprina/sericata* along with a number of other calliphorid species), feed on carrion while the larvae of hoverflies have varied lifecycles depending on the species. Narrow-yellow hoverflies *Symosyrphus grandicornis* and black-orange hoverflies *Melangyna viridiceps*, two species collected visiting macadamia flowers, have larvae that consume aphids.

Several beetle species were also collected visiting macadamia flowers. One of the most common was the pest species, red shouldered leaf beetle (*Monolepta australis*). Adults feed on leaves and flowers, while larvae feed on plant roots, particularly grasses. The lycid (or net-winged) beetle, *Metriorrhynchus rhipidius*, has previously been considered as a likely pollinator of macadamia (Vithanage and Ironside 1986), and this has now been verified in the current study. Lycid larvae are typically found in leaf litter or under bark where they predate other insect larvae. The soldier beetle, *Chauliognathus flavipennis*, is another species that has been verified as a pollinator. Soldier beetle larvae also predate other ground and leaf dwelling soft bodied insects. The brown flower beetle, *Glycyphana stolata*, has also been shown to move relatively large amounts of pollen between flowers (to styles and close to stigmas). Although it is a likely pollinator, it has yet to be verified as a whether it transfers pollen directly to stigmas. Larvae of brown flower beetles lives in soil or rotting logs feeding on decaying plant material.

Flower visitor abundances and variation

Despite the range of flower visiting insect species collected or observed from macadamia flowers, in most orchards species richness was low and abundance variable. This has been noted by other researchers in previous studies on macadamia in Australia (Heard and Exley, 1994), and even within orchards between seasons (Vithanage and Ironside, 1986). However, our findings on overall flower visitor composition across orchards were similar to Heard & Exley's (1994) study, despite the development of new orchards. From their survey of 15 orchards located across the Northern Rivers and Gympie-Glasshouse regions between 1985-87, they reported honey bees as the most abundant flower visiting species representing 60.5% of all insect visits to flowers followed by stingless bees 35.8% (*T. carbonaria*), with all other insects very low in abundance. We found honey bees to be even more dominant (77.9%) followed by stingless bees (13.8%) with all other species representing 9.2%. The dominance of honey bees was highest in the Bundaberg region (86.7%) and lowest in the Northern Rivers (55%). Numbers of the pest species, the red shouldered beetle, were relatively high in the Northern Rivers representing 31.6% of all flower visitors (maximum 46.9% in one orchard block).

The numbers of flower visitors were highly variable between orchard blocks and this was the case for all insect taxa. Variability in honey bee abundance within orchards may reflect numerous factors including variable numbers of honey bee hives and their positioning within orchards, as well as variable strength of hives [influencing number and behaviour of workers (Manning 2006)] and relative attractiveness of other flowers surrounding the orchard. Even with consistent allocation of honey bee hive numbers and their placement, the abundance of honey bee foragers can remain highly variable within many crops due to the presence of other preferred flowering plants in the surrounding landscape that reduce the number of foragers on the target crop (Goodwin 2012).

Variability in stingless bee numbers between orchard blocks is likely reflected in their placement as managed hives in some blocks and not others. In those blocks where they are not placed they are wild pollinators, similar to all other observed flower visiting species. As such, their numbers can be highly influenced by variation in orchard management (e.g pesticide application, pruning) and the surrounding land use. Heard & Exley (1994) found stingless bee numbers within macadamia orchards were correlated with the percentage of *Eucalyptus* spp. in surrounding forest.

Flowering intensity and insect counts

Our surveys showed large variation in the flowering intensity (number of fully flowering racemes present on trees) between blocks on survey days. This flowering intensity may also have potentially influenced pollinator abundance and therefore rate of flower visitation to racemes. For example, in brassica crops, the abundance of pollinators has been found to track closely with flowering intensity (Mesa et al. 2013; Sihag & Khatkar 1999). Using insect counts in addition with quadrat data on flowering racemes collected on the day, we converted our counts to an estimated count of flower visiting insects per 100 fully flowering racemes. Regional comparisons found Bundaberg orchards blocks recorded the highest flowering intensity on survey days and Gympie-Glasshouse the lowest whereas insect counts were opposite, strongly suggesting regional differences rather than flowering intensity playing a key role in flower visitor abundances. Within orchards that contained multiple blocks of different varieties, their flowering intensity was not obviously correlated with insect abundances. Likewise, Heard & Exley (1994) found no significant differences in the visitation rates of honey bees and stingless bees with varying flowering intensity of trees within orchards, although it did influence visitation rates of other flower visiting insects. These findings suggest that for honey bees and stingless bees, factors other than variation in flowering intensity are more influential in determining flower visitor abundance.

Varietal differences

Despite Rhodes (Rhodes (1986) noting a difference in the abundance between honey bee numbers foraging on two cultivars, we did not find any obvious pattern in the numbers of pollinator visiting racemes (per 100 fully flowering racemes) between different macadamia varietal blocks grown on the same farm. We did observe difference in pollinator abundances between some varieties on specific farms but when we compared data between different farms containing the same varieties, we did not observe any consistent pattern. Assessments to compare the relative attractiveness of cultivars contained in different orchard blocks are complicated by factors that may be more influential in pollinator preferences such as flowering intensity variation at a given time (although flowering intensity did not appear to reflect flower visitation rates in our surveys), positioning, age and management of compared blocks.

Diurnal patterns of flower visitation, location of racemes and activity under variable weather

Our data found a trend of increasing abundance from morning (survey start 8:30 am), to midday (12:00 pm) and afternoon (2:30 pm) surveys in all three regions. However, this was not a consistent pattern within all orchard blocks. The trend was most apparent for Bundaberg orchards compared to the other regions. This trend did not match closely with Heard & Exley (1994) who found pollinator abundance generally peaked around midday, with stingless bees peaking late morning to midday and honey bees just after midday. In contrast Vithanage & Ironside (1986) found insect abundances peaking twice throughout the day, mid-morning (around 10 am) and early afternoon (around 2 pm)

Honey bee and stingless bees were found more frequently visiting racemes located on or near the outer canopy as opposed to the inner canopy, a finding previously noted by (Heard and Exley, 1994). This may relate to the higher light intensity. Despite this, both species still visited inner racemes at around half the rate of outer racemes. We did not have enough data to assess other pollinating species, however, some pollinators such as blow flies readily forage under low light conditions (Howlett 2012) and therefore may potentially be less influenced by racemes positioned in the inner versus outer canopy.

We assessed air temperature, humidity, light intensity and average wind speed for various flower visiting insect taxa observed during the surveys. The range of weather variation was limited by the frequency of weather variability as well as insect abundance. Despite this, we found that many taxa foraged under a wide range of weather conditions. Honey bees and stingless bees were observed visiting flowers close to the extreme range of each weather variable recorded across the surveys. For example, at temperature of around 18°C to just over 30°C. The temperature of 18°C is considered to be about the minimum at which stingless bees will forage (Heard and Hendrikz, 1993). Although we saw activity for many taxa under a wide range of weather variability, many of the taxa may have much broader activity range. Many blow flies including *Calliphora stygia* will forage on flowers at temperatures below 10°C, under low light and high humidity conditions, while honey bees prefer higher light intensity, and low humidity although under some circumstances may forage at temperatures as low as 10°C (Howlett et al. 2013).

Pollinator effectiveness

For the orchard blocks where we assessed the presence and removal of pollen from the florets of racemes following survey completion (after 3 pm), there appeared to be a higher proportion of self-pollen was removed from racemes in those blocks with higher flower visitor abundance. This finding suggests that insect abundance may be important for the removal of self-pollen from stigmas. Further understanding of this relationship may provide information on the rates of insect flower visitation resulting in pollen removal and potentially pollen flow. However, as stingless bees were observed readily collecting pollen as opposed to honey bees and many non-bee pollinators that were

predominantly visiting flowers for nectar foraging, an understanding of these differences would be valuable to further glean the relationship between pollen removal and pollen flow.

To our knowledge, our study is the first to quantify and compare the efficiency and effectiveness of multiple flower visiting species of macadamia. We found nectar collecting honey bees to be the most important pollinating species across all three regions. Even so, their contribution across regions and between blocks within regions was highly variable, reflecting variability in their abundance. The greater efficiency of pollen collecting stingless bees as pollinators resulted in them being almost as effective as honey bees in the Gympie – Glasshouse region. Other insects that were found to be capable of pollinating macadamia had negligible effectiveness as pollinators due to very low abundances. The efficiency of these species indicates they have potential to be effective pollinators if populations can be increased within orchards.

Macadamia florets have fully exposed stigmas following the removal of self-pollen. A broad array of insects that either collect or feed on pollen at the stigmatic region of the floret or alternatively, forage for nectar but contact the stigma while moving into, within and from racemes are capable of pollinating macadamia.

Improving pollinator effectiveness

Many orchard blocks currently consist of single variety plantings that limit the opportunity for cross pollination within these blocks. This is highly likely to significantly reduce the effectiveness of all insect pollinators that forage within trees and between neighbouring trees. The inclusion of multiple macadamia varieties that flower at similar times will increase the potential for pollinators to move cross pollen to stigmas.

Honey bees and stingless bees are currently the most effective pollinators and both species can effectively transfer pollen to stigmas.

Not all macadamia growers appear to have a strategy for accessing and placing honey bee hives within their orchard. Cross pollination could be improved with more attention to the placement and spacing of honey bee and stingless bee hives, as well as adhering to consistent standards of hive strength. The effectiveness of these species and other wild pollinators will be the greatest in orchards with mixed varieties that overlap in flowering. They are not likely to be as effective in large single varietal plantings.

To increase cross pollination within single varietal blocks, growers may want to consider removal and replacement of weak or dead trees with trees of a different variety (pollenizer trees) that flowers at a similar time to the block trees. The placement of multiple pollenizer varieties that flower at similar times within the orchard may reduce the impact of unpredictable or variable off-sequence inter-varietal flowering, thereby maximising the opportunity for cross pollination to occur. The pollenizer trees may be kept small to maintain light at the location (both honey bees and stingless bees prefer to forage on outer racemes exposed to higher light intensity) and to encourage pollinator movement between pollenizers and orchard trees.

To improve cross pollination, growers should consider planting multiple varieties within the block when developing new blocks. Cultivars that flower at similar times could be planted in repeated sequence within the row to minimise distances between trees of different varieties.

This study has shown that other wild pollinators are capable of contributing to pollination and, if their visitation rates increased, could contribute more significantly to pollination. The ability to increase the abundance of these pollinators is currently limited due to either a lack of information on their lifecycle requirements or, for those with predacious larvae (eg, some fly species, lycid and soldier beetles), a reliance on prey species. Maintaining native vegetation near to macadamia blocks can improve the contribution of wild pollinators within crops, however, this could also potentially support potential pest

species. Heard & Exley (1994) found nearby eucalypt forests was correlated with higher numbers of stingless bees in Macadamia orchards. There is potential to promote wild pollinators and other beneficial insects without supporting pest species, but this requires further knowledge of the interactions of beneficial and pest species with the potential mix of plant species that will support or inhibit populations. Similar research in New Zealand has resulted in the establishment of designed on farm plantings (Howlett et al. 2013).

On farm practices should also be considerate to pollinators. For example, trying to avoid the use of pesticides during flowering. If unavoidable then choosing pesticides and applying them in the evening when bees are not active. Unfortunately, the impact of pesticides on beetle and fly pollinators may not be minimised at any time if they are residing within orchards during the day and night.

Key Points

- Despite the collection of many macadamia flower visiting species (including bees, flies, beetles and wasps), honeybees and stingless bees were by far the most abundant and effective pollinators.
- The open flower structure of macadamia flowers and the demonstration that beetle and fly species are capable of pollinating macadamia indicate that wild pollinators can be effective pollinators. However, this can only occur by significantly increasing their numbers within orchards.
- A lack of detailed knowledge on the lifecycles of wild pollinating species currently limits the ability to develop strategies to increase their numbers. Eucalypt forest near to macadamia orchards has been correlated with stingless bee abundance and, in other crops, native vegetation is known to support wild pollinator populations and their occurrence in neighbouring crops.
- Honey bees and stingless bees are pollinators of macadamia and placement of managed hives within orchard blocks is recommended. Particularly in mixed varietal plantings.
- Trees of more than one variety should be incorporated in orchard block design to encourage cross pollination to increase nut yields These may be planted in various ratios, however, preferably not in lines of the one variety as this will limit the crossing of pollinators between trees. If a single variety planting is preferred then inclusion of trees of another variety to act as pollenizers is recommended. In large established blocks, the removal of poorly performing trees to create an opening to place trees of different varieties (pollenizers) should increase the rate of cross pollination.
- Avoid spraying pesticides during flowering if possible. If unavoidable choose short acting pesticides that will limit bee deaths. Apply in the evening when bees are not active (Australian Macadamia Society n.d).
- Prune trees to maximise light to racemes to encourage bee visitation.

3.3.5 References

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4 Avocados

4.1 Relationships between avocado flower phenology and temperature

4.1.1 Introduction

Plant & Food Research (PFR) has collected a multi-year dataset in New Zealand on the relationship between overnight minimum temperatures and the timing of the opening of female 'Hass' flowers. Avocado flowers open first as functionally female, then close before opening as functionally male on subsequent days. Pollination only occurs when female flowers are open and receptive, and when there is pollen available for transfer (either from male 'Hass' or other cultivars). Avocado cultivars are grouped in to those that flower as female in the morning and male in the afternoon (such as 'Hass') and those that flower as female in the afternoon and male in the morning. However, this description is highly simplified, as lower overnight minimum temperatures, along with other environmental factors, can delay the opening of female flowers. Furthermore, depending on the temperature, there can be considerable overlap in between male and female stages in the middle of the day.

Using a system of multiple time-lapse cameras, we have determined the relationship between overnight minimum temperatures and mean first female flower opening time (note that this represents the earliest female flower opening for a given day (Figure 4.1.1). Over multiple years, the relationship is consistent. Using this model we are able to predict when female flowers are likely to open. We have also found that the majority of flowers that open later than 16:00 remain open all night long and only close the next day.

An important implication of this finding is that on different days and different locations, pollination will be required at different time of the day. Pollinators also exhibit daily activity patterns, and to ensure optimum pollination, it is important to consider whether sufficient pollinators are active during the time of female flower receptivity.

Our aim in this study was to conduct an initial assessment of orchard temperatures in the Tri-State area and compare this with our flowering model to determine whether we needed to consider different groups of pollinators with different activity patterns, including nocturnal species.

4.1.2 Methods

Using our flowering model, we identified broad groups of temperatures and flowering times to categorise flowering patterns (Figure 4.1.1). Overnight minimums of 4–8°C were classified as 'late afternoon', 8.5–11°C were classified 'early afternoon' and 11.5–16°C were classified as 'morning' (Figure 4.1.1).

The names and contact details for 30 avocado growers in the Tri-State area were provided by PHA, HIA and horticultural consultants. Emails seeking involvement in the trial were sent to all addresses, and nine responded to say they would like to be involved. All nine were sent HortPlus Temperature Microloggers in early October 2014. The loggers were set to record temperatures every 30 min until mid-December 2014, and were housed in a Stevenson radiation screen. The growers were then asked to retrieve the loggers and return them to PFR's Brisbane office.

As the loggers were located in the same room for over 10 days prior to shipping, these data were used to confirm that the loggers were calibrated so that the variation in minimum temperature recorded was less than 1°C.

We plotted maximum and minimum temperatures for each orchard, and recorded the number of nights that the overnight temperature fell within each of the three flowering temperature ranges ('morning', 'early afternoon' and 'late afternoon'). Overnight temperatures greater than 16°C were recorded as 'morning'. Temperatures lower than 6°C were further classified as 'Probably nocturnal'.

In addition to this data collection, field surveys of flowering at orchards in the Tri-State area were conducted to assess whether the phenomenon of nocturnal flowering occurred in this region following temperatures below 6°C.

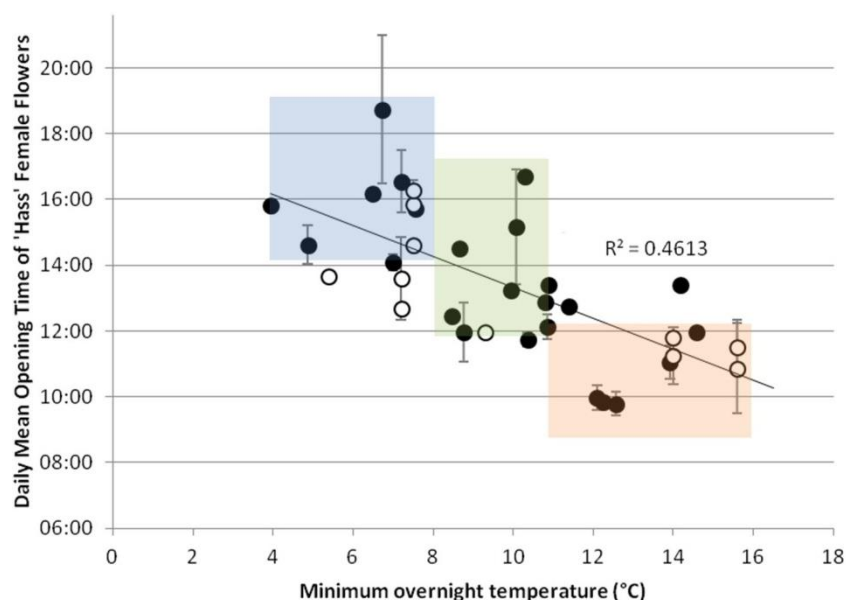


Figure 4.1.1. The relationship between overnight minimum temperature and mean first female opening time in New Zealand orchards in 2011 (open circles) and 2013 (closed circles). Blue square indicates temperatures classified as 'Late afternoon', green is 'early afternoon' and orange is 'morning'. Error bars indicate SEM, regression line is for combined dataset for both years.

