Final Report

Healthy bee populations for sustainable pollination in horticulture

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Delivery partner:

Western Sydney University

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Content

Summary

Insect pollinators are declining globally, threatening horticultural production. In particular, the Honey bee plays a key role in Australian horticulture, but is expected to decline severely if the *Varroa* mite establishes. This creates an urgent need to better understand the role of Honey bees and native pollinators (both bees and other insects) in pollination of Australian crops. We also need to understand the key factors - such as floral resources, climate change and disease – that may limit the persistence of valuable wild pollinator populations in agroecosystems. This project addressed these issues through both targeted research and grower engagement.

Our research in apple and cherry orchards confirmed a major role for Honey bees, and Honey bees are the only significant pollinator in almonds. Currently, the relative role of wild Honeybees versus rented hive bees is essentially unknown and we developed a marking and survey approach to investigate this at farm level. In apple and cherry systems, some native bees are as good or better pollinators than Honey bees at the individual insect level, but occur in lower numbers. An exception involves wild stingless bees, which can be very abundant when present and may already play a major role in pollination. Other native bees could provide resilience if Honey bees do collapse due to *Varroa* mite or other factors, and land management can influence their numbers greatly.

Pollinators require non-crop floral resources for food, especially in production systems where the main crop has a short bloom period. We found that the diversity of pollinators and/or crop visitation by pollinators increased with the diversity of non-crop floral resources in both apple and cherry orchards. Planting extra floral resources is one option for growers and we screened the nectar production and flowering phenology of a panel of native plants whose seed is available through Greening Australia. Native perennial plants provide a good option to support resident pollinators around the year, while commercially available exotic annual plant seed mixes may provide nectar and pollen resources that attract greater numbers of Honey bees during crop bloom. Climate change could influence plant/pollinator in several ways and we leveraged ongoing experiments to investigate this. Over three flowering events, elevated CO_2 did not significantly change the nectar of *Eucalyptus tereticornis* trees, but Honey bee numbers were consistently lower in elevated CO_2 treatments. In warming experiments, plant responses were highly variable between the 10 species studied, making generalisation difficult, although changes in flowering phenology were noted.

Australia's main Honey bee-associated viruses were all detected in Honey bees in apple and cherry orchards with black queen cell virus the most common. Most viruses were also detected in native pollinators, but were far less prevalent and it is not yet clear if they cause symptomatic diseases. Next generation sequencing studies confirmed these PCR-based screening results and did not detect major new insect-specific viruses in native pollinators. However, they did reveal that Honey bees and native pollinators may potentially vector some significant plant viruses, notably apple stem grooving virus.

To engage growers in pollination issues, we ran a number of local workshops and field days, and produced fact sheets, industry magazine articles, podcasts and videos. In addition, we organised a grower study tour of India, focused on crop pollination issues, including the use of managed Honey bees and stingless bees. Links established during this tour have led to parallel collaborative work on e.g. apple and almond pollination in India.

Public summary

Not applicable – as above

Keywords

Pollination; fruit quality; yield; native bees; Honey bee; alternative pollinators; floral resources; apple; cherry; almond; climate change; bee diseases, virus.

Introduction

Insect pollinators provide vital pollination services for Australian horticultural industries, valued at around $1.2 billion/year. However, there is growing concern about global declines of insect pollinators (Sanchez-Bayo et al 2019; Vanbergen 2013), driven by factors such as habitat modification, loss of floral resources, pests and diseases, and intensive pest management (Goulson et al. 2015). Globally, the Honey bee is the most important crop pollinator, but faces pressure from the devastating Varroa mite and associated viruses (Wilfert et al. 2016). Varroa is yet to establish in Australia, but this is expected to happen at some point, leading to major declines in wild and managed Honey bee colonies, as seen in New Zealand (Iwasaki et al. 2015). Consequently, there is an urgent need to better understand the role of native bees (>1500 species in Australia – Houston 2018) and other insects (Cook et al. 2020) pollinating our horticultural crops (Garibaldi et al 2013; Rader et al., 2016).

A key issue for pollinators is decline in floral resources. In Australia, managed Honey bees may be moved repeatedly between good foraging areas, e.g. for eucalypt flowering events. However, wild pollinator populations rely on local floral resources that have been reduced in many areas through agricultural intensification or urbanization (Goulson et al. 2015). We therefore need to develop planting schemes that help sustain local pollinator populations. However, key knowledge gaps include the seasonal patterns of floral resource use by bees, the value of particular floral species for bees, and the effects of targeted floral enhancements in cropping environments. Moreover, climate change is already known to affect other aspects of plant performance and may also impact the quality, quantity and seasonality of nectar and pollen resources (Phillips et al. 2018).