4.1.3 Results

Of the nine temperature loggers that were sent, four were returned to PFR for analysis. Only two growers recorded when the loggers were placed in the orchard and retrieved. Most loggers were placed into the orchards on 15 October. Data collection stopped on 8 November for all loggers. Based on the temperature record, one logger (Robinvale 1) appears to have remained inside a building until 1 November (Figure 4.1.2C). For this orchard, all data prior to this date were not analysed.

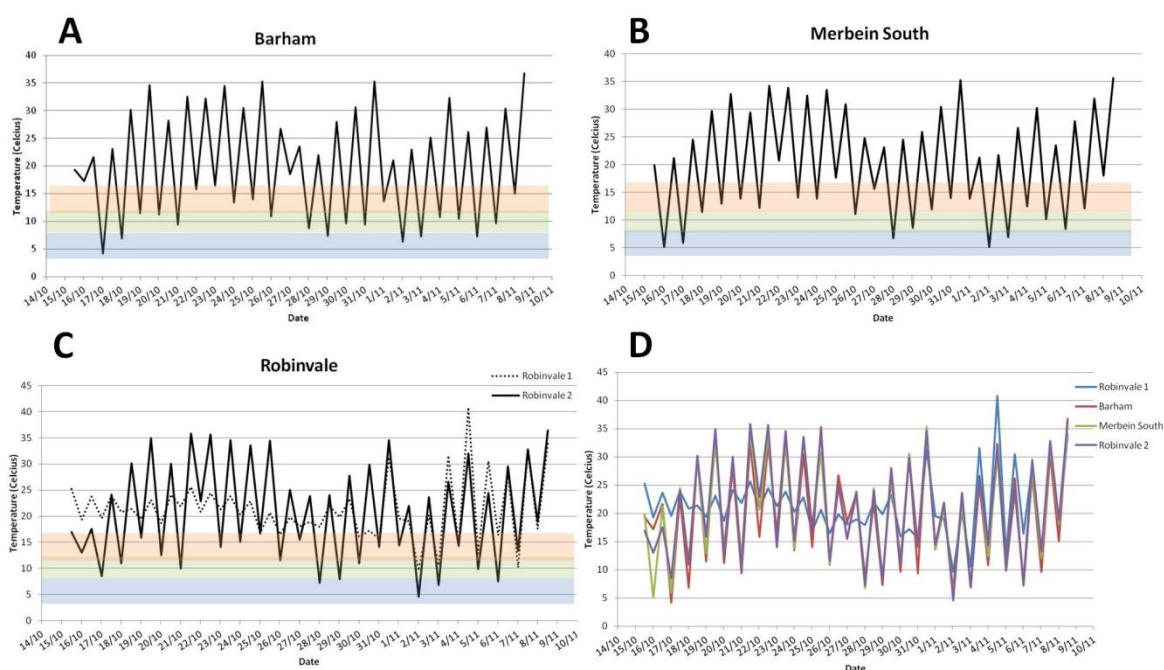


Figure 4.1.2. Temperature record from 15 October 2014 to 8 November 2014 from microloggers installed in four orchards in the Tri-State region, showing A) Barham, B) Merbein South, C) Two orchards at Robinvale and D) all orchards combined. Blue square indicates temperatures classified as 'late afternoon', green is 'early afternoon' and orange is 'morning'. Vertical positioning of coloured bars is approximate. See text for actual figures.

Temperatures across all orchards in the 24-day period varied between 4.2°C and 40.8°C (Figure 4.1.2). Apart from Robinvale 1 (Figure 4.1.2C), all orchards experienced all categories of flower opening time (Figure 4.1.2, Table 4.1.1). More than half of the nights at three sites were warmer than 11.5°C, which is predicted to lead to morning flowering (Table 4.1.1), whereas Barham had 62.5% of nights predicted to be afternoon flowering (Table 4.1.1, Figure 4.1.2A). The same flowering classification was recorded across all sites on 10 out of the 24 days, with 8 of those classified as morning flowering.

Nocturnal flowering was predicted to be probable at all sites apart from Robinvale 1. Nocturnal flowering was confirmed at Merbein South by observation on two nights following low overnight minimums.

Table 4.1.1. Classification of time of female 'Hass' flowering based on recorded overnight minimum temperatures for each day monitored at four orchards in the Tri-State area. Number in parentheses indicates nights when flowering was 'Probably Nocturnal'.

	Robinvale 1	Robinvale 2	Barham	Merbein South
Morning	5	14	9	14
Early Afternoon	3	5	9	5
Late Afternoon	0	5 (1)	6 (1)	5 (3)
Totals	8	24	24	24

4.1.4 Discussion

We found considerable variation in overnight minimum temperatures, and thus timing of female flowering, between and within sites over the course of 24 days. While the orchards would have experienced a majority of morning flowering days, the spread of times (including nocturnal flowering) suggests that pollination during other flowering periods of time as well.

Grower participation was low and the sampling period was short, which could have disguised the true variability in temperatures in the region. However, these data are sufficient to show that overnight temperatures and therefore flowering vary significantly across this region during the period of avocado flowering.

We strongly recommend that any study of avocado pollination in the Tri-State region consider the role of pollinators at different times of the day, including nocturnal pollination where possible.

Key point

The broad variation in minimum overnight temperatures will greatly alter the daily pattern of female flower opening times within orchards in the Tri-State region. The presence of diverse wild pollinators that are active at different times and weather will maximise the chances of pollination in these orchards.

4.2 Efficiency of avocado pollinators

4.2.1 Introduction

Honey bees are often considered key pollinators of avocado in commercial orchards (Dixon 2006; Perez-Balam et al. 2012; Ish-Am & Lahav 2011; Wysoki et al. 2002) despite a range of wild flower-visiting species having been recorded visiting flowers (Perez-Balam et al. 2012; Ish-Am et al. 1999). In Australia, these include syrphid (hover) and calliphorid flies (Evans et al. 2011; Vithanage 1986). Earlier work in New Zealand avocado orchards by our team noted that the assumption of honey bees as key pollinators was correct, but our first visits to avocado orchards in the Tri-State region of Australia indicated a very much wider range of flower-visiting insects. This suggested similarities to that of other plant pollinator systems.

Wild pollinators play an important role in increasing the yields of many insect pollinated crops (Garabaldi et al. 2013), but often their identity and specific efficiency as pollinators is not known. An indication of their potential has been demonstrated in the crop (*Brassica rapa*), where a recent study has demonstrated that many wild pollinating species can be efficient pollinators, with other bee and fly species depositing comparable amounts of pollen onto stigmas and moving between flowers at comparable rates to honey bees (Rader et al. 2009). As a whole, wild pollinating species have been shown to contribute more than 50% of the pollination in some fields (Rader et al. 2012), while the non-bee pollinators alone provide a sizeable proportion of pollination to a wide range of crops (Rader et al. 2016).

As with many crops, knowledge of the efficiency of wild pollinating species within avocado orchards is poorly known. A Mexican study by Perez-Balam et al. (2012) examined aspects of the pollination efficiency of honey bees, flies and wasps. These authors examined rates of movement, deposition of pollen and abundance to find that flies are capable of contributing at a similar level as honey bees with the contribution of wasps lower. This study did not identify the flower visitors to the species level.

Avocado pollination is further complicated by the complex flowering system. Avocado flowers first open as functionally female (viable stigma with pollen yet to dehisce) for several hours (commonly but not restricted to 3–4 hours) then close before opening functionally male flowers, again usually for several hours, a reproductive system known as protogynous dichogamy (Sedgely 1987). Within a cultivar, the timing of these phases is synchronous, but the timing varies between cultivars and also depending on environmental conditions such as air temperature (Davenport 1986).

Although some growers plant blocks of the one variety, many interplant a smaller number of other cultivars as pollenizers. Cultivars are usually grouped into either 'Type A' cultivars where the female flowers usually open in the morning, or 'Type B' when the female flowers open in the afternoon. This breeding system is adapted to maximise cross pollination, with male flowers open and releasing pollen while female flowers on a different type cultivar are receptive. Most cultivars are self-compatible, so in the right environmental conditions self-pollination within a cultivar can occur during the overlap between the closing female phase and the opening male phase. This is known as close pollination (Ish-Am & Lahav 2011). In tropical regions, some cultivars also have flowers that can be pollinated in their male phase (Davenport et al. 1994). The Tri-State has climatic conditions more akin to the Mediterranean. Therefore, when studying the role of insect pollinators, it is important to ensure the study is conducted when both pollen is available on male flowers and female flowers are open.

Quantification of the efficiency of wild pollinating species is necessary to assess their importance as pollinators. Ideally, this should be done at the species level so that management strategies can be developed for specific effective species. In this study we assess the efficiency of several avocado flower-visiting dipteran and coleopteran species, and compare them against honey bees. For our

calculation of efficiency we focussed only on time periods when overlap occurred between the female and male phases of a single cultivar, 'Hass' (type A cultivar). The availability of pollen was determined by visually inspecting open male phase flowers to assess whether the anthers had dehisced and pollen had been released. This study therefore assesses the movement of pollen within 'Hass' trees during periods when the two phases overlap. Cross-pollination from polliniser cultivars (which requires longer distance movements) was not considered. We assess the ability of insect flower-visitors to move pollen to stigmas, their rates of movements between flowers, and their rate of contact with anthers and stigmas. Using fluorescent powder we also assess the degree to which insects move between neighbouring trees and distance moved. We then discuss the relative efficiency and effectiveness of each of these pollinators that are present in Tri-State orchards.

4.2.2 Materials and methods

Pollen deposition onto avocado stigmas by flower-visiting insects

Budding inflorescences that were to be a source of test flowers were initially bagged to ensure the opening flowers had not been exposed to insect visitors. These bags were removed briefly to abscise test flowers when needed before being replaced. Each test flower was exposed either to an insect that was foraging on flowers in the female flowering phase, or to an insect foraging on flowers in the male phase. This was done by slowly moving the test flower to a position of approximately 1–2 cm from the foraging insect to avoid disturbing the insect's behaviour. Care was also taken to ensure that the test flower did not contact any flowers or other plant material from the tree on which the insect was foraging, but was of similar position to other open flowers within the inflorescence. The insect was then allowed to move (walk or fly) across to the test flower and forage for nectar or collect/consume pollen. Once a target insect species had visited the test flower, the stigma and style were abscised using forceps. The style and stigma were then placed on a microscope slide with a cube of gelatin-fuchsin melted at a temperature of approximately 30°C. A cover slip was then mounted and pressed onto the gelatin-fuchsin and stigma/style and left to solidify. Control flowers were also prepared using the same technique, except that flower visitors were not allowed to contact the stigma/style. For each insect visit, we recorded the insect taxa, time of visit, insect behaviour (pollen or nectar collecting) and weather variables at the time of visit (temperature, humidity, light intensity and wind speed).

Movement of insects between flowers and trees

To evaluate pollinator efficiency, an important variable to assess is the rate of movement among flowers, inflorescences and trees. This information is required to evaluate the likely frequency of pollen transfer by different flower-visiting taxa. We used audio methods to record insect movements and determine how frequently insects moved between flowers, within and between inflorescences, and between trees. Initial Information recorded included orchard identity, tree variety, time, date and flower-visiting species/taxa observed. Flower visitors were then followed to record visits to individual flowers, whether each flower visited was female (pistillate) or male (staminate), an estimate of distance travelled to the next flower, movements to new inflorescences, and movements to new trees. Insects were followed for periods of up to 30 min if movement between flowers were infrequent. Following each recording, the temperature, humidity, light intensity to the north (held horizontally), south and towards the sun, and the maximum and minimum wind velocity over a 15-s period were also recorded. The audio records were transcribed for subsequent analysis and interpretation.

Estimation of pollinator efficiency of different flower visitors

We combine single pollen deposition data for common flower visiting species with rates of movement between flowers to compare their relative efficiency. We only assess the proportion of movements from male to female flowers that lead to successful pollen transfer in 'Hass' avocado. Although a

proportion of flower visits between female flowers by pollinators can deliver pollen to stigmas, our sample sizes were too small to calculate values with certainty. Values range from one (pollen transferred on every flower visit) and zero (insect does not deliver pollen to stigmas as it moves between flowers). We then use the mean numbers of flowers visited per minute by the different pollinators and divide this value by $\frac{1}{4}$. This is determined based on the assumption that the ratio of male to female flowers open during the cross-over of flowering phases is 1:1 and that male to female movements are $\frac{1}{4}$ of movement combinations (i.e. other possible movements are male to male, female to female and female to male). We also assume that each flower visitor does not discriminate between male and female flowers as they forage. Our calculation of efficiency for each species is therefore the number of female flowers receiving one or more pollen grains per minute.

4.2.3 Results

Pollen deposition onto stigmas by different flower visiting insects

We collected a total of 445 styles from flowers visited by 23 different flower visiting insects (Table 4.2.1) from two 'Hass' orchards near Mildura 34.175°S, 142.031 °E. In year 1 a total of 262 styles with stigmas from test flowers were collected after being visited by a single insect. Of these, 110 insects had moved from a flower in the female phase and 145 from flowers in the male phase. The most common insects sampled moving to test flowers were honey bees (13 from male phase flowers – M, and nine from female phase flowers – F); rhiniid flies (20 M, 10 F); Australian sheep blow flies (13 M, 13 F); large green blow flies (19 M, 16 F); brown calliphorid flies sp. 1 (17 M, 24 F); narrow yellow hover fly (7 M, 10 F); sarcophagid flies sp. 1 (7 M, 11 F); lesser brown blow flies (21 M, 9 F); tachinid flies sp. 1 (9 M, 4 F); banded pumpkin beetles *Aulacophora hiliaris* (9 M, 4 F); and transverse ladybird beetle (5 M, 1 F).

In year 2 we collected a further 183 styles/stigmas visited by individual insects and matching control stigma/styles. Of these, 66 insects had moved from a flower in the female phase and 114 from flowers in the male phase. Collections were made within two 'Hass' orchards near Mildura 34.175°S, 142.031°E (same orchards as year 1) and one near, Dareton 34.109°S 142.05°E. The most common insects sampled moving to test flowers in year 2 were narrow yellow hover fly (7 from male phase flowers – M, and 24 from female phase flowers – F); dark green calliphorid flies (13 M, 10 F); rhiniid flies (11 M, 8 F); Australian sheep blow flies (10 M, 9 F); large green blow flies (7 M, 2 F); brown calliphorid flies sp. 1 (5 M, 0 F); sarcophagid flies sp. 1 (0 M, 3 F); lesser brown blow flies (13 M, 1 F); tachinid flies sp. 1 (10 M, 3 F); and transverse ladybird beetle (6 M, 3 F).

The majority of the abundant species were identified to the highest taxonomic level possible by staff at Plant & Food Research. Dr Barry Donovan (Donovan Scientific Insect Research) has provided additional assistance on the identification of bees and wasps. Further identification of the flower visiting species is currently being conducted by the University of New England (led Dr Romina Rader and Bryony Wilcox). Where we have been able to identify an insect to species level, we have used morphospecies (e.g. Bristle fly sp. 1). This categorises insects based on our observation of morphological differences, but may include multiple species within the grouping. In a minority of cases, where individuals within a taxonomic grouping appear variable without an obvious dominant morphospecies, we have grouped individuals into a taxonomic grouping (e.g. Tephritidae spp.).

Table 4.2.1. Numbers of stigmas and styles collected from test flowers following individual movements by different insect species from male and female phase flowers.

Insect	Species	Family	Flower moved from	
			female	male
Honey bee	<i>Apis mellifera</i>	Apidae	9	19
Thynnid wasp sp. 1	Thynnidae sp. 1	Hymenoptera	3	9
Brown blow fly	<i>Calliphora stygia</i>	Calliphoridae	24	22
Lesser brown blow fly	<i>Calliphora augur</i>	Calliphoridae	10	34
Large green blow fly	<i>Chrysoma rufifacies?</i>	Calliphoridae	22	23
Australian sheep blow fly	<i>Lucila cuprina</i>	Calliphoridae	18	26
Dark-green calliphorid fly	Calliphoridae sp. 1	Calliphoridae	8	13
Rhiniid fly	Rhiniidae? sp. 2	Rhiniidae	18	31
Oxysarcodexia sp. 1	<i>Oxysarcodexia</i> sp. 1	Sarcophagidae	14	7
Bristle fly sp. 1	Tachinidae sp. 1	Tachinidae	3	11
Bristle fly sp. 3	Tachinidae sp. 3	Tachinidae	3	9
Unidentified bristle fly	Tachinidae spp.	Tachinidae	0	3
Yellow abdomen muscid fly	Muscidae sp. 2	Muscidae	0	2
Spotted wing fly	<i>Anthomyia punctipennis</i>	Anthomyiidae	1	3
Narrow yellow hover fly	<i>Simosyrphus grandicornis</i>	Syrphidae	34	14
Black-orange hoverfly	<i>Melangyna viridiceps</i>	Syrphidae	3	4
Yellow hover fly	<i>Eristalis punctulatus</i>	Syrphidae	2	5
Green soldier fly	<i>Odontomyia</i> sp.	Stratiomyidae	0	3
Tephritid fruit fly	Tephritidae spp.	Tephritidae	0	2
Transverse ladybird beetle	<i>Hippodamia variegata</i>	Coccinellidae	4	11
Variegated ladybird beetle	<i>Coccinella transversalis</i>	Coccinellidae	1	1
Banded pumpkin beetle	<i>Aulacophora hiliaris</i>	Chrysomelidae	5	11
Lycid beetle	Lycidae sp. 1	Lycidae	0	1

Overall, the mean number of pollen grains delivered by the various pollinator species moving from male phase flowers to test flowers was greater than their movement from female phase flowers to test flowers, although most were found capable of transferring pollen from male and female phase flowers to test flowers (Figures 4.2.1 A & B). In a few species we did not record any pollen deposition, but these were also species with very low abundance.

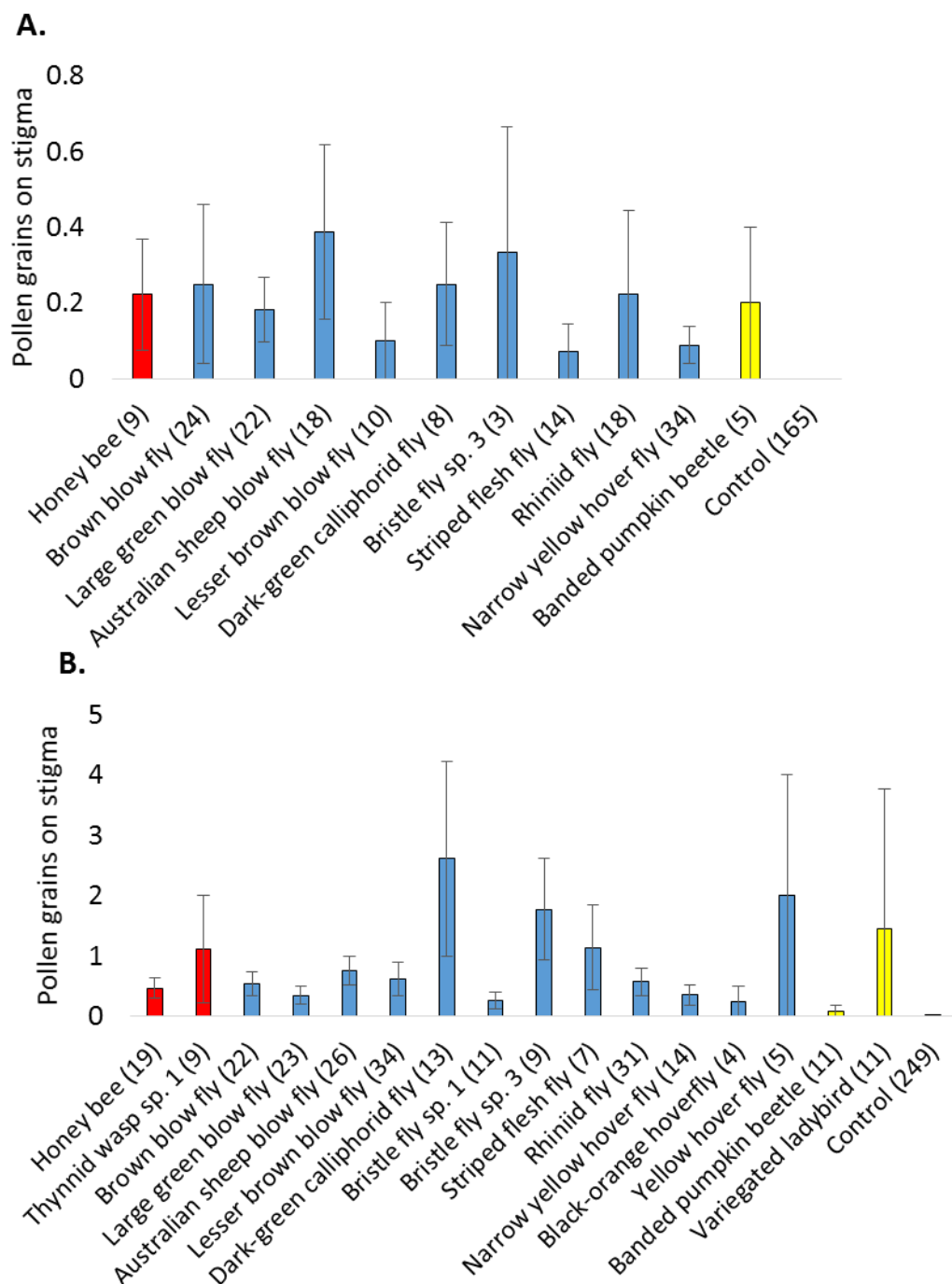


Figure 4.2.1. Pollen grains deposited on test female phase flowers following visits by different flower visiting species moving from A. female phase flowers and B. male phase flowers. The x-axis are insect species (number of individual assessed). Bar colours indicated taxonomic groups: red = Bees and Wasps (Hymenoptera), blue = Flies (Diptera), yellow = Beetles (Coleoptera).

In terms of the numbers of visit that successfully delivered pollen to stigmas, the majority of visits did not result in pollen delivery (Figure 4.2.2 A & B). The one exception was the bristle fly sp. 3, that successfully delivered pollen 56% of the time when moving from male phase flowers to test flowers. Individual numbers sampled of many species was low (in some cases less than ten), and therefore it is difficult to compare the relative efficiency of species regarding pollen transfer. Overall, the various

pollinating species delivered pollen at a higher percentage to the stigmas of test flowers when moving from male phase flowers (Figure 4.2.2 A & B).

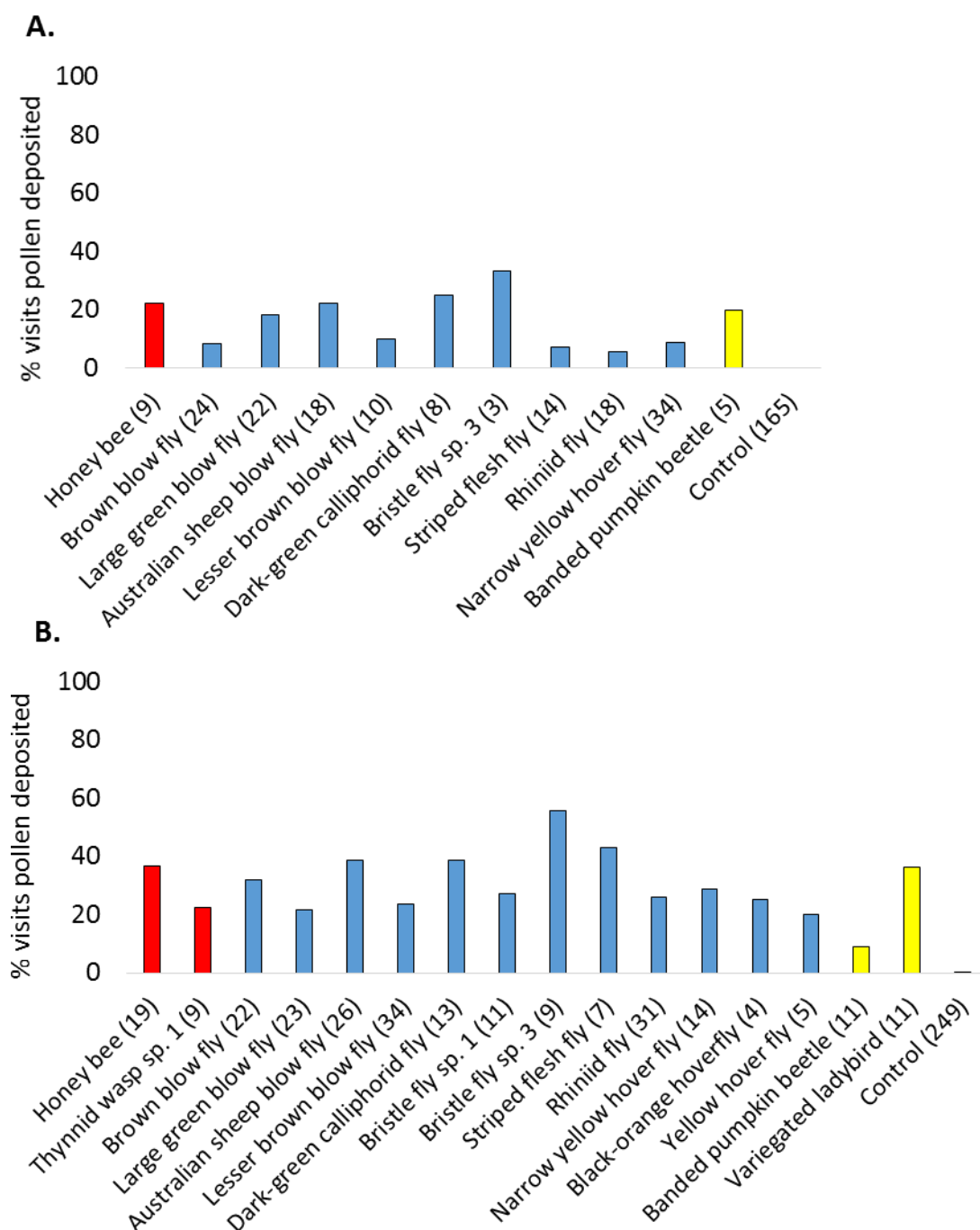


Figure 4.2.2. The percentage of visits by different flower visiting species that successfully delivered one or more pollen grains to stigmas of test female phase flowers. Insects moved from A. female phase flowers and B. male phase flowers. The x-axis are insect species (number of individual assessed). Bar colours indicated taxonomic groups: red = Bees and Wasps (Hymenoptera), blue = Flies (Diptera), yellow = Beetles (Coleoptera).

Pollinator movement

A total of 373 audio recordings noting the movement and behaviour of pollinating insects were conducted across two seasons and eleven farms. In 2014, recordings were conducted from three orchards near Mildura while in 2015, these were conducted across seven orchards located near Mildura and four orchards near Robinvale. Recordings totalling 7.14 h were conducted in 2014 and 19.03 h in 2015. All recordings were conducted between 8.15 am and 6.15 pm. Temperatures varied from 12.1 to 38.0°C, light intensity (towards sun) from 106 to 1280 W/m², humidity from 19 to 66%, and wind from 0 to 16 kmh. Dipteran and honey bee flower visitors were the most frequent insects observed. Table 4.2.2 outlines the numbers of individuals of each species observed, the flowering phase (male, female or together) and the total amount of time observing.

Table 4.2.2. The number of individual insect pollinators observed on avocado trees in different flowering phases, and the total observation time recording movements and behaviour.

Insects	Tree flowering phase				Total mins.
	Observed	Female	Male	Both	
Honey bee	64	10	39	15	210.39
Thyniid wasp sp. 1	21	4	6	11	47.05
Ichneumonid wasps	2	1	1	0	10.38
Meat ant	3	2	1	0	17.73
Unidentified hymenoptera	1	1	0	0	2.94
Brown blow fly	37	14	17	6	153.40
Lesser brown blow fly	27	8	18	1	83.30
Large green blow fly	25	2	21	2	120.20
European green blow fly	24	5	18	1	83.35
Dark-green calliphorid fly	17	8	6	3	150.43
Blue blow fly	2	1	1	0	13.86
Bristle fly sp. 1	4	3	1	0	9.52
Bristle fly sp. 2	1	1	0	0	0.35
Bristle fly sp. 3	6	2	2	2	41.60
Unidentified bristle fly	2	1	1	0	4.62
Striped flesh fly	11	4	4	3	25.53
Rhiniid fly	22	7	9	6	74.36
Nose fly	12	2	10	0	38.26
Narrow yellow hoverfly	58	24	26	8	239.91
Black-orange hover fly	7	1	3	3	17.23
Unidentified hover fly	1	0	0	1	1.07
Unidentified hover fly sp.2	1	0	0	1	8.49
Transverse ladybird	13	4	8	1	107.96
Unidentified ladybird	8	3	5	0	81.39
Red shouldered leaf beetle	2	0	1	1	8.45
Lycid beetle	2	1	1	0	18.72

Proportion of time visiting open flowers

All of the pollinating species were observed visiting buds and leaves as well as open flowers (Figure 4.2.3). Honey bees almost exclusively visited flowers and buds while the other species spent some time visiting leaves and stems. Just the wasp (Hymenopter asp. 1) and narrow yellow hover flies spent a higher proportion of time visiting open flowers than honey bees, although some of the fly species – dark green calliphorid flies, rhiniid flies, Australian sheep blow flies and large green blow flies – spent a similar proportion of their time visiting flowers.

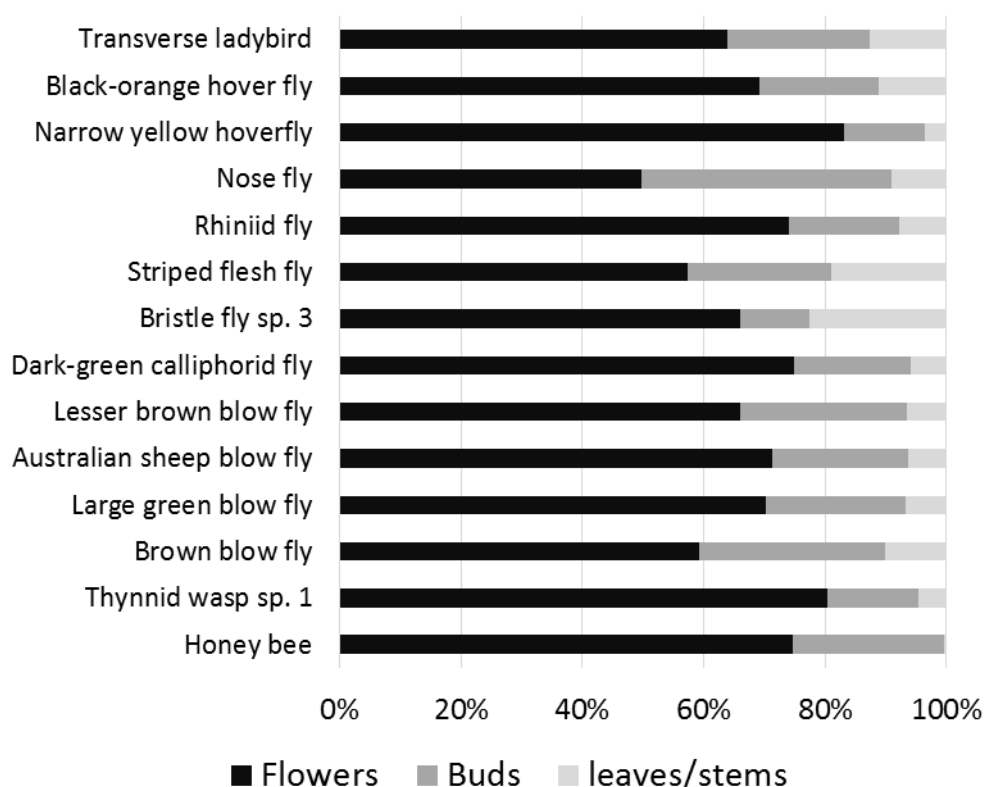


Figure 4.2.3. Proportion of time spent visiting open flowers, buds, leaves and stems within avocado trees by flower-visiting insects.

Flower visitation rate

Honey bees visited more flowers per minute on average than other flower visiting species, although Thynnid sp. 1, larger blow flies (brown blow fly, lesser brown blow fly) and bristle fly sp. 3 also visited more flowers per minute than other species (Figure 4.2.4).

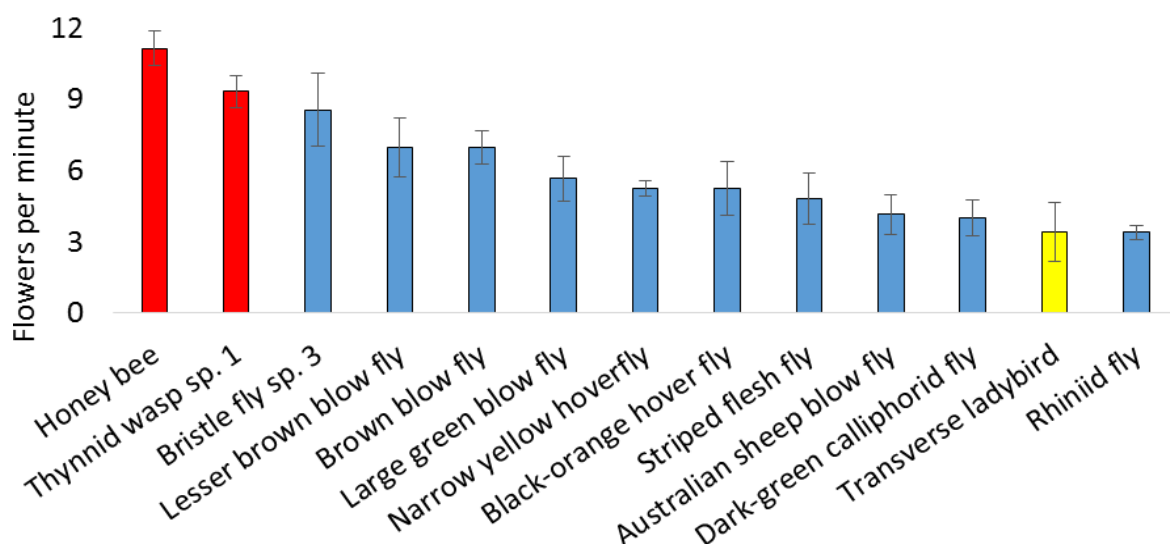


Figure 4.2.4. Mean numbers (\pm Std. Err.) of flowers visited per minute by flower visiting species. The data includes trees in all flowering phases (female flowers, male flowers and trees with both female and male at the same time). Bar colours indicated taxonomic groups: red = Bees and Wasps (Hymenoptera), blue = Flies (Diptera), yellow = Beetles (Coleoptera).

Rhiniid flies visited flowers in male flowering phase at a marginally faster rate than flowers in female flowering phase trees. In contrast, brown blow flies visited flowers in female flowering phase trees at a faster rate than flowers in male flowering phase trees. There was no evidence for the other insects of differences in their visitation rates across female flowering phase, male flowering phase and the phase when both male and female flowers were present (Figure 4.2.5).