Another key threat to pollinator populations is disease. Honey bees have a wide range of pests and pathogens (particularly RNA viruses), some of which can devastate colonies. Varroa mite is a key pest, and RNA viruses a key group of pathogens, and recent work suggests their combined presence is particularly damaging (Martin et al. 2012). However, major knowledge gaps include whether these Honey bee viruses also infect other pollinators, and which other viruses exist in native pollinators. We need to understand disease biology in wider pollinator communities rather than focus on Honeybees as if they were isolated from other bees (Manley et al. 2015).

Overall, then, there is a need to better understand which insects (both wild and managed) are providing pollination services in different crops and regions (Wilcox et al. 2019), and how to better support these pollinators through adequate floral resources. Better understanding of diseases and other threats to pollinator populations is also a priority (Goulson et al. 2015; Manley et al. 2015). Consequently, there is a need to educate growers about pollinator biology and crop pollination services and encourage on-farm practices that promote, or at least limit harm to, beneficial pollinators.

To address these knowledge and action gaps, the project focused on five key work packages:

1. Crop pollination --- Identifying pollinator species present on farms and which species visit and pollinate horticultural crops in field situations.

2. Floral resources --- Determining how bees use different floral resources across seasons and devising and testing appropriate farm-level floral enhancement schemes.

3. Climate change --- Testing how experimentally manipulated climate changes affects the timing, quality and quantity of nectar and pollen available to bees in key forage plants such as *Eucalyptus*.

4. Bee health --- Determine what microbial diseases (especially viruses) infect insects in Australian pollinator communities and which of these are shared across native and Honey bees.

5. Grower involvement and adoption --- Inform and educate growers and land managers about bee population health - floral resources, diseases, soil and pest management - and appropriate actions that they can take to maximise bee health and pollination services in their own horticultural context.

Methodology

The main on-farm project focus was on apple and cherry production areas in NSW, across regions with contrasting degrees of human disturbance surrounding orchards. The study regions (Bilpin, Orange, Young) were necessarily within a few hours drive of each other, because crop bloom is brief and overlaps between regions.

1. Crop pollination.

1.1 Pollinator surveys. On six farms in Bilpin and six in Orange, we surveyed flower visitors (potential pollinators) on Pink Lady apples, Granny Smith apples and, more opportunistically, on available cherry varieties. Surveys were undertaken during early (king) and full bloom to test for effects of resource availability, documented by flower counts, on pollinator visitation. For each crop cultivar at each orchard, two rows were surveyed. Within each row, the first five trees were disregarded to avoid edge effects and the subsequent 15 trees surveyed. Surveys were carried out in the 2017, 2018 and 2019 seasons.

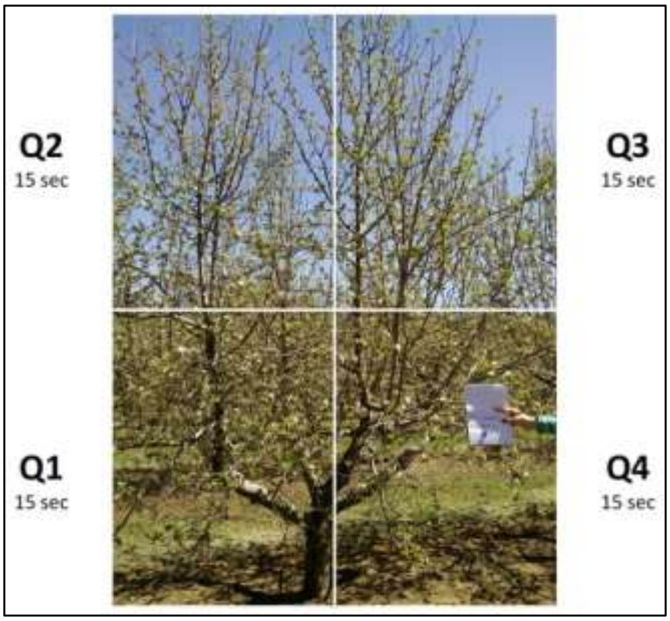


***Figure 1**. Scan sampling procedure for insect visitors to crop flowers*

1.2 Pollinator efficiency. To characterise the efficiency of different pollinators, we sampled insects visiting apple flowers and determined by microscopy how much crop versus non-crop pollen they carried. We then used bags to exclude insects from unopened flowers, allowed them to open and tested how much pollen was transferred to the apple stigmas by a single insect visit, for different insect species. We also filmed flower visits and studied the footage to determine the on-flower behaviour of different insect species and its potential to cause pollination.