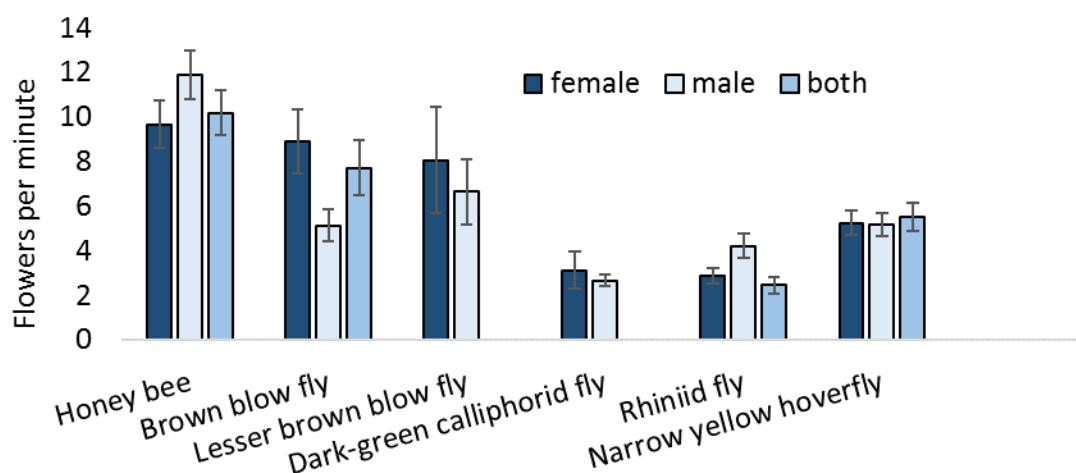


Figure 4.2.5. Mean numbers (\pm Std. Err.) of flowers visited per minute by flower visiting species to female flowering phase trees, male flowering phase trees and trees with both male and female flowers open together within the tree.

Estimated efficiency of different pollinating species

Our calculations of the rate of pollen delivery to stigmas when 'Hass' avocado trees are presenting both open male and female flowers shows bristle flies and honey bees to be most efficient. They deliver more pollen to the stigmas of female flowers per minute than other flower visiting species.

However, a wide range of flies and the transverse lady beetle are also important pollinators (Figure 4.2.6).

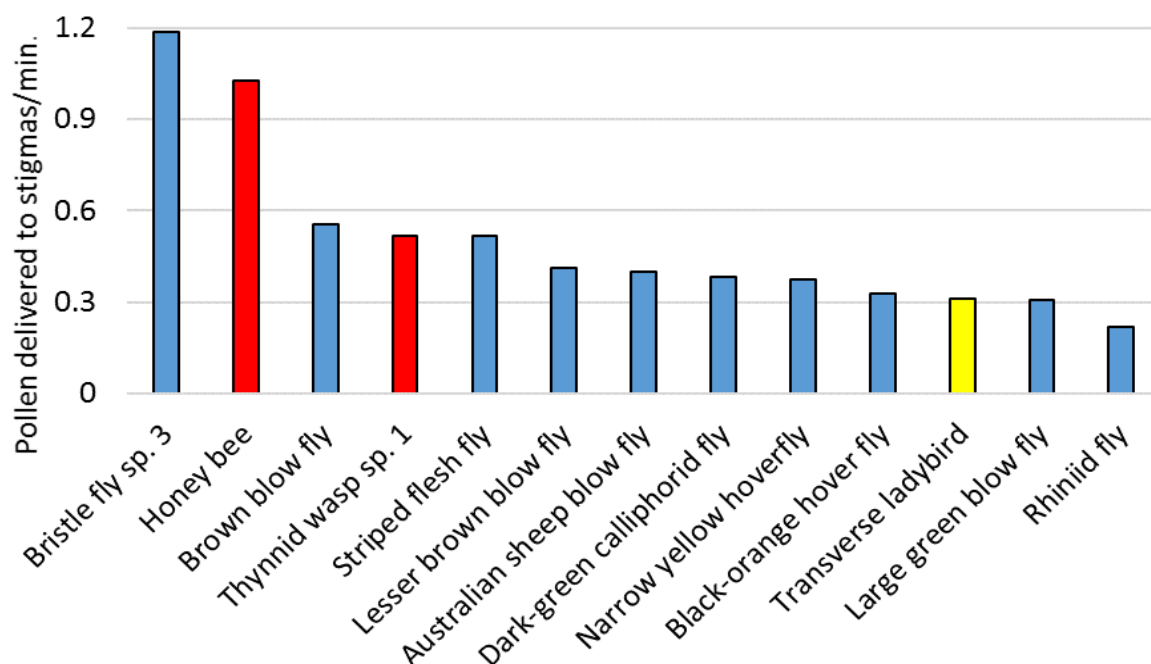


Figure 4.2.6. Estimate of the efficiency of various pollinators visiting tri-state avocado orchards during the period when both male and female flowers are open on trees. Bar colours indicated taxonomic groups: red = Bees and Wasps (Hymenoptera), blue = Flies (Diptera), yellow = Beetles (Coleoptera).

4.2.4 Discussion

To our knowledge, this study of Tri-State avocado orchards has evaluated and quantified the pollination efficiency of the broadest range of flower visiting species to date. For avocado, wild pollinating species can be of particular benefit for pollination due to the nature of its floral reproductive system. The floral biology of atypically provides limited and variable opportunities for pollination to occur. In the right conditions there can be a brief period (~1 h) of overlap where both male (with pollen being released) and female flowers are open on the same plant, allowing pollen to be transferred by insects from male to female flowers. The timing of flower opening is highly dependent on weather conditions, particularly overnight temperatures, which cause female flowers to open at different times during the day or night. Plant & Food Research has successfully modelled the relationship between female flower opening and overnight temperatures. The high variability in overnight temperatures experienced by orchard growers in the Tri-State region increases the risk of pollination not occurring if pollinators are inactive when the female flowers are open. The presence of multiple pollinating species that are active at different times and under different weather conditions (Howlett et al. 2013; Rader et al. 2013) can ensure pollination occurs under the highly variable weather conditions experienced in the Tri-State region.

Pollen deposition onto stigmas by different flower visiting insects

A number of common flower visiting species were found capable of transferring pollen to the stigmas of avocado. Many of the species assessed transferred similar or more pollen grains to test flowers when moving from male phase flowers than when moving from other female phase flowers. These were the wasp species (Thynnid wasp sp. 1), brown blow flies, Australian sheep blow flies, lesser brown blow flies, dark green calliphorid flies, bristle flies sp. 3, striped flesh flies, rhiniid flies, yellow

hover flies and transverse ladybird beetles. Many of these species also transferred pollen to test flowers when moving from flowers in the female phase, but pollen counts were low and infrequent making it difficult to compare with accuracy among species. Female flowers do not release pollen. Thus, pollen transferred by insects on these occasions is most likely to have originated from their earlier visits to male-phase flowers, either newly opened 'Hass' male flowers or male flowers on a different cultivar. Alternatively, pollen that had previously been moved to other floral parts by insects may have inadvertently been picked up on the bodies of these insects and transferred to test flowers.

We also considered the proportion of visited flowers that successfully resulted in pollen deposition on stigmas. When moving from male phase flowers, the proportion of visits leading to successful pollen deposition on stigmas was fairly similar across many of the species tested. The bristle fly sp. 3 was the only species that had a success rate higher than 50%. Honey bees were similar to the broad range of fly species that were assessed along with the transverse ladybird beetle. Banded pumpkin beetles had the lowest transfer success of all species test. With regards to movements from female phase flowers, successful pollen deposition to stigmas was less than 25% (with exception of bristle fly sp. 3 but only three slides were collected). For most species, a low proportion of samples showing pollen deposition (often just one or two) hinder accurate assessments of pollen transfer from female to female flowers. These data demonstrate that a broad range of pollinating species can still transfer pollen when moving between female flowers, meaning that a single visit to a male flower from either another 'Hass' or a different cultivar can result in the pollination of more than one female 'Hass' flower.

Pollinator movement

A factor that can reduce pollinator efficiency are behaviours that limit direct movements between flowers. These include visiting non-flowering plant structures such as buds, leaves, stems. All of the pollinating species assessed visited non-flowering structures. Honey bees visited a relatively high proportion of flowers compared to most other pollinators; however, they also occasionally visited buds (or closed flowers). The reason as to why they (and other pollinating species) visited these structures was not examined. All other flower visitors differed from honey bees in that they visited higher proportion of leaves. Many of the fly species were observed grooming themselves or resting on these structures. Other species observed visiting a relatively high proportion of open flowers versus non-flowering plant structures were the wasp (Hymenoptera sp. 1) and narrow yellow hover flies.

Honey bees were found to visit the most flowers per minute followed by Hymenoptera sp. 1. Although slower, a number of fly species visited flowers at or above half the rate of honey bees. These included bristle flies sp. 3, brown blow flies and lesser brown blow flies. We also found some differences in the visitation rate of flowers depending on the phase of the flower. For example, honey bees visited male flowers at a faster rate than female flowers, averaging about two extra flowers per minute. In contrast, brown blow flies visited female flowers at a rate more than three flowers per minute faster than male flowers. The differences in visitation rate were not explored but factors might include different responses to varying olfactory and visual cues and rewards; for example, differing amounts or attractiveness of nectar and pollen resources. Another factor could be weather. For brown blow flies, the mean temperature across recordings of their interaction with female flowers was 27.7°C compared with 20.7°C for male flowers. For honey bees it was 24.3°C for female flowers and 26.4°C for male flowers.

Pollinator efficiency

Using means of the proportion of visits that lead to pollen deposition on stigmas and rates of flower visitation per minute, we estimated that the Bristle fly sp. 3 was the most efficient species in delivering pollen to flowers. Honey bees were the second most efficient. A group of muscoid flies that include the blow flies, the stripped flesh fly and the dark green calliphorid fly, were between a third to just over half the efficiency of honey bees. Rhiniid flies were the least efficient being about one fifth as efficient

as honey bees. The species assessed are just a fraction of the insect species observed visiting avocado flowers and many of these species may also be very efficient. These findings greatly extend our knowledge on the range of avocado pollinating species, an area of research where there is little published information.

It is important to note that we used the mean proportion of successful pollen delivery to flowers and the rate of flowers visited per minute. The data have varying degrees of coarseness across the assessed insect species due to variable sample sizes. The calculations are based on the assumptions that insects do not discriminate between male and female phase flowers and that even numbers are presented by the plant. In addition, the calculation does not consider the possibility of pollen transfer by insects moving from female to female flowers, although we do show this occurs.

We have also not considered the rate of pollinator movement between plants of different cultivars, which is how cross pollination occurs. Cross pollination between cultivars is recognised as a key contributor to avocado yields for two reasons. Firstly, there is a greater period of time when polliniser cultivars are male simultaneously with 'Hass' female phase than when 'Hass' male and female phases overlap (although insect tend to move much less frequently between trees). Furthermore, it has been shown that cross-pollinated fruit are less likely to be dropped during periods of water stress than self-pollinated fruit (Degani & Goldring 1989). So the majority of the harvested crop can be due to pollination that occurs between cultivars. The species that we have studied here may vary in their propensity to move between trees or cultivars rather than within a tree or cultivar, so these behaviours could also have an important influence on their relative importance.

However, we consider that these data on self-pollination within 'Hass' trees provide a useful estimate of the relative potential pollen deposition rate of different flower visiting species. Our results highlight the potential value of maintaining or building numbers of these various species, particularly as these species may be active at different times and move pollen through differing visitation patterns.

Key points

- It has often been assumed that honey bees are key pollinators of avocado. To our knowledge, this study has assessed and compared the pollination efficiency of the broadest range of avocado flower visiting insect species.
- Our findings show that many insects are capable of transferring pollen to the stigmas of avocado. They include a range of fly species, predominantly but not exclusively blow flies and hover flies, beetles (lady bird beetles and the banded pumpkin beetle) a thynnid wasp species as well as honey bees.
- In general, honey bees visited more flowers per minute than other species; however, a number of the species still visited at around or more than half the rate of honey bees.
- Overall, the bristle fly sp. 3 and honey bees were deemed to be the most efficient pollinators, calculated to successfully deliver pollen to the highest number of stigmas per minute. A range of other species (nine flies, a beetle and a wasp) delivered pollen to stigmas ranging from 0.53 to 0.21 the rate of honey bee pollen deposition per minute.
- Strategies to encourage the presence a range of pollinating species within orchards including some of the species assessed here are likely to benefit avocado pollination. This includes increasing the likelihood of pollination occurring under variable weather conditions (different pollinators are active under different weather conditions) and through increased variation in pollen flow patterns between flowers corresponding to the differing flower visitation patterns between pollinating species.

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4.3 Abundance and distribution of avocado insect flower visitors in the Tri-State region

4.3.1 Introduction

Although wild pollinating insects are acknowledged as playing a significant role in the pollination of many crop species, the identity of pollinating species, along with their occurrence and abundance is often poorly documented. Outside of Australia, avocado flowers are visited by a range of species including honey bees, stingless bees, fly and wasp species (Perez-Balam et al. 2012; Ish-Am et al. 1999). In Australia, Queensland, Western Australia, Victoria and South Australia are the major production states. However, information on the identity of flower-visiting species across these regions is limited to a small number of localised studies. For example, a study near Toowoomba (Qld) recorded honey bees, syrphid flies and other flies visiting avocado flowers (Evans et al. 2011a) while at Coomealla (NSW), predominantly syrphid and calliphorid flies were noted (Vithanage 1986).

Despite visitation by wild insect species, managed honey bees have often been considered key pollinators in commercial avocado orchards with hives introduced to facilitate pollination (Dixon 2006; Perez-Balam et al. 2012; Ish-Am & Lahav 2011; Wysoki et al. 2002). However, honey bees may not be strongly attracted to avocado flowers with studies indicating that nectar compounds or concentrations of particular minerals are repellent to bees (Afik et al. 2006; Afik et al. 2007). The lack of attraction of honey bees to avocado has been considered a key reason for highly variable visitation rates within orchards (Ish-Am & Eisikowitch 1998).

Broad-scale studies have indicated that it is usually a small number of common and widespread insect species that contribute the bulk of crop pollination (Kleijn et al. 2015). However, on-farm management practices such as the use of pesticides during the flowering period can impact pollinator abundance at a localised level (Williams & Kremen 2007). For wild pollinators in particular, the presence of native habitat near to crops not only increases pollinator diversity (Chacoff & Aizen 2006; Senbeta & Denich 2006) but also results in increased stability of pollination services (Garibaldi et al. 2011) and can improve crop yield (Klein et al. 2012; Vergara & Badano 2009). Weather conditions and insect diurnal activities can also influence the abundance of different flower-visiting species that are present (Howlett et al. 2013; Rader et al. 2013). For avocado, these factors that influence spatial, temporal and diurnal (and likely nocturnal) variation in pollinator abundances, coupled with the variable flower opening times of avocado, Evans et al. (2011a) suggest the need to have multiple pollinating species present to maximise pollination.

In this study we examine the abundance and distribution of flower-visiting species within and between 26 'Hass' avocado orchards in the Tri-State region, encompassing orchards along approximately 300 km of the Murray River. Orchards were within 50 km of four locations: Waikerie (South Australia), Renmark (South Australia); Mildura (Victoria and New South Wales) and Robinvale (Victoria). We examine whether pollinator communities across the region consist of the same or similar taxa by assessing uniformity across:

- Orchards located within and between the four locations
- 'Hass' trees when in male and female flowering phases
- 'Hass' versus pollenizers trees
- Orchard flowering intensity and pollinator abundance.

We also assess the range of weather variables (temperature, humidity, light intensity and wind speed) that common pollinating species were active under. We also estimate the effectiveness of those species within regions and orchards for those species where their pollination efficiency has been

calculated (Section 4.2). We then discuss the role of specific wild pollinator species and honey bees in the pollination of orchards across the Tri-State region.

4.3.2 Materials and methods

Preparation of a reference collection

Flower-visiting insects were opportunistically collected from flowering avocado orchards during 2014 and 2015. The methods of collection were the same as those for *Macadamia* (Section 3.3) with flower visitors collected directly from flowers either by a specimen container or a sweep net. In 2014, specimens were collected from three orchards located in Mildura. In 2015, a much broader collection was made as orchards were surveyed across the four locations (Mildura, Robinvale, Renark and Waikerie). Identification of specimens is currently ongoing and the collection is currently lodged at the University of New England (Armidale, NSW).

Insect flower visitor surveys

Selection of orchards

Surveys were conducted from 8 to 28 October 2015, in fine weather (sun or light cloud) in South Australia (Renmark and Waikerie), New South Wales (Near Mildura) and Victoria (Mildura and Robinvale), within 30 km of the Murray River. Five orchards were surveyed near Waikerie (34.19°S, 139.98°E) five orchards near Renmark (34.16°S, 140.80°E), eleven orchards near Mildura (34.14°S, 141.98°E) and five orchards near Robinvale (34.57°S, 142.77°E). Most orchards were surveyed on a single day, but six orchards were surveyed twice on two separate days.

Survey design

The survey design contained nine observation trees per orchard for orchards without pollinisers. Where pollinisers were present, three were additionally surveyed. Three observation trees were marked to form a set of trees. Two were located in the same row but separated by a single tree with third marked on an additional row but in a position adjacent to the unmarked centre tree. The first set of three trees were located in a corner of the block, while another set of three in the opposite corner. The other set of trees were located within the centre of orchard to form a diagonal across the orchard (Figure 4.3.1). When present, each polliniser tree selected for observation was located as close to each set of three fruiting trees as possible.

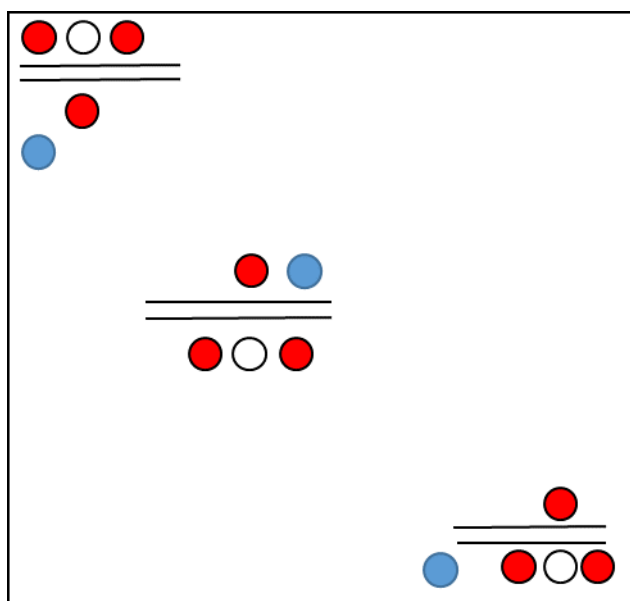


Figure 4.3.1. Flower visitor survey design indicating pattern of trees observed per orchard. Observations were conducted on nine ‘Hass’ trees (red circles) and where present, polliniser trees (blue circle). Double lines between circles represent the inter-row space.

Survey method

For each survey, trees were observed three times during the day beginning at 9am, 12pm, and 3pm. The flowering phase of the trees were also recorded. During each survey a 1.5 m long measuring pole was held with the base at a vertical height of 0.5 m above the ground (determined by an attached tape). This was used to restrict insects counts on flowers to a specified area of the canopy. The observer then counted flower-visiting insects around the entire circumference of each tree on flowers located from the outer canopy to the trunk of the tree. This was possible due to the open nature of the canopy and the tendency for inflorescences to be located nearer to the outer canopy of the tree than buried in foliage within the tree. Care was taken to minimise brushing the foliage or inflorescences with the pole. Where a flower-visiting species was particularly abundant, a hand-held counter was used to record numbers. Insects were recorded on a paper spreadsheet to species level if possible; however, where flower visitor abundances were particularly high, counts were recorded onto an audio recorder and later transcribed directly into Excel spreadsheets. If the species were unfamiliar to the observer, an attempt was made to capture the specimen for identification purposes. Based on the flower-visiting insect species collected across several orchards in 2014 and their subsequent examination, it was predicted that the most abundant species observed would be fly species, particularly from the families Calliphoridae, Syrphidae, Tachinidae, Muscidae as well as beetles from the Coccinellidae. Flower-visiting insects collected in 2014 formed the basis for developing a spreadsheet containing likely flower visitors for the 2015 surveys.

Flowering Intensity

Flower visitor surveys were coupled with flowering intensity count surveys that were conducted on the same day and on the same trees. Two separate counts for each tree were conducted during the day, one when trees were in their female flowering phase and the other during their male phase. Counts were conducted on four sides of the tree. These were the two opposite sides of the tree facing directly into the rows and the two opposite sides within the row. Thus, the four survey points were separated from each other by 90°. To conduct counts, a 75 x 75 cm quadrat was placed at a height of 1.5 m at each survey point of the tree. A piece of tape attached the quadrat assisted in determining the correct height. All inflorescences (budding, flowering, completed flowering) were counted within the area of the quadrat to the tree trunk. Three inflorescences were then selected within the quadrat. These were: visually closest to the upper left hand corner, closest to the centre and closest to the lower right hand corner. To avoid sampling only inflorescences nearest to the quadrat (that was placed on the outer canopy), we chose the inflorescences that were deemed to be, closest to our quadrat points based on

our field of view. Therefore, inflorescences close to the trunk could be selected if they were visually closest to the quadrat points.

Once inflorescences within the quadrat were determined, they were marked with tape and the number of open flowers counted and their phase noted. During the second survey, open flower counts were conducted on the same inflorescences.

Effectiveness of abundant pollinating species

We calculate the effectiveness of thirteen of the more abundant wild pollinating species and compare them with honey bees. We use:

1. Pollinator efficiency data (calculated as pollen delivered to stigmas per minute) that was calculated in Section 5 for honey bees, thynnid wasps, brown blow flies, lesser brown blow flies, large green blow flies, Australian sheep blow flies, dark green calliphorid flies, rhiniid flies, bristle flies (sp. 3), striped flesh flies, narrow yellow hoverflies, black-orange hoverflies and transverse ladybird beetles.
2. Counts of each species across nine 'Hass' trees in each orchard. We select one of the three survey data sets from each orchard survey day (surveys conducted during morning, midday and evening). A key selection requirement is the survey having been conducted during the female phase, prior to the male phase flowers opening. In some orchards, two of three data sets collected were during the female flowering phase, therefore we select the set that we considered closest to the time male flowers were predicted to occur. We only use female phase survey data because pollination can only occur at this time, and it represents the insects that are most likely to be present during the short period of phase overlap when both male and female flowers appear together (this is the typical progression from female to male phase flowering).

To calculate pollinator effectiveness, we determine the mean number of individuals per tree within each orchard. The mean is calculated from the nine surveyed trees within each orchard. We then use the mean time taken to survey each tree to then calculate the numbers of each insect species observed on each tree per minute. We then combine the pollinator efficiency data to determine the amount of pollen delivered to stigmas per tree by each pollinator species.

4.3.3 Results

Avocado reference collection

A reference collection of flower-visiting insect species has been developed to assist in the identification of key pollinating species of avocado in the Tri-State region (the border between South Australia, Victoria and New South Wales). Flower visitors were collected opportunistically on insect survey days from orchards located near Robinvale, Mildura and Waikerie. The aim of the collection was to identify flower visitors to species level where possible, train staff and transfer knowledge to growers of the key flower-visiting species. Identified insects are listed below.

Diptera

Syrphidae: five morphospecies, eight specimens

Eristalis punctulatus

Melangyna viridiceps

Simosyrphus grandicornis

Calliphoridae: seven morphospecies, 23 specimens

Calliphora stygia

Calliphora augur

Lucilia cuprina

Tachinidae: seven morphospecies, 11 specimens

Stratiomyidae: one morphospecies, two specimens

Sarcophagidae: two morphospecies, two specimens

Rhiniidae: two morphospecies, nine specimens

Stomorhina discolor

Anthomyiidae: one morphospecies, one specimen

Anthomyia punctipennis

Lauxanidae:

Sapromyza sp. (two species)

Hymenoptera (bees and wasps)

Thynnidae sp. 1

11 morphospecies, 12 specimens

Hemiptera

two morphospecies, six specimens

Coleoptera

five morphospecies, 14 specimens

Epilachna vigintioctopunctata

Hippodamia variegata

Harmonia testudinaria

Aulacophora hilaris

Lepidoptera

one morphospecies, one specimen

Flower visitor surveys

Flower visitors

All orchards were surveyed in 2015. A total of 12,683 flower-visiting insects across eight Orders were observed across all orchards in the Tri-State region. The mean number of flower-visiting individuals observed per survey day in each location were Mildura were 562.5 (13 survey days; 11 orchards);

Robinvale 551.2 (6 survey days, 6 orchards); Waikerie 339.4 (5 survey days, 5 orchards) and Renmark 40.7 (9 survey days; 5 orchards). The three most common insect orders were Diptera, Coleoptera and Hymenoptera (Figure 4.3.2) representing 99.3% of all flower visitors.

Honey bees were observed less frequently on flowers than wild species at all locations. They represented just 4.1% of visitors in orchards around Mildura, 7.5% in Robinvale and 20.4% in Waikerie orchards and 0.14% in Renmark.

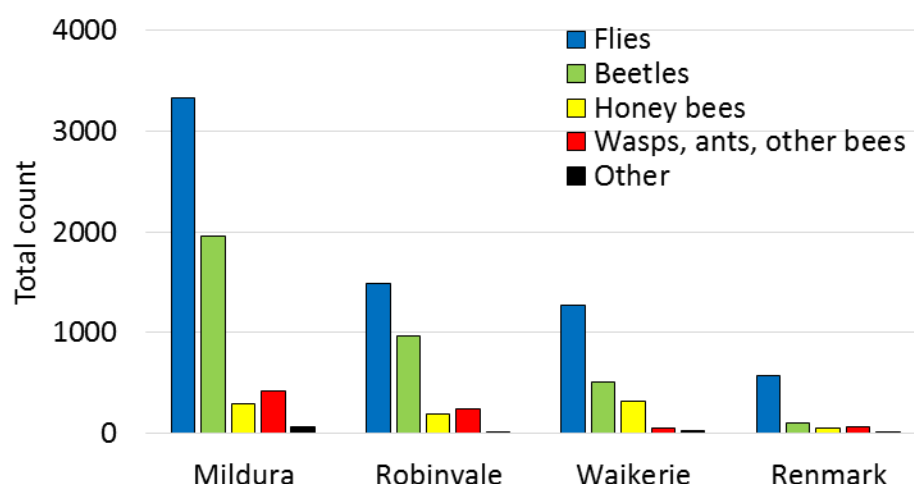


Figure 4.3.2. Total count of insects within orchards across five locations. Mildura orchards surveyed (s)= 11, Number of surveys (n) = 13; Renmark s = 5, n = 9; Robinvale s = 6, n = 6; Waikerie s = 5, n = 5.

Flower visitor abundances varied greatly across the 11 orchards surveyed near Mildura (Figure 4.3.3). Flies were the most abundant flower visitor counted in 9 of the 13 survey days while beetles were most abundant in two. Honey bee abundances on flowers exceeded 50 individuals on only four survey days in three separate orchards.

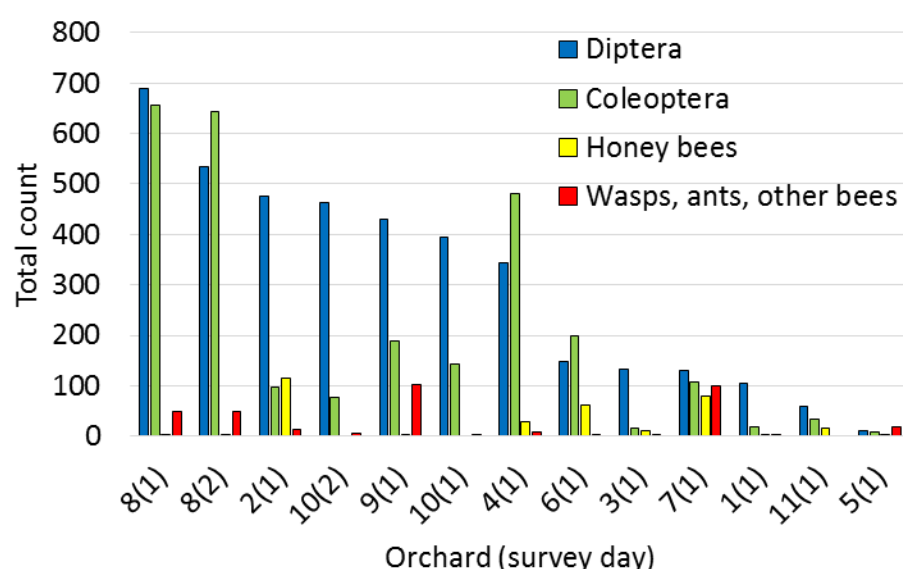


Figure 4.3.3. Abundances of avocado flower visitors across orchards near Mildura. Orchard number is presented on the x-axis (non-bracketed number). Bracketed numbers represent survey day. Orchards 8 and 10 were surveyed twice.

At orchards near Robinvale, flies were the most abundant flower visitor in three orchards (each orchard surveyed once), beetles in two and honey bees in one (Figure 4.3.3). Orchard six in Robinvale was one of only three orchards where honey bee counts on avocado flowers exceeded 100 (total count 105).

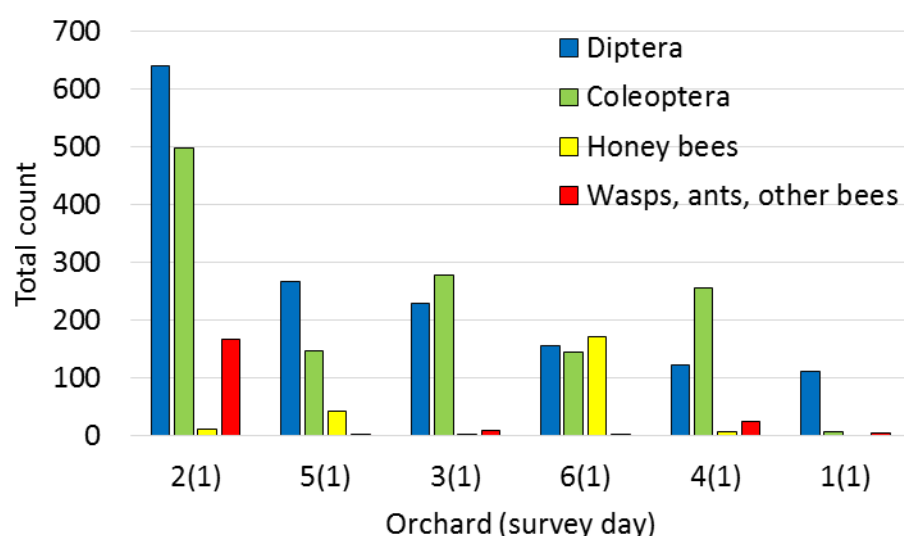


Figure 4.3.3. Abundances of avocado flower visitors across orchards near Robinvale. All orchards were surveyed once. Orchard number is presented on the x-axis (non-bracketed number). Bracketed numbers represent survey day.

At orchards near Waikerie, flies were the most abundant flower visitor counted in all orchards except orchard 5. Orchard 5 was the only one of two orchards across all 33 separate survey days where honey bee counts (54 counted) exceeded counts of flies or beetles; however, this orchard recorded the lowest number of flower visitors in the location (Figure 4.3.4).

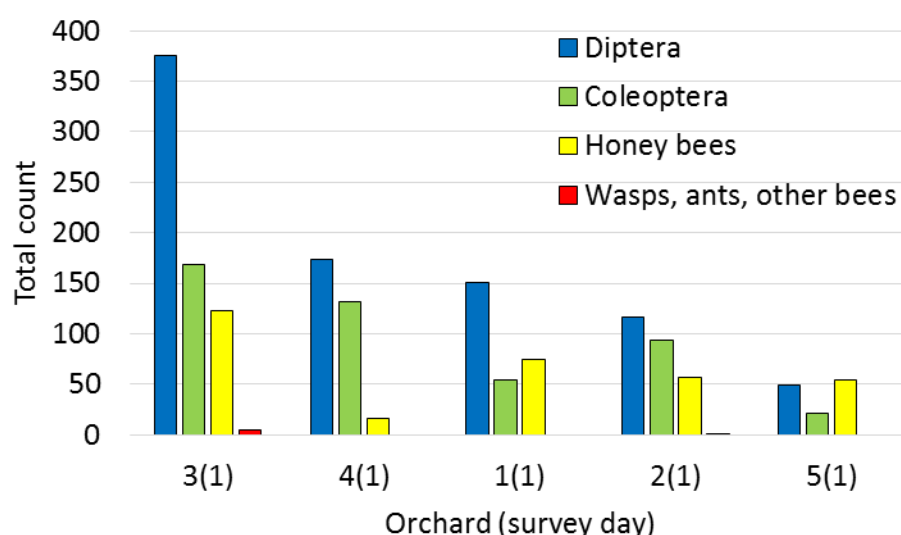


Figure 4.3.4. Abundances of avocado flower visitors across orchards near Waikerie. Orchard number is presented on the x-axis (non-bracketed number). Bracketed numbers represent survey day.

At orchards near Renmark, flies were by far the most abundant insects observed visiting avocado flowers across all orchards (Figure 4.3.5). Counts of flower visitors overall were lower than in other

regions and may reflect the lower flower density than the other districts at the time surveys were conducted.

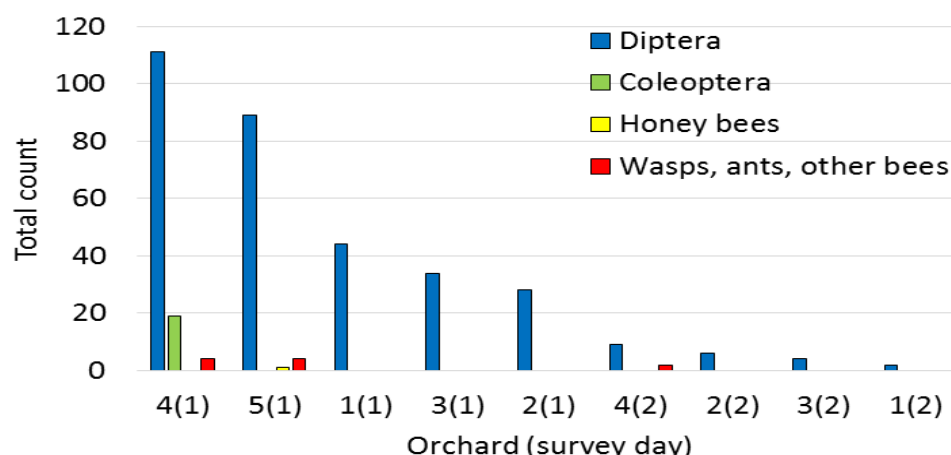


Figure 4.3.5. Abundances of avocado flower visitors across orchards near Renmark. Four of five orchards were surveyed twice. Orchard number is presented on the x-axis (non-bracketed number). Bracketed numbers represent survey day.

We identified 69 different flower visiting taxa. Species that we assessed for pollination efficiency were among the most common (Table 4.3.1). Overall there was a great deal of similarity in the flower visiting insects present between orchards across Mildura, Renmark, Robinvale and Waikerie with exception of a few species that were more locally abundant. For example, dark green calliphorid flies were abundant in Mildura and Robinvale but not in Renmark or Waikerie, while the two most common ladybird beetles (Variegated and Tranverse ladybirds) were uncommon in Renmark compared to other locations (Table 4.3.1).