1.3. Pollen transport. We also designed an experiment to assess pollen transport across an entire orchard using next generation sequencing (NGS) techniques. First, we developed a genomic plant reference data set for 15 crop varieties (13 apple, 2 cherry) and 79 Blue Mountains native plants, which will permit us to identify pollen carried by pollinators. We then sampled the two main pollinator species – Honey bees and stingless bees – on apple flowers using a 20m x 20m grid approach (Figure 2) at three times during crop bloom. The next phase of the genomics lab work will identify the pollen carried on the bodies of the bees. This still requires approximately 3 weeks of additional lab work at The University of Adelaide's Braggs Laboratory, followed by data analysis. This work has been delayed due to COVID interruptions to travel and Adelaide colleagues' long service leave during the only window of opportunity in 2021. We hope to complete this in 2021 but it depends on COVID restrictions. Hence the full results of these experiments will be included in the final report of PH20002, which addresses bushfire effects on pollination services in the same cropping areas.

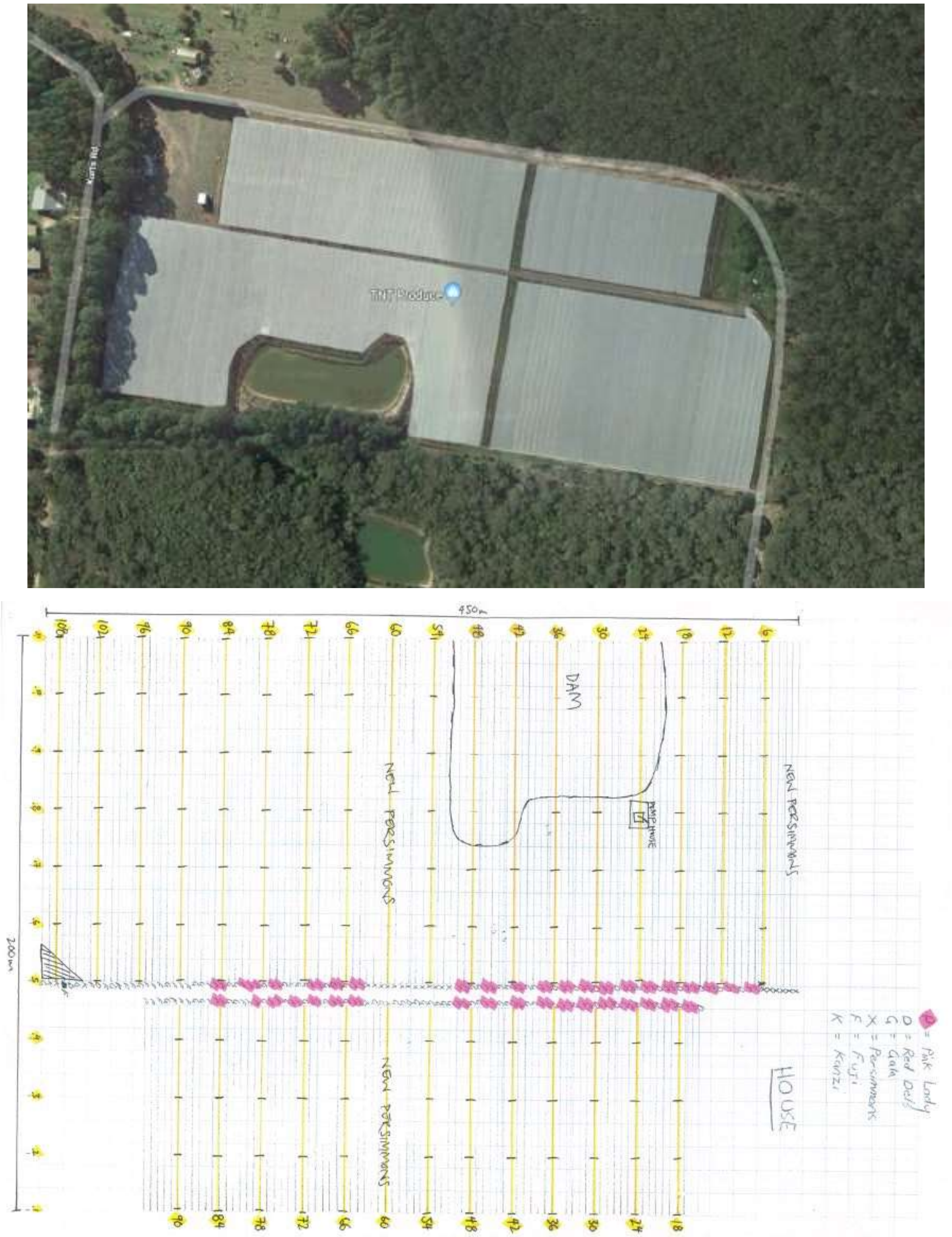


***Figure 2: Top** - aerial view of protected orchard 4-BM (dimensions ~400m x 250m), which is surrounded by the Blue Mountains National Park to the West and South. **Bottom** - Sketch map of orchard 4-BM consisting of 110 rows, showing the 18 sampled rows at 20m grid-point intervals. Legend indicates cultivars in each row (e.g. P denotes Pink Lady).*

1.4. Rented hives. Many growers rent Honey bee hives during crop bloom and wonder how much these bees contribute to crop pollination. We designed and trialled an experiment to assess this by marking bees using trays of fluorescent dust that they must crawl through to enter or leave the hive. We then recorded the proportion of marked (out of all) Honey bees on apple flowers across the orchard, providing a direct estimate of the contribution of marked bees from the on-farm hives to apple pollination.

2. Floral resources.

2.1. Floral resources. To assess non-crop floral resources in cropping landscapes we set up a series of survey plots both on-farm and in adjacent habitat. On-farm surveys were undertaken every 6 weeks (outside crop flowering season) and at three time points during crop flowering season (early, peak and late bloom) at farms in Bilpin, Orange and Young for at least 2 full years.

2.1. Floral plantings. We assessed the value for pollinators of a panel of native floral species grown by Greening Australia in their seed production area (SPA) in Richmond NSW. Each species was assessed in terms of the nectar it produced, the pollinators that fed upon it, and the seasonal phenology of its flowering. In addition, we planted and compared (insect visitors, seasonal patterns) floral strips of either native (using species native to Cumberland Plain Woodland in Sydney) or exotic (using commercially available pollinator friendly seed mix) plants within an apple orchard in Bilpin and a horticultural area in Richmond.

3. Climate change.

We used the unique experimental facilities at WSU Hawkesbury campus to test the effects of climate change manipulations on floral resources and their use by pollinators.

3.1. EucFACE. We investigated the effect of elevated CO_2 on the quantity and quality of floral resources produced by *Eucalyptus tereticornis* using the EucFACE facility (Figure 3). Flowers were sampled in three flowering events (2017,2018,2019) and analysed for three sugars using High Performance Liquid Chromatography. Pollen samples were also prepared and are awaiting analyses due to major delays in sourcing reagents due to COVID-19. Reagents are now available and as we are able to analyse samples in the WSU laboratories, we expect to complete this work within a few months of laboratories being reopened, following the latest Sydney COVID restrictions. As it is not clear when this will be, due to the current lockdown, the full results of these experiments will be included in the final report of PH20002.

Figure 3. *The Western Sydney EucFACE facility (left photo) is within a mature eucalypt woodland where Eucalyptus tereticornis is the dominant canopy tree. There are six large rings equipped with cranes for canopy access. Right photo shows flower counting and sampling from the canopy, accessed via the crane. Air is circulated within the rings and this has either ambient qualities (3 control rings;* CO_2 *= 410 ppm) or elevated* CO_2 *(3 experimental* eCO_2 *rings = 560 ppm) to simulate predicted changes in atmospheric carbon dioxide.*

3.2. Glasshouse experiment. To investigate the effect of elevated temperature on the flowering phenology and floral resources of11 species known to be important species for a range of pollinators, a glasshouse experiment with two temperature treatments, ambient = 30°C (T_A) and elevated = 34°C (T_E), was conducted.

4. Bee health.

4.1. Honey bee virus survey. During spring 2017, approximately 500 individual insects were caught foraging on apple/cherry flowers in orchards in Bilpin and Orange. 200 of these, representing the dominant pollinator species were then screened for five key Honey bee – associated viruses using molecular methods. The focal viruses were those most common in a recent survey of managed Honey bees in Australia (Roberts et al. 2017). All samples were also tested for two fungal pathogens of concern to beekeepers - *Nosema apis* and *N. ceranae*.

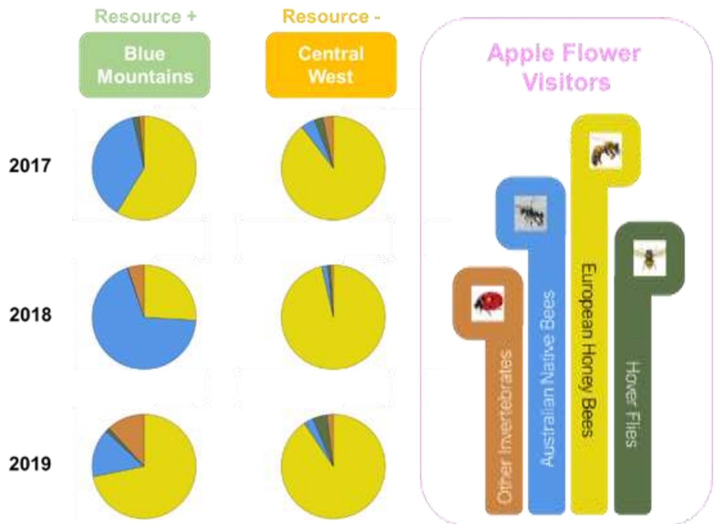
4.2. New virus discovery. In addition to screening for known Honey bee viruses, we also used transcriptomics to test whether these were the main viruses in Australian pollinators and to potentially discover novel, previously undescribed viruses or host associations. To this end, high throughput sequencing was carried out on 12 individual insects (3 Honey bees, 3 stingless bees, 3 reed bees and 3 hoverflies).