Table 4.3.1. Total counts of the most common flower visitors that were observed visiting avocado flowers within orchards across four locations in the Tri-State region. Orange shaded insects are those with data available on their efficiency.

Insect	Location				Total
	Mildura	Robinvale	Waikerie	Renmark	
Transverse ladybird	1663	756	295	3	2717
Narrow yellow hover fly	1186	483	103	182	1954
Variegated ladybird	824	540	168	2	1534
Dark green calliphorid fly	843	407	9	0	1259
Honey bee	317	235	325	1	878
Rhiniid fly	412	94	29	11	546
Black-orange hover fly	105	191	201	45	542
Lesser brown blow fly	192	32	301	2	527
Brown blow fly	391	57	52	0	500
Meat ant	161	85		3	249
Large green blow fly	123	40	4	3	170
Australian sheep blow fly	75	74	2	3	154
Small striped muscid fly	102	26	14		142
Unidentified Ant	24	97			121
Thynnid wasp sp. 1	110	2			112
Striped flesh fly	68	19	19	1	107
Tephritid fruit fly	72	1	11	17	101
Nose fly	64	10	24		98
Bristle fly sp. 1	52	8	23	1	84
Unidentified bristle fly	62	6	4	1	73
Redshouldered leaf beetle	54	5		1	60
Spotted wing fly	39	2	13	4	58
Bristle fly sp. 3	26	20	6	4	56
Others	348	117	94	82	641
Total	7313	3307	1697	366	12683

Pollinator visitation of 'Hass' and pollenizers trees and male and female phase flowers

Of the 27 orchards surveyed, 17 had pollenizer trees present. These were observed flowering in the opposite phase to the 'Hass' trees during all the surveys that were conducted. However, at the 6 survey times where 'Hass' had both male and female phase flowers present, pollenizers were in the male phase. Despite 'Hass' and pollenizers being in opposite flowering phase, we did not see a large difference in the mean (\pm S.E.) pollinator counts per tree ('Hass' 48.6 ± 11.4 ; pollenizers 63.2 ± 11.4). Likewise, the average abundance on Hass and pollenizer trees showed a similar pattern across three locations (Surveyed Renmark orchards did not have pollenizers) despite often highly variable abundances within orchards (Figure 4.3.6).

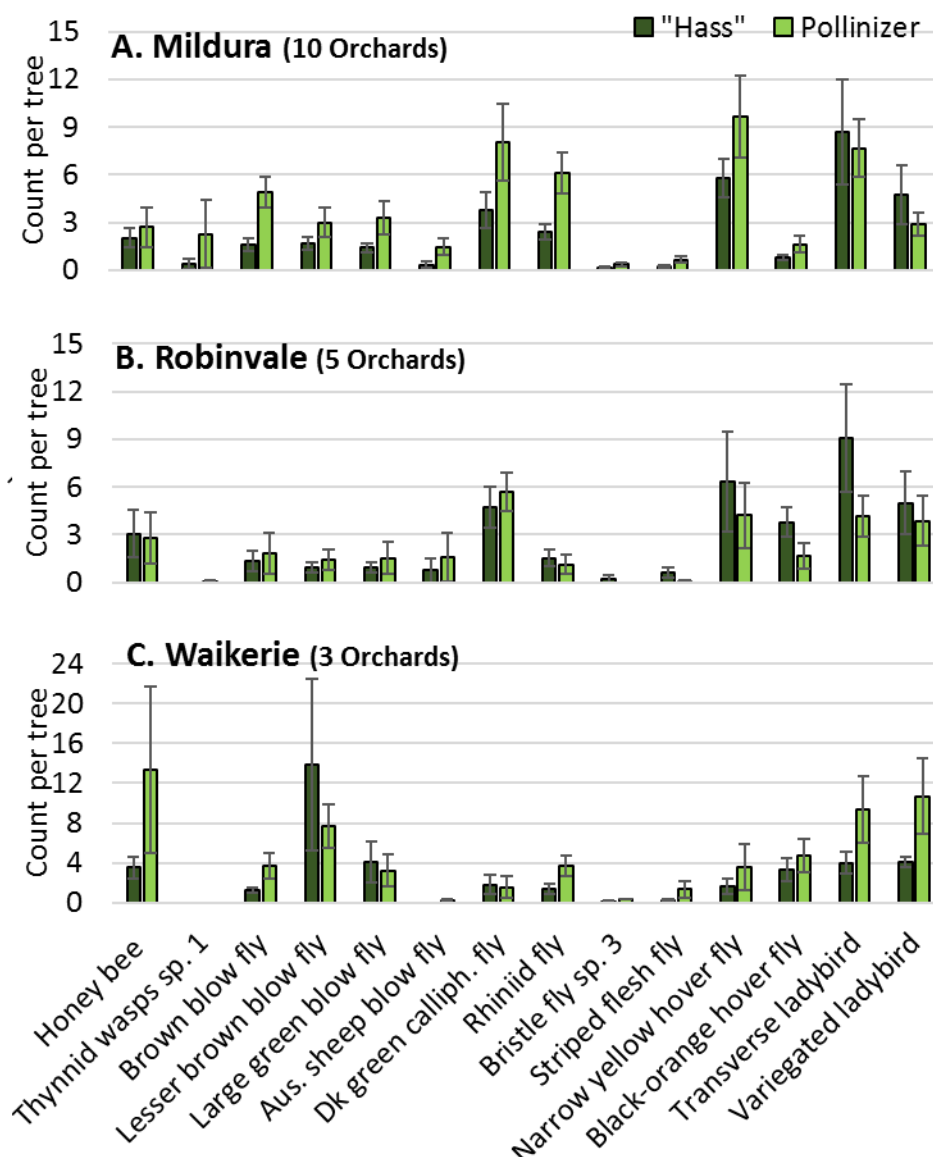


Figure 4.3.6. Average counts (\pm S. E.) of pollinators observed on 'Hass' and pollinizer trees across three locations.

Pollinators visiting 'Hass' at different flowering phases

The total number of surveys conducted while 'Hass' was flowering in female phase was 44 compared to 46 surveys where flowers were in male phase and 6 surveys where flowers were in mixed phase (male and female together). No flowers were open on six occasions and therefore counts were not obtained. For the female phase surveys, 18 were morning, 15 midday and 11 afternoon surveys. For male phase surveys there were 12 morning, 15 midday and 19 afternoon surveys. For the mixed phase four of the surveys were midday and two were afternoon surveys.

Insect abundances for each species observed on 'Hass' male and female phase flowers was similar for most species across regions (Figure 4.3.7). There were some examples where the mean insect counts per survey period were different. An example is the counts of the two ladybird beetles that were higher when trees were in the male flowering phase in both Mildura and Robinvale orchards. This pattern was not apparent in Waikerie or Renmark orchards.

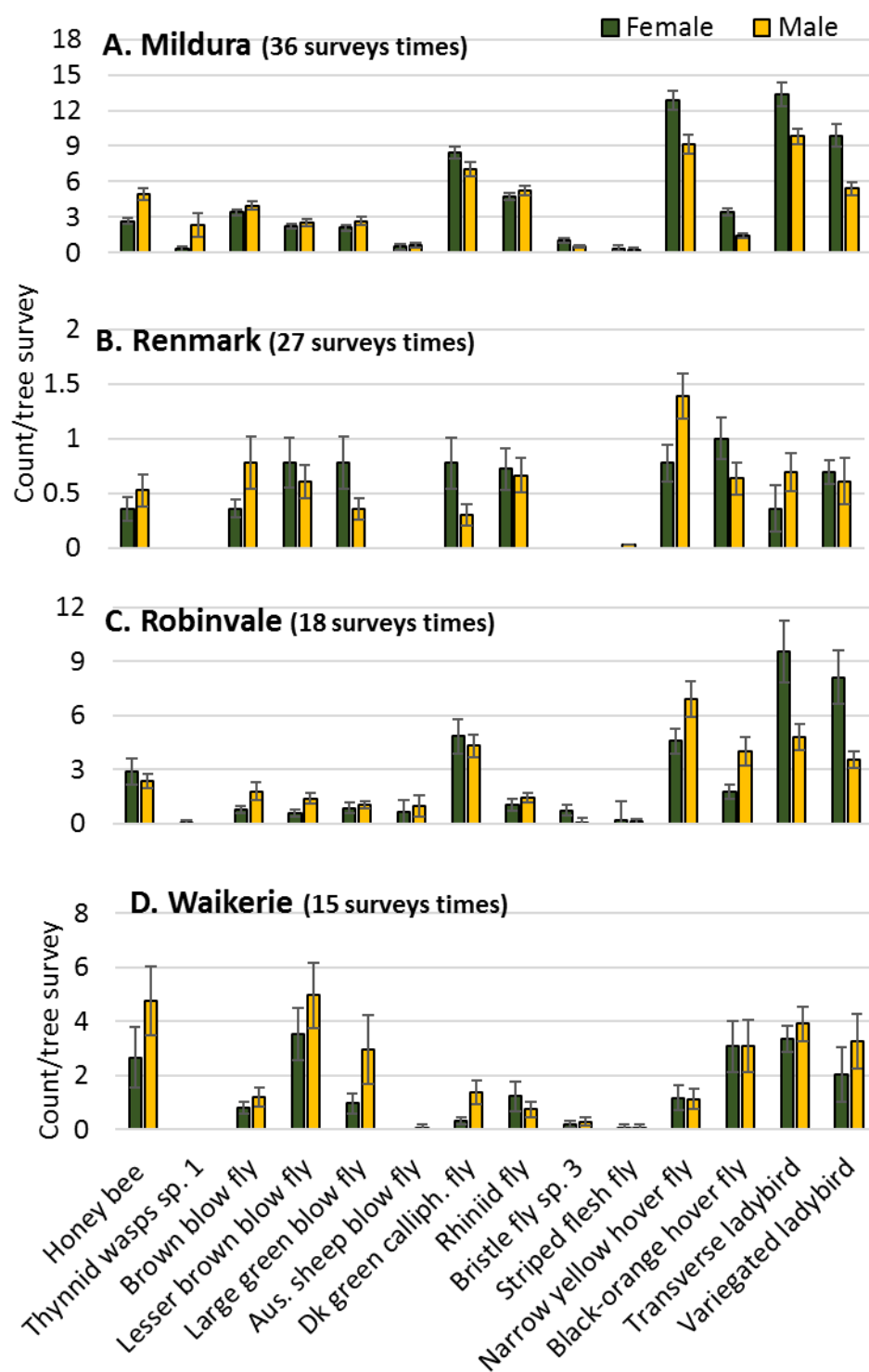


Figure 4.3.7. Average counts of pollinators visiting female and male phase flowers for each tree survey period (\pm S. E.) across four locations.

Weather and pollinator activity

Temperatures at survey times varied from 13.8°C to 36.1°C (av. 26.6°), Light intensity from 100 to 1150/W.m² (av. 754), humidity from 17 to 93% (av. 37.0%) and average wind speed from 0.0 to 10.5 km/h (av. 2.31 km/h). Table 4.3.2 shows the range of weather variability at which various insect species were observed actively visiting flowering racemes. These may not fully reflect the range of weather variability that these insects may be active under as the variability reflects the frequency of weather measures recorded across all surveys and the abundance of the specific taxa.

Table 4.3.2. Range of weather variability that flower visitors were observed visiting flowering macadamia racemes.

Insect	Observed	Temperature (°C)	Light Intensity (W/m ²)	Humidity (%)	Wind speed (km/h)
Honey bee	856	13.8 - 36.1	100 - 1150	17 - 62	0 - 10.5
Hymenoptera sp. 1	103	21 - 33.8	115 - 1100	21 - 0.69	0 - 6.2
Brown blow fly	506	13.8 - 36.1	100 - 1150	17 - 62	0 - 10.5
Large green blow fly	469	13.8 - 36.1	100 - 1150	17 - 62	0 - 10.5
Australian sheep blow fly	125	17.5 - 36.1	130 - 1100	21 - 46	0 - 10.1
Lesser brown blow fly	790	13.8 - 36.1	100 - 1150	17 - 62	0 - 10.5
Dark-green calliphorid fly	1065	13.8 - 36.1	100 - 1150	17 - 62	0 - 10.5
Bristle fly sp. 3	42	17.5 - 36.1	110 - 1100	21 - 46	0 - 8
Striped flesh fly	111	15 - 36.1	829 - 1005	21 - 62	0 - 10.1
Rhiniid fly	628	13.8 - 35.4	100 - 1150	17 - 62	0 - 10.5
Nose fly	79	15 - 36.1	115 - 1100	21 - 62	0 - 6.2
Narrow yellow hover fly	1525	13.8 - 35.4	100 - 1150	17 - 62	0 - 10.5
Black-orange hover fly	701	13.8 - 36.1	100 - 1150	17 - 62	0 - 10.5
Banded pumpkin beetle	49	18.6 - 34	100 - 1076	22 - 46	0 - 10.1
Transverse ladybird	1974	13.8 - 36.1	110 - 1120	17 - 62	0 - 10.5
Variegated ladybird	1365	13.8 - 36.1	100 - 1150	17 - 62	0 - 10.5

Flowering intensity and flower visitor abundance

The flowering intensity of orchards surveyed varied across the locations at the time of survey (Figure 4.3.8). The flowering intensity of orchards surveyed at Renmark were much lower than other locations. These orchards also had low numbers of flower visitors (Figure 4.3.8). The average numbers of flower visitors counted per orchard survey day was highest in Mildura and Robinvale that also corresponded with the highest flowering intensity on the days of survey (Figure 4.3.8).

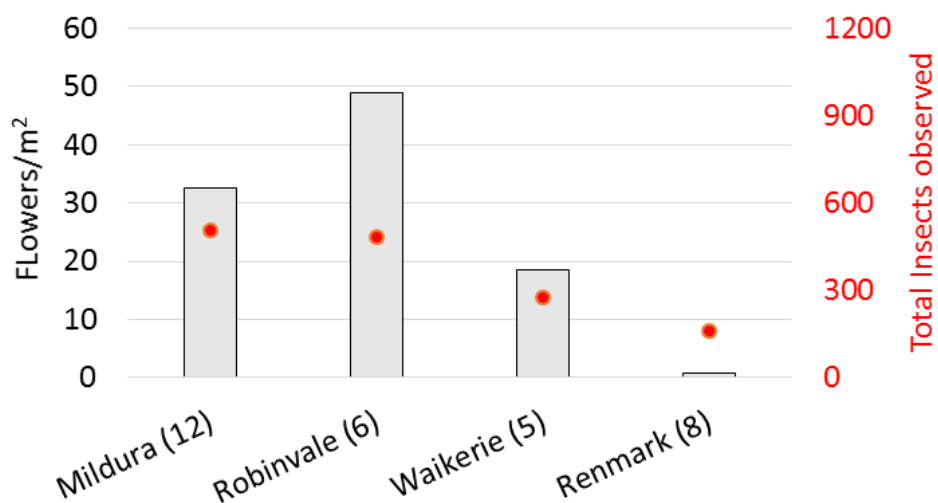


Figure 4.3.8. Average flowering intensity and total insect observed per orchards at four locations in the Tri-State region.

At the location level, orchards that had high flowering intensity also tended to have higher numbers of flower visitors (Figure 4.3.9). The relationship was particularly pronounced for Waikerie orchards (Figure 4.3.9D) but not for the Robinvale orchards (Figure 4.3.9C).

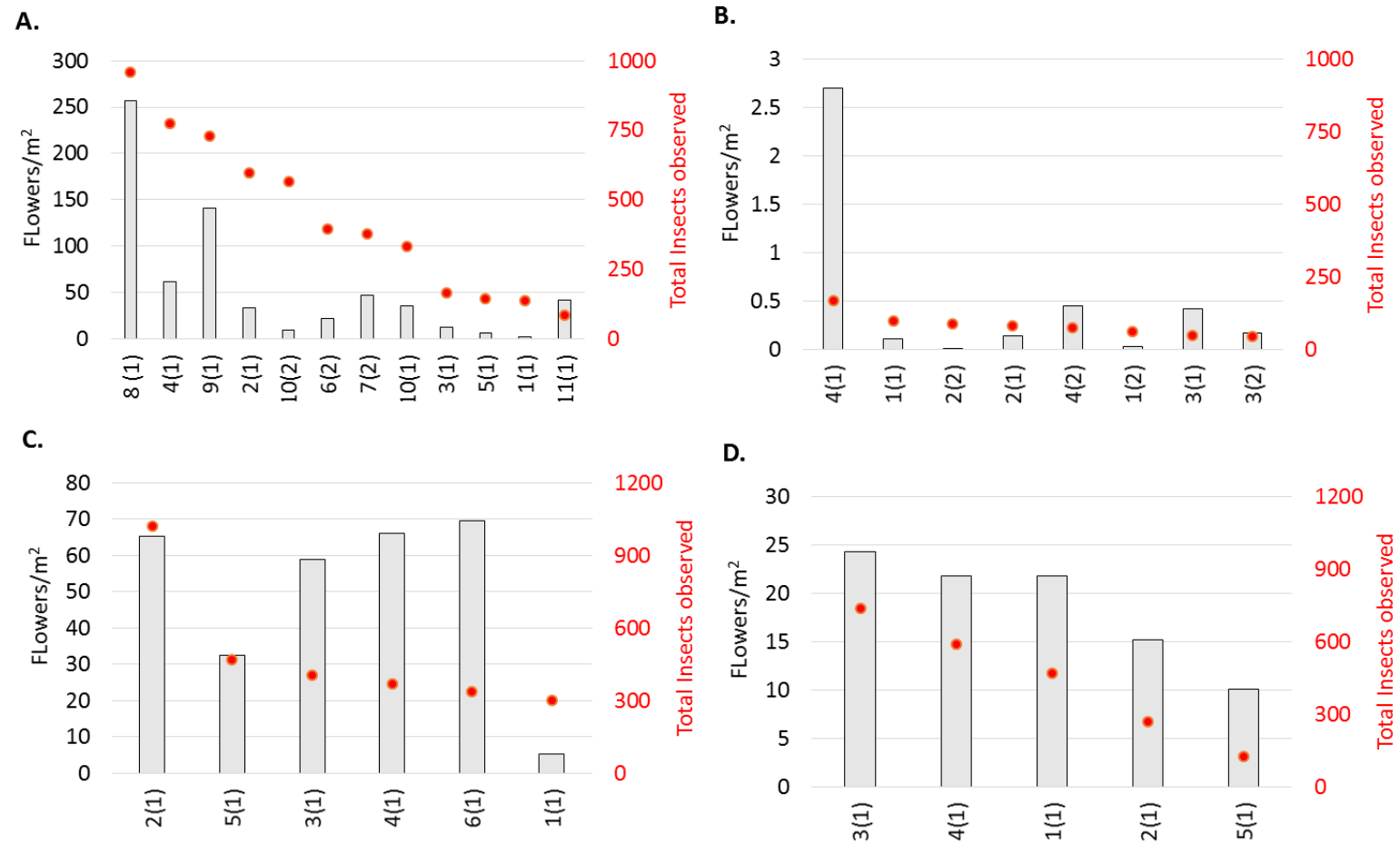


Figure 4.3.9. Flowering intensity and total insect observed per orchards within four locations in the Tri-State region. The x-axis are individual orchards with the survey day bracketed to account for orchards where additional survey days were completed.