5. Grower involvement and adoption. We set up a project reference group (PRG) involving all co-investment partners plus grower representatives, including Bill Shields (Pome and stone fruit, Bilpin NSW), Lachlan Donovan (Avocadoes, Childers QLD) and Ed Fagan (Cucurbits, Cowra). The WSU team hosted 6-monthly meetings to update members on project progress and receive feedback and suggestions for future plans. In addition, we presented project findings and pollination recommendations at grower workshops in the main study regions at different stages of the project, and produced factsheets and videos that have been made available through a range of outlets. Further, as a variation to the initial contract we organised and led two study visits to India for growers, beekeepers and researchers to discuss pollination issues in a series of workshops and view cropping, beekeeping and pollination management practices in India in comparison to management of the same crops in Australia. A further aim was to lay the ground for links with India for collaboration on horticultural research.

Outputs

1a) Database on pollinator species visiting horticultural crops under field conditions

Apple: Over three spring apple bloom periods (2017-2019), 581 insect surveys comprising 8,715 minutes of observations were undertaken on Pink Lady (n = 465) and Granny Smith (n = 116) apples during crop flowering (king-bloom n = 297; full bloom n = 284) in the Blue Mountains (BM n = 356) and Central West (CW n = 225) of NSW. These surveys recorded 69,354 visits from five insect orders and one arachnid order: Hymenoptera (96.4% of visits), Diptera (1.84%), Hemiptera (1.4%), Coleoptera (0.3%), Lepidoptera (0.04%) and Araneae (0.02%).



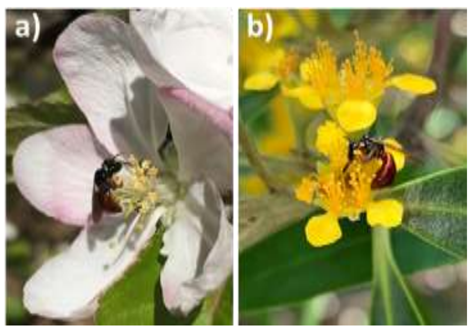


Figure 4. Visitors to apple flowers in two regions with contrasting natural resources. Pie charts show proportions of the most common apple flower visitors in two regions of NSW from 2017-19. Each pie chart summarises annual data from six orchards per region. Blue Mountains orchards (light green) are surrounded by protected sandstone sclerophyll forests - a region rich in natural resource (Resource +). In contrast, the eucalypt forests surrounding orchards in the Central West have been extensively cleared for agricultural land use - a region poor in natural resource (Resource -). Photographs show a native allodapine bee (Exoneura) foraging on flowers of: (a) apple orchard; and (b) native water gum (Tristaniopsis) in the surrounding Blue Mountains sclerophyll forest (photo credit: O. Bernauer).

The database reveals that bees are by far the dominant group, representing 93.5% of all visits to apple flowers, and including four of the five Australian bee families (see Table 1). At the regional scale, Australian native bees were more frequent visitors (59%) than the European Honey bee (41.5%) in Bilpin; the reverse was true in Orange, where European Honey bee was by far the dominant bee species (97%). Indeed, the bee was the most frequent visitor to apple flowers across all surveys combined (53.7%), although it was not possible to distinguish between wild and managed Honey bees. The vast majority of native bee visits in Bilpin were from the stingless bee, *Tetragonula carbonaria*, which was absent from Orange. In contrast, halictid bees were the main native bees in Orange; they were very diverse (27 species) but had only low visitation rates (2% of total).

While there were striking differences between regions in which species of native bees visited apple flowers, there were also interesting patterns within regions. Previous milestone reports have detailed these, but in short:

(i) visitation by both stingless bees and Honey bees increases with temperature, but there is a threshold effect for stingless bees such that they are virtually absent below 20C but dominate above 22 C (Figure 5).

(ii) Bilpin orchards exhibited higher apple floral densities than those in Orange, which may explain greater native bee visitation rates; although there are other possible confounding factors.

(iii) Cavity-nesting native stingless bees showed decreasing abundance with distance from natural resources.

(iv) In contrast, ground-nesting halictid bees showed positive relationships with distance from natural resources, which agrees with other studies suggesting that nesting abundance increases with ground disturbance.

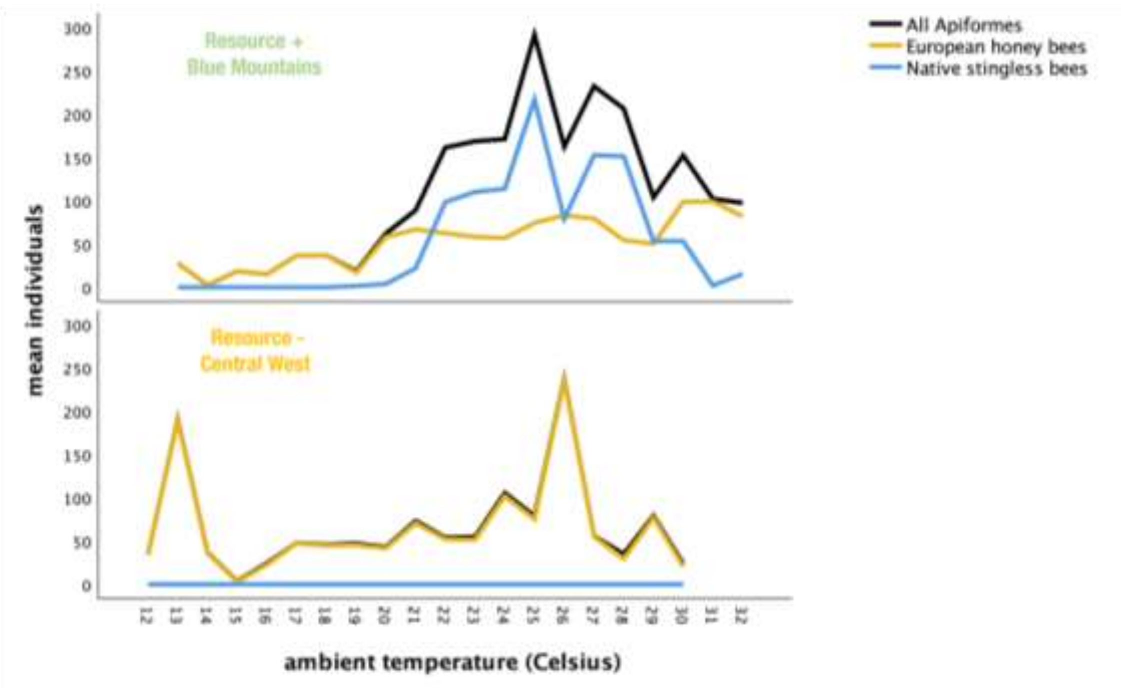


***Figure 5: Effect of temperature on bee visitation frequency in two regions with contrasting natural resources**. Line graph plots the mean number of individual bees visiting apple orchard flowers as a function of temperature (C), summarised for: All bees (Apiformes - black);*

European Honey bees (yellow); Native stingless bees (blue). Top Panel: Blue Mountains orchards are surrounded by pristine protected sandstone sclerophyll forests - a region rich in natural resource (Resource +). Bottom Panel: Central West orchards where surrounding upland eucalypt forests have been extensively cleared - a region poor in natural resource (Resource -). *The Central West region is beyond the distributional range of stingless bees.*

Cherry: We also conducted a similar set of studies (to apple work reported above) for sweet cherry, with orchard studies in Young, Bilpin and Orange in the flowering seasons of 2017, 2018 and 2019. The general approach was essentially the same as for apples, and indeed some orchards had both apples and sweet cherries, with some overlap in flowering, though cherry generally started and finished flowering a week or two earlier than apples at the same farm.

Overall, the suite of insects visiting cherry flowers (Figure 6) was very similar to that visiting apple flowers, dominated numerically by Honey bees, which were abundant in all three regions, and by stingless bees but only in Bilpin. Other native bees were a smaller component of the pollinator community, with *Lasioglossum* bees the most notable group (Figure 6). Hoverflies were again widespread across farms and regions but in relatively small numbers (Figure 6).

Despite the clear dominance of Honey bees, followed by native bees, in the overall visitation patterns, on some days we found large numbers of insects from other groups, such as ants and beetles. However, these tended to be short-lived local cases of high abundance and further results on pollen transport (see next section) suggest that they are of limited significance for cherry pollination.