Effectiveness of abundant pollinating species

Wild pollinators versus honey bees

Our calculation of pollinator effectiveness in the Tri-State found that in all four locations, wild pollinators are more effective pollinators than honey bees (Figure 4.3.10). Although honey bees were found to be among the most efficient pollinators of avocado, the much greater numbers of the 12 wild pollinator species assessed meant they were the most effective across orchards overall.

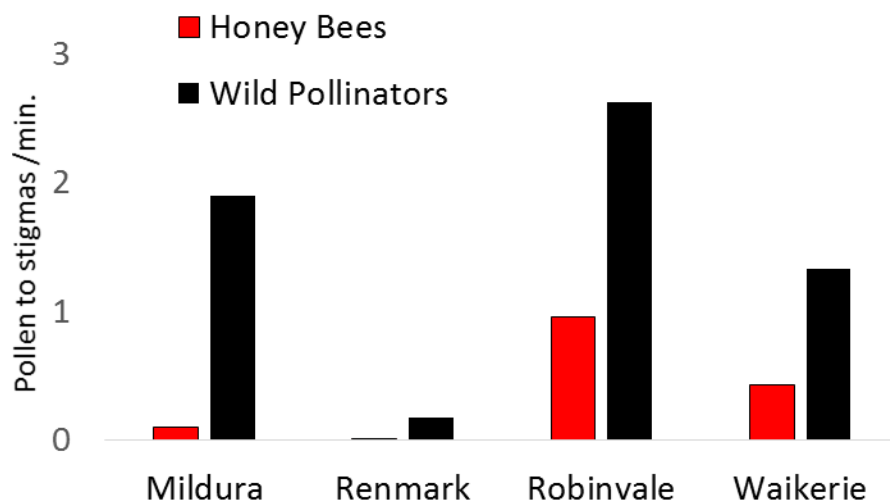


Figure 4.3.10. The effectiveness of honey bees and thirteen wild pollinating species combined across avocado orchards located in four locations in the Tri-State region. Values are estimated hourly rate of pollen deposition to stigmas within individual trees by honey bees and wild pollinators.

At the orchard level, there was much variation in pollinator effectiveness of both wild pollinators and honey bees both within and between locations. Pollination within several orchards were almost exclusive conducted by wild pollinators (Figure 4.3.11). In only three orchards was the pollinator effectiveness of honey bees greater than the wild pollinators assessed across the 30 survey days conducted across 24 orchards (Figure 4.3.11).

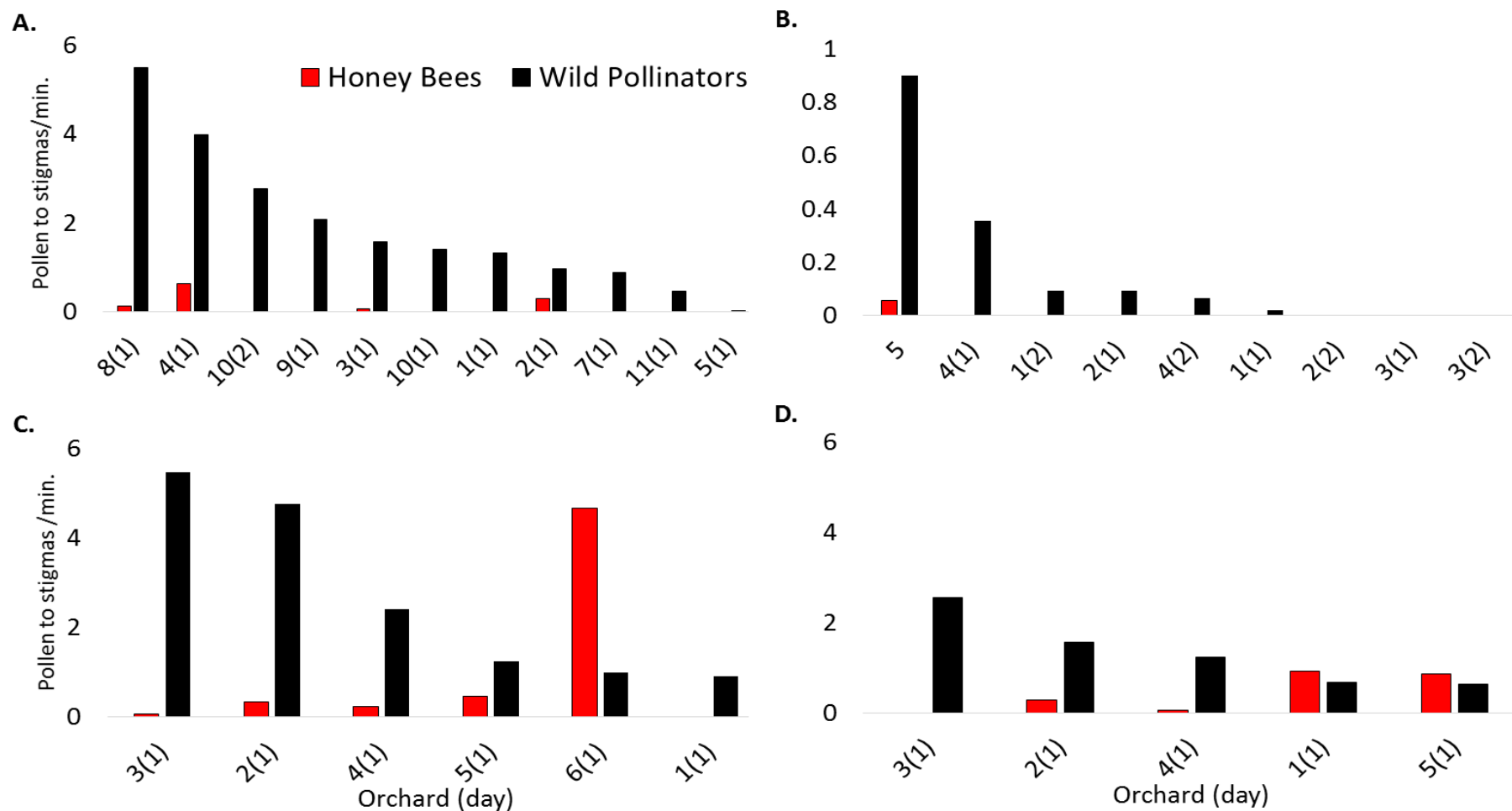


Figure 4.3.11. The effectiveness of honey bees and 12 wild pollinating species across individual avocado orchards across four locations A. Mildura, B. Renmark, C. Robinvale and D. Waikerie in the Tri-State region. Values are estimated hourly rate of pollen deposition to stigmas within individual trees by each taxa.

Effectiveness of different wild taxa

Blow flies and other calliphorid species (Calliphoridae).

Several calliphorid fly species were common flower visitors in Tri-State orchards. The combination of five common species were more effective than honey bees across orchard survey days in Mildura (Figure 4.3.12) and Waikerie. Of the five species, lesser brown blow flies contributed the highest rate of pollen deposition to stigmas within trees in Waikerie orchards, whereas large green blow flies were most effective in Robinvale and Mildura orchards. Four of the species are pictured in Figure 4.3.13.

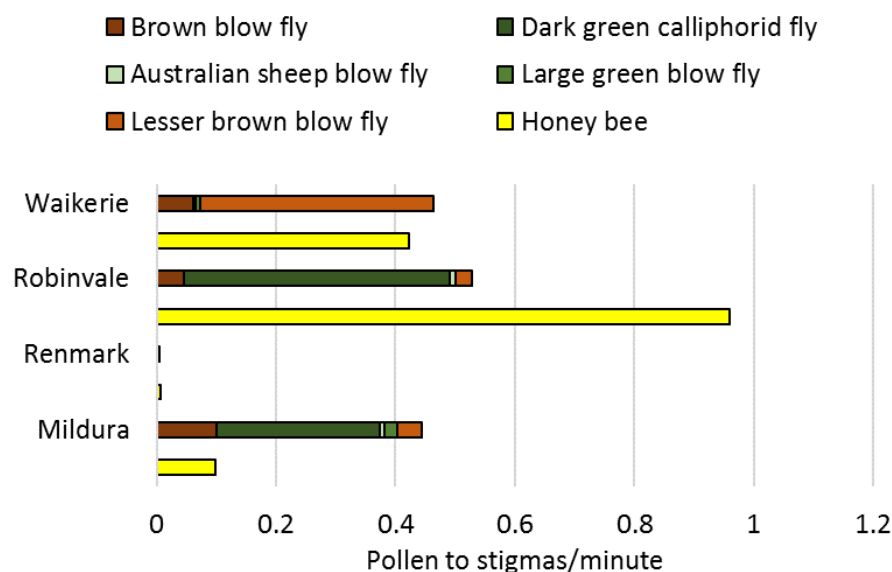


Figure 4.3.12. A comparison of the effectiveness of five calliphorid species and honey bees across orchards at four locations in the Tri-State region. Values are estimated pollen deposition rates for individual trees.



**Lesser brown
blow fly
(*Calliphora
augur*)**



**Brown blow
fly
(*C. stygia*)**



**Australian
sheep blow
fly (*Lucilia
cuprina*)**



**Dark green
calliphorid fly**

Figure 4.3.13. Four common calliphorid species.

Hover flies

Several hover flies were observed visiting avocado flowers with two species, narrow yellow hoverflies and black-orange hoverflies, being the most common. Together they were more effective pollinators than honey bees on the survey days in orchards at three locations (Figure 4.3.14). The two species are pictured in Figure 4.3.15.

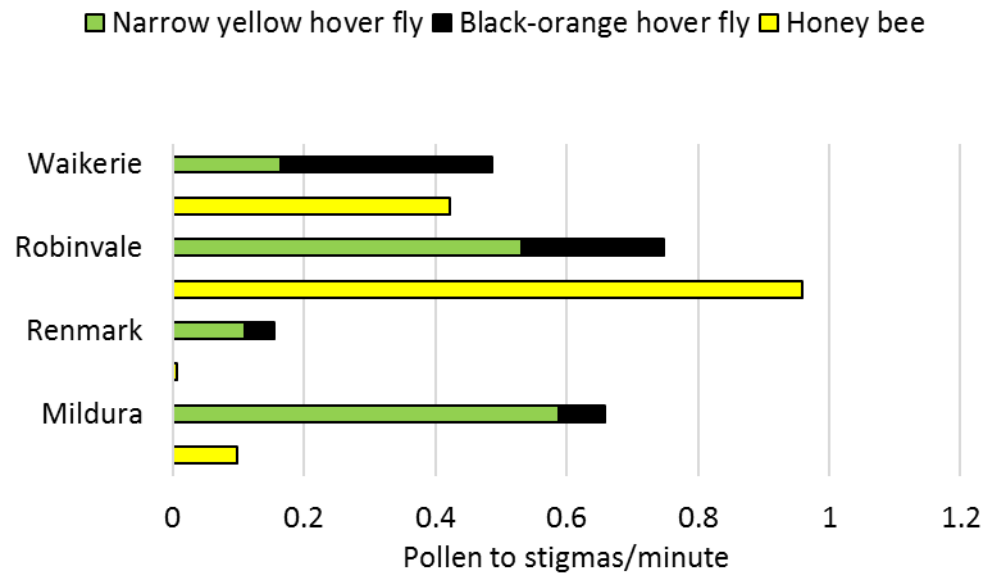


Figure 4.3.14. A comparison of the effectiveness of five calliphorid species and honey bees across orchards at four locations in the Tri-State region. Values are estimated pollen deposition rates for individual trees.



Figure 4.3.15. The two most commonly observed hoverfly species – Top: Black-orange hoverfly *Melangyna viridiceps*; Bottom: Narrow yellow hover fly *Simosyrphus grandicornis* (photograph of *S. grandicornis* Victoria Potdevin, University of New England).

Other Muscoid flies: Bristle flies; Striped flesh flies; Rhiniid flies

Other Muscoid flies (a superfamily that includes) blow flies (Calliphoridae), bristle flies (Tachinidae), house flies (Muscidae), and rhiniid flies (Rhiniidae); besides, calliphorids were also abundant pollinators. Summing the four species – bristle fly sp. 3, striped flesh fly and Rhiniid fly – found them to be of similar but low effectiveness across survey days in Renmark and more effective in Mildura (Figure 4.3.16). A striped flesh fly is pictured in Figure 4.3.17.

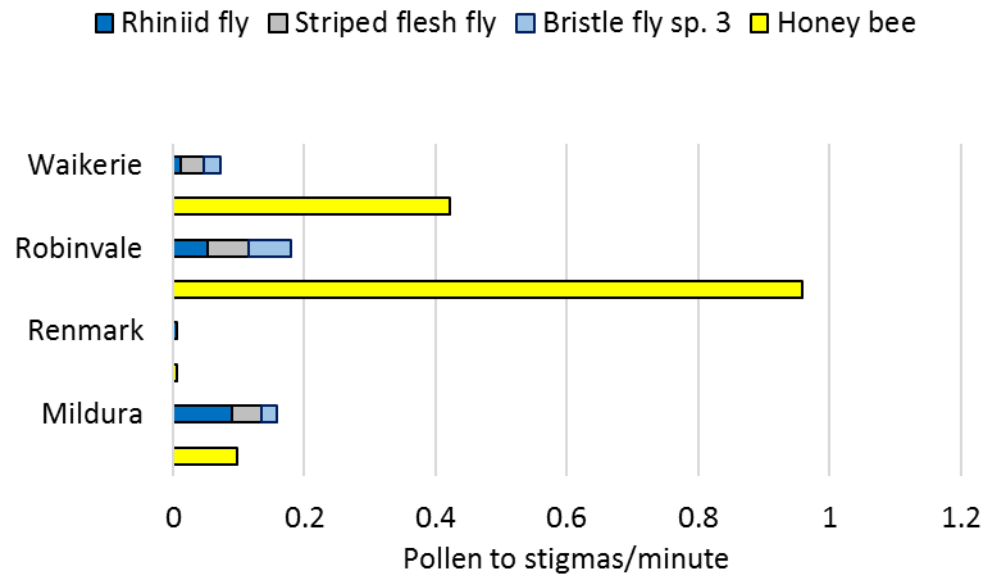


Figure 4.3.16. A comparison of the effectiveness of four muscoid species and honey bees across orchards at four locations in the Tri-State region. Values are estimated pollen deposition rates for individual trees.



Figure 4.3.17. Striped flesh fly (*Oxysarcodexia* sp.?)

Ladybird beetles

Ladybird beetles were by far the most common beetle species observed. Two were very common: the transverse ladybird beetle and the variegated ladybird beetle. The abundance of the transverse ladybird beetle across most orchards on the survey days has resulted in it being one of the more effective pollinators. We did not collect enough data to accurately assess the effectiveness of the variegated ladybird beetle. It is of similar size to the transverse ladybird beetle and potentially of similar efficiency. If this is the case, it would also be one of the more effective pollinators in Tri-State avocado orchards (Figure 4.3.17). Photographs of the two species are presented in Figure 4.3.18.

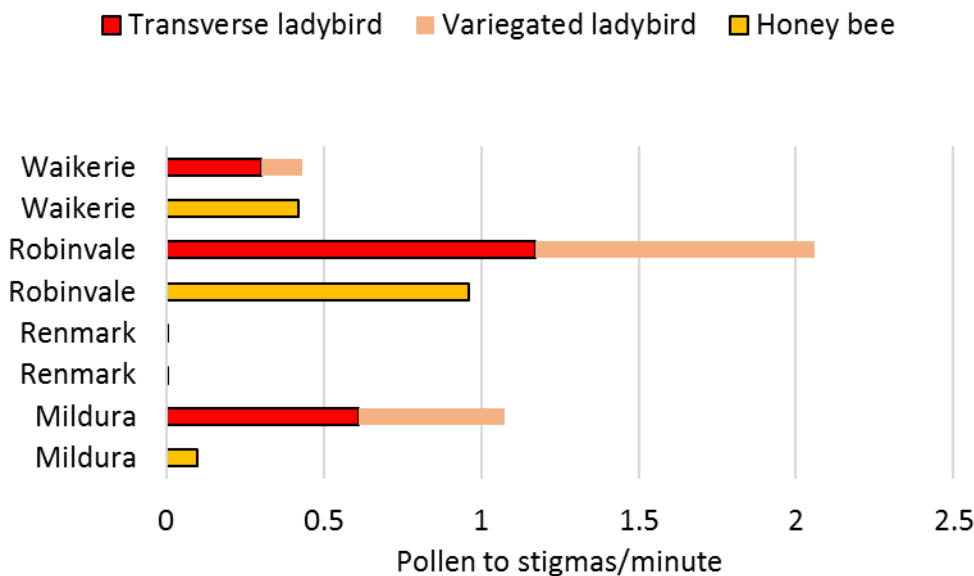


Figure 4.3.17. A comparison of the effectiveness of transverse ladybird beetles and honey bees across orchards at four locations in the Tri-State region. Values are estimated pollen deposition rates for individual trees. We also present the possible pollinator effectiveness of the variegated ladybird beetle (in non-outlined pale red bars) assuming it were to have similar pollinator efficiency as the transverse ladybird beetle.