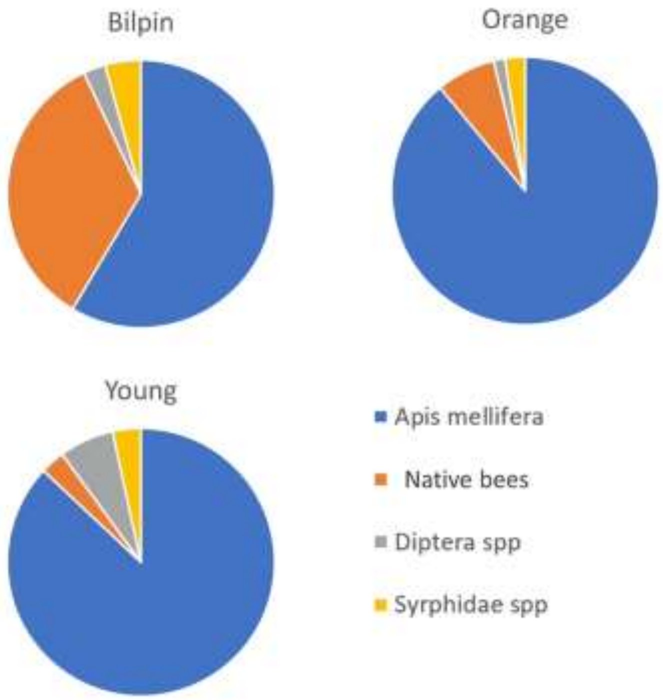


Figure 6. Overall patterns of cherry flower visitation in three production regions of NSW from 2017-2019. *The Honey bee (Apis mellifera) is the dominant visitor in all three regions, but this represents a mixture of wild and managed bees in unknown proportions. Many growers rent Honey bee hives during pollination season. Native bees are the second most numerous group, represented mainly by stingless bees in Bilpin and by solitary bees such as Lasioglossum species in the other regions. Hoverflies were also found at all orchards, but numbers of fly visits were generally quite low.*

1b. Assessment of which native pollinator species transfer pollen between plants of selected crops, and the impact of their services on crop yield / quality.

Apple: A range of native insects (especially bees) contribute to apple pollination, however, their effectiveness varies between species. Exclusion (bagging) experiments showed that apple flowers unequivocally depend on animal pollination, that single-visits do not result in adequate fruit set/quality and that ambient insect visitation resulted in pollination deficits (lower fruit set than we achieved through manual pollination).

Collections of 1,197 insect visitors to apple orchards (2018-19 Blue Mountains & Central West) yielded 171 different insect species, half of which (52%) possessed no loose body pollen. We assessed pollen loads for the 31 commonest species (> 5 individuals collected), and found that native bees (allodapine, halictine, meliponine) and introduced Honey bees carried more body pollen than any other insects, and also showed high degrees of pollen load purity (range 71-99% apple pollen, n = 9 species from 5 bee genera). It is important to note that body pollen counts do not necessarily correlate to pollen deposition rates, as indicated in Figure 7. While Honey bees and stingless bees carried the most loose-body-pollen, halictine and allodapine bees deposited more pollen per visit. In fact, *Exoneura* allodapine bees deposited the most pollen in a single visit. Pollen deposition rates also correlated positively with time spent on flowers during a legitimate visit (i.e. one where the bee physically contacted the flower stigma). These results collectively highlight the importance of undertaking detailed behavioural experiments in concert with body pollen assessments.

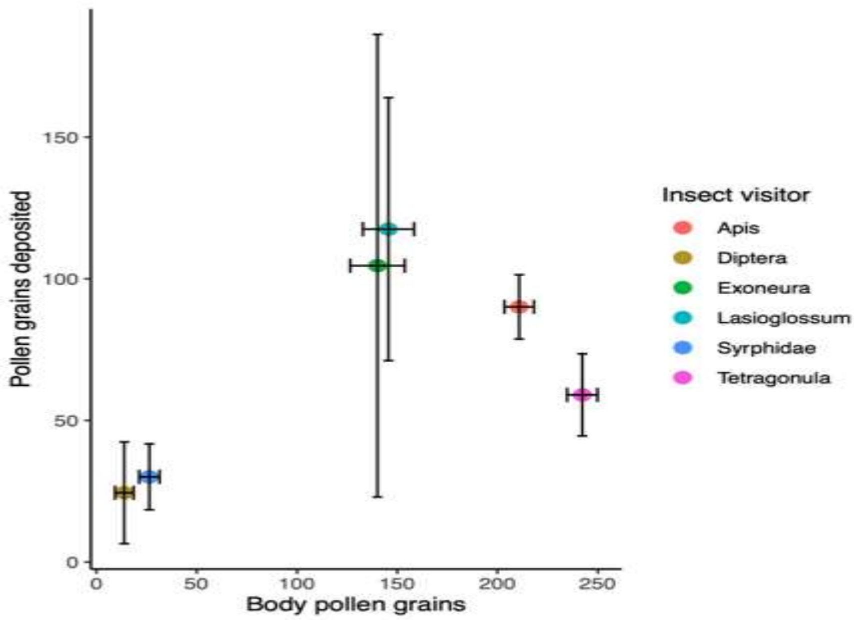


Figure 7. Pollen transfer efficiency by insects visiting apple flowers. *The number of pollen grains per insect visit (Y-axis) does not simply reflect the amount of pollen on the body (X-axis) but also depends on insect behaviour on the flower. Native bees (Lasioglossum and Exoneura) are the most efficient, followed by Honey and stingless bees. Flies (Diptera, Syrphidae) deposited less than half the amount of pollen grains cf. bees.*

Overall, we conclude that **Honeybees and stingless bees are the main current contributors to apple pollination services** due to their combined individual efficiency and high abundance. However, **other native bees, *Exoneura* and *Lasioglossum*, are even more efficient at the individual level** (Figure 7) and may be important bees in a number of apple-growing regions, e.g. both are also significant apple visitors in the Yarra Valley (Dr. Julian Brown, personal communication). *Exoneura* bees nest in dead stems (e.g. tree fern or similar) and may be amenable to husbandry, or at least to colonisation of extra nests provided, to enhance numbers locally.