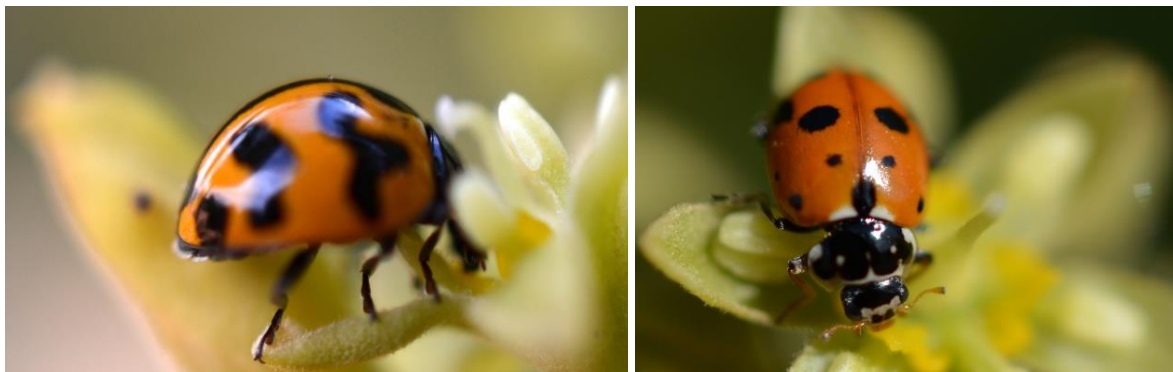


Figure 4.3.18. Left – Transverse Lady Beetle *Coccinella transversalis*; Right – Variegated Lady Beetle *Hippodamia variegata* (photographs of *C. transversalis* by Victoria Potdevin, University of New England).

4.3.4 Discussion

Our study highlights the very important role of wild pollinating species in the pollination of Tri-State avocado orchards. Previous studies have suggested that wild pollinators may be playing a useful role as pollinators with flies in general being considered efficient pollinators (Perez-Balam et al. 2012). To our knowledge, this is the most comprehensive study of avocado flower visitors globally. We have calculated the effectiveness of a broad range of wild pollinators (13 species) and compared them with honey bees, a pollinator that is commonly relied on for the provision of pollination service in avocado orchards around the world. By quantifying the efficiency and effectiveness of wild pollinator species, this study provides a foundation for developing strategies to sustain and build the populations of key pollinating species that can support avocado pollination within these orchards.

Honey bee and wild pollinator effectiveness

Although numerous flower visiting species have previously been noted in Australian orchards (NSW and Queensland) (Evans et al. 2011a; Vithanage 1990), most studies have focussed on the importance and benefits of honey bees as pollinators. (Vithanage 1990; Isham & Lahav 2011). Elsewhere, honey bees have been noted as the most frequent or important pollinators including for Florida (Davenport et al. 1994) and Israel (Eisikowitch & Ish-Am 1996). In New Zealand, approximately 85% of flower visitors to avocado orchards were found to be honey bees (Howlett unpublished). Our survey of 24 orchards across four locations in the Tri-State region found that the wild pollinators we assessed were, together, more effective pollinators in all locations and in all but two orchards. Moreover, our study under represented the effectiveness of wild pollinators as we assessed just a portion of the wild pollinating species (we identified 69 different taxa). Some of these were abundant across locations while others were more abundant at specific locations.

The insects that we identified as efficient pollinators of avocado were largely found to occur across locations and the numerous orchards within each. Exceptions were the wasp – *Thynnid* sp. 1 that was common in one orchard near Mildura but rarely observed at other orchards. The dark-green calliphorid fly was a more abundant flower visitor of orchards in Mildura and Victoria, but not in Renmark or Waikerie. The survey was conducted over a single season and populations of some of these species may fluctuate significantly between seasons, as is the case for many wild pollinator species (Committee On Status Of Pollinators In North America 2007).

A broad range of the pollinating species we observed were found to be foraging under a wide range of conditions. For example, 10 of the 16 most common species were observed on flowers at the extreme ends of the weather variables (air temperature, humidity, light intensity and average wind speed) measured during the surveys. A number of these could therefore be active even outside of the range of each variable measured. For example, various blow fly species can forage at temperatures below 10°C and under low light intensity, whereas, honey bees are less active under these conditions (Howlett et al. 2013). The comparatively high species richness of pollinating species within avocado orchards in the Tri-State, compared with other growing regions such as New Zealand, is likely to increase the opportunity for pollination to occur in orchards under the highly variable weather conditions typical of the Tri-State region.

Pollinator visitation of ‘Hass’ and pollenizers trees and male and female phase flowers

In other crops, pollinators are known to develop preferences between flowers of different cultivars or within a cytoplasmic modified cultivars that produce a combination of sexually functional and non-functional flowers (Erickson et. al 1979; Evans et. al 2011b). Pollinators can even discriminate between male and female flowers on the same plant (Mamut et al. 2014), potentially reducing their efficiency as pollinators. Discrimination between male and female flowers may also vary between pollinating taxa. For example, within the plant

Eremurus anisopterus, halictid and honey bees favoured visiting flowers that produced pollen over flowers that did not, whereas syrphid flies did not discriminate (Mamut et al. 2014). In avocado orchards with pollinizer cultivars present, it is possible that some pollinator species may prefer to visit the pollinizer or alternatively the fruit tree, reducing the potential for pollen movement between the two. In this study, we did not explore the movements of insects between pollinizers and 'Hass' trees; however, the pollinator assemblages and insect abundances within each tree type were similar. There were examples in some locations where we counted more individuals of a pollinator species on either the pollinizer or the 'Hass' trees (e.g. Transverse and Variegated ladybird beetles in Waikerie orchards); however, the patterns were never consistent across locations. We did not assess the relationship between different pollinizer cultivars and 'Hass' trees and it may be that some and not others may be preferred by pollinators over 'Hass'. This is a worthwhile examining as it has valuable applications regarding orchard design. However, determining whether pollinator preferences are determined by cultivar type is further complicated by the timing of resources between tree cultivars (these may vary throughout the day) and the timing of flowering (that at times may not match fully); these factors may alter pollinator preferences between two interplanted varieties.

Our assessments of the abundances of different pollinating species in response to male and female phase flowering within 'Hass' also did not reveal any potential preferences. As with the cultivar versus 'Hass', we found some species being more abundant on flowers in either male or female flowering phase (e.g. Transverse and Variegated ladybird beetles were more abundant on flowers in the female phase in Robinvale orchards). However, there was no consistency of patterns across locations. Overall, the abundances of common flower visiting species was reasonably consistently independent of flowering phase.

Flowering intensity and pollinators

Flowering intensity across orchards varied widely, both within and between regions. Pollinator abundance is often closely tied with flowering intensity (Mesa 2011). In this study our counts of pollinators were often higher in orchards with larger numbers of flowers per unit area. In Renmark, our observation surveys were conducted after peak flowering and therefore flowering intensity was low along with pollinator abundance. Because of this, our data for Renmark orchards are not representative of the likely pollinator abundance and diversity at peak flowering. The survey did however show that many pollinator taxa present in these orchards are similar to the other locations.

For Robinvale, flowering intensity and pollinator abundances did not correspond very closely. For orchards within this location, flowering intensity did not vary as greatly as other locations. Therefore, other factors such as orchard management practices, tree age, and surrounding landscape usage may have been more important contributors to pollinator abundances than flowering intensity.

Honey bee abundances

In some regions, honey bees are considered particularly valuable pollinators for avocado. For example, in NSW, Vithanage (1990) concluded that honey bees play a leading role in the pollination of avocado for improving fruit yield, while in Israel, the presence of honey bees are important for yield (Ish-Am & Eisikowitch 1998). This study also found them to be an efficient pollinator, even though their contribution to the pollination of orchards in the tri-state was highly variable. In some orchards they were very rarely observed on avocado flowers. Although they may have been present at higher numbers on days we did not conduct our surveys, the overall low numbers across nearly all orchards points to them being a minor pollinator compared with wild species. We did note several growers had placed hives within, or next to, their orchards, but this did not correspond to an obvious increase in the number of honey bees visiting flowers. We did not examine why honey bees were often infrequent visitors to these orchards; however, competing bloom from citrus trees, which are often grown on the same or neighbouring farms in most locations, may be a factor. As in the Tri-State, Israeli avocado orchards also flower at the same time as citrus orchards and their competing bloom is

considered a key factor in poor visitation rates of honey bees to flowering avocado (Ish-Am & Eisikowitch 1992). Further, Ish-am & Eisikowitch (1992) note that late blooming avocado varieties that do not correspond with the peak blooming period of citrus can receive much greater visitation by honey bees than those varieties that bloom at the same time.

Key pollinating species

Blow flies

Blow flies and other calliphorid flies (brown blow flies, large green blow flies, Australian sheep flies and dark-green calliphorid flies) were among the most abundant flower visiting species. Although honey bees were estimated to be more efficient pollinators than these species (1.9–2.7 times), in combination, they were more effective pollinators in Mildura and Waikerie than honey bees. Although on average they were less effective than honey bees in Robinvale orchards, they deposited pollen to stigmas at a similar rate compared to Mildura and Waikerie orchards (Figure 4.3.12).

Large green blow flies and dark-green calliphorid flies still require identification to the species level. The other three species, *Calliphora stygia*, *C. augur* and *Lucilia cuprina*, have simple lifecycles with larvae feeding on protein sources, particularly meat products. They are important species forensically (Day & Wallman 2006; Parry et al. 2011) and can be problematic to the livestock industry by causing sheep fly strike. Due to their simple lifecycle, avocado growers may be tempted to place animal carcasses within their orchards to build populations; however, careful consideration of their potential impact on surrounding livestock is required before this practice is used. Our research did not assess why blow flies were abundant visitors to avocado throughout the Tri-State region, but such knowledge could determine landscape and management practices that support populations and predict local abundances.

Hoverflies

Several different hoverfly species were observed on avocado flowers. The most abundant species were *Symosyrphs grandicornis* and *Melangyna viridiceps*. These species were identified from our collection. Other species of similar morphological structure (e.g other *Melangyna* spp.) may have been present and grouped with *M. viridiceps* during observation surveys. Descriptions of these species in published literature is limited; however, they are verified predators of aphids Soleyman-Nezhadiyan & Laughlin (1998) and possibly the caterpillars of Lepidoptera (Bowie et al. 2001). Both adults and larvae and their aphid prey have been found to be locally abundant in wheat fields in NSW (Bowie et al. 2001). During our survey, large numbers of *S. grandicornis* were occasionally observed visiting unidentified grasses neighbouring avocado orchards. We did not observe for the presence of hoverfly larvae or aphids. Further research is required to assess whether alternative plants including certain varieties of grasses could be grown to support non-problematic prey such as aphids and therefore populations of these syrphid species. One grower (Western Australia) has started trialling a combination of barley (to provide an aphid source), phacelia, coriander, alyssum to provide nectar and pollen sources based on the findings of our research.

Other species of hoverfly, for example *Eristalis*, *Eristalinus* and *Helophilus* species, have larvae (rat tailed maggots) that live in stagnant water feeding on organic material. Drone flies (*Eristalis tenax*) and metallic blue hoverflies (*Helophilus hochstetteri*) have been found to be efficient pollinators of avocado in New Zealand (Howlett unpublished) and we are working with the vegetable seed growers with the aim of increasing numbers using within field rearing methods. In this study, we found the yellow hoverfly (*Eristalinus punctulatus*?) (Figure 4.3.19) to be capable of pollinating, with one of five individuals placing pollen on the stigmas of test flowers when moving from male flowers to test flowers and one of two individuals moving from female phase to test flowers. Yellow hoverflies and drone flies were observed on avocado flowers in Tri-State orchards but were relatively uncommon (seven and three specimens recorded respectively).



Figure 4.3.19. Yellow hoverfly (*Eristalinus punctulatus?*) visiting a female phase avocado flower. (photograph by Lisa Evans, Plant & Food Research).

Muscoid flies

Muscoidea is a superfamily of flies that encompasses a number of families. These include Calliphorids (e.g. blow flies), Muscids (House flies), Tachinids (Bristle flies), Sarcophagids (Flesh flies) and Rhiniids (Nose flies). Their lifecycles are diverse and often complicated by being associated with other insect hosts. Bristle flies are very difficult to identify to species level and there is ongoing alteration to their taxonomic groupings. This family of flies were relatively common and the one morphospecies we assessed was estimated to be the most efficient pollinator of avocados. Bristle flies are parasitoids and their larvae feed within the host body cavity of Arthropods (Cantrell 1986; Stireman et al. 2006). The potential management of bristle flies is complicated by a lack of knowledge of taxonomy and their host requirements.

Flesh flies are often common in open pastoral areas (Henning et al. 2005) particularly where cattle are grazed (Barratt et al. 2001). Their larvae feed on a range of organic material including rotting vegetation (Miller & Walker 1984), sheep and cattle dung (Bishop 1998). They also feed on meat products and can be forensically important (Archer & Elgar 2003). As with blow fly species, supplying appropriate feeding substrates for larvae may build populations in orchards.

Rhiniid flies are another species that was found to be an abundant pollinator of avocado in some orchards. The larvae have been found living in ant and termite nests.

Ladybird beetles

Transverse and variegated ladybird beetles were amongst the most common flower visitors. The transverse ladybird beetle alone was calculated to be more effective than honey bees in Mildura and Robinvale orchards, largely due to their large numbers. If we assume that the variegated ladybird beetle (similar size) is of similar efficiency, then ladybird beetles would be of similar effectiveness as honey bees in Waikerie orchards.

Both of these species have adults and larvae that feed on aphids and lepidopteran caterpillars. The transverse ladybird beetles have a preference for aphids, laying more eggs on this diet than moth caterpillars (Evans 2000). Immatures and adults will feed on several aphid species but develop better on some aphids (e.g. the cotton aphid, *Aphis gossypii*) than others (Omkar & James 2004).

The variegated ladybird was first found at Gatton, QLD, Australia in 2000 and has since been noted feeding on multiple aphid species and at least one psyllid (Franzmann 2002). It is also a known predator of noctuid moth caterpillars, leafhoppers (Franzmann 2002 and references within). Ladybird beetle populations could potentially be boosted by planting preferred hosts of their prey; however, careful consideration is necessary to minimise the spread and eruption of pest species such as aphids within the orchard or in local crops. Moreover, aphid populations tend to fluctuate rapidly that can then significantly alter the abundance of pollinating ladybird beetles (Hemptinne & Dixon 1997).

Key points

- To our knowledge, this is the largest study conducted assessing pollinator identity, abundance and effectiveness of avocado pollinator species within a region. The study assessed 24 orchards in the Tri-State region near Mildura (Vic), Robinvale (Vic), Renmark (SA) and Waikerie (SA).
- Our surveys recognised 69 different insect species/morphospecies that visit avocado flowers in the Tri-State region. The number is conservative as it includes unidentified groupings at the order and family level and the possibility of additional species that were grouped under the one morphospecies.
- We assessed the pollinator effectiveness of 13 different species/morphospecies; these were honey bees, ten fly species and a ladybird beetle species. Together, the wild species were the most effective pollinators in all locations. Honey bees were more effective than the 12 wild pollinators assessed in just two of the 24 orchards. We sampled just a subset of the wild flower visiting assemblage, therefore our calculation is an underestimate of the contribution of wild pollinating species.
- There was a large degree of variability in pollinator effectiveness between orchards, between locations and within locations. The effectiveness of pollinators at Renmark orchards were amongst the lowest (six or less pollen grains to stigmas within a tree per hour) but this is most likely due to the low flowering intensity at the time of the surveys. Mildura orchards were the most variable ranging from 0.01 to 5.50 pollen grains delivered to stigmas within a tree per minute.
- Calliphorid and other muscoid flies (bristle, flesh, rhiniid flies), hoverflies and ladybirds are key contributors to the pollination of tri-state avocado orchards. Some of the species that have less complex lifecycles, e.g. blow flies, some hoverflies and flesh flies, have higher potential for developing management strategies within orchards; however, consideration of the impacts on neighbouring properties need to be considered.
- Although effective pollinators of avocado, honey bees largely appear to be unreliable pollinators in the Tri-State region, even though many growers place hives near their orchard. Competing citrus bloom is likely to be a factor reducing honey bee abundances within orchards.
- Due to the importance of flies and beetles as pollinators in Tri-State orchards, the impact of pesticide use during flowering of Tri-State may be particularly large and unpredictable. Product use regulations rarely consider potential impacts on pollinators other than bees. Flies and beetles may be present in orchards both day and night and therefore application of these products based on minimising bee loss may still cause significant reductions of these key pollinators.
- We did not determine why Tri-State orchards contained such high diversity and abundances of wild pollinating species; surrounding vegetation and land use are factors known to influence wild pollinators. Better understanding of these factors and the lifecycles of specific species is required to further increase management options for growers.

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