Consequently, we also studied the nesting biology and annual life cycle of *Exoneura angophorae* in a population adjacent to our Bilpin study orchards. These bees exhibit facultative social organisation and unlike most bees progressively rear young feeding-stage larvae in stem-nest linear burrows that lack brood cell divisions. Figure 8 shows that larvae are present in nests at the same time period as Bilpin apple orchards are in bloom, suggesting that these bees make use of apple pollen as a nutritional resource for their growing offspring. Members of the genus *Exoneura* are frequent visitors to certain orchards especially on warmer days. Their foraging activity shows positive relationships with increasing ambient temperature, like other native bees in the Blue Mountains. They exhibit a mean foraging temperature of ~24°C, which is 1.5°C below that of European Honey bees (MS111).

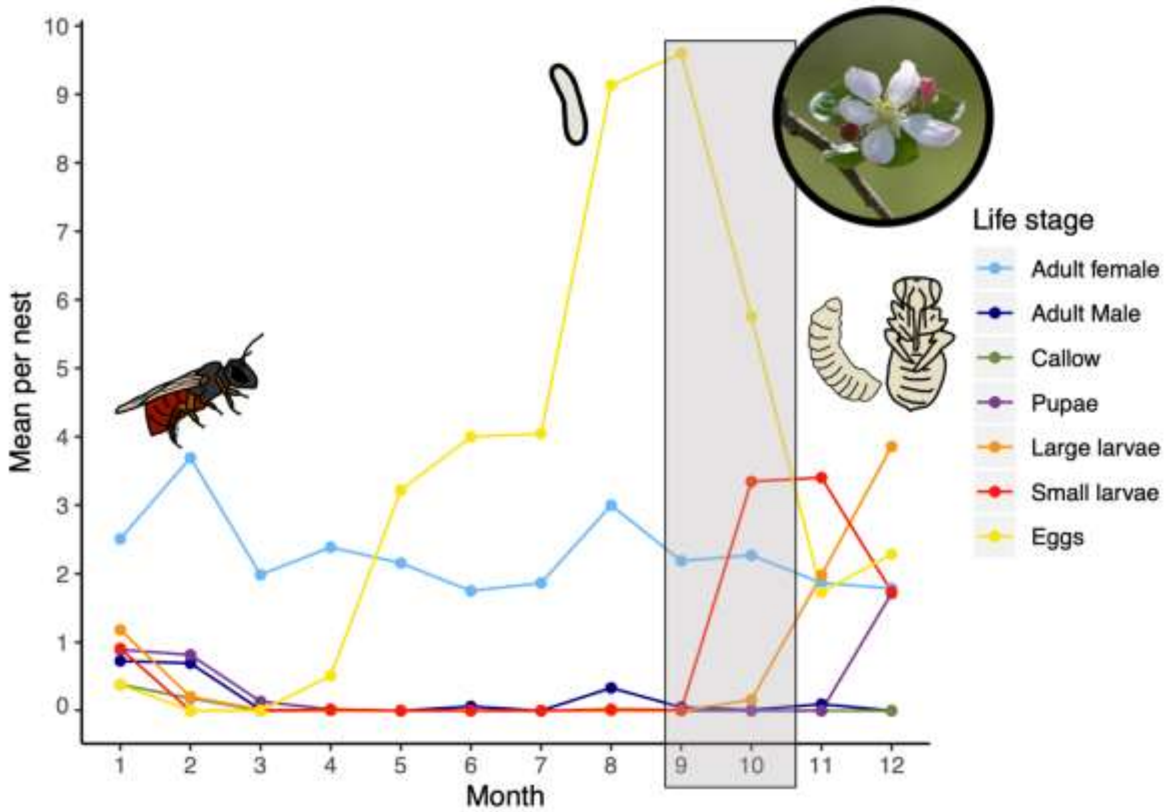


Figure 8. Exoneura native bee nest composition over an annual period. *Monthly mean adult and brood developmental stages per nest, denoted by colour: adult females (black); adult males (orange); pupae (grey); larval instars - prepupae (green); large larvae (dark blue); medium larvae (blue); small larvae (light blue); eggs (yellow). Light green box indicates apple bloom period – overlapping with feeding-stage larval instar presence in nests.*

Cherry: To test which cherry flower visitors were likely to be the most important pollinators, we assessed the amount of cherry pollen carried by a target sample size of 20 insects per group. Results varied somewhat between regions and years, but the major pattern was very consistent. Although quite a range of insect visit cherry flowers (e.g. flies, beetles, ants) only bees were found to carry substantial amounts of cherry pollen. Although we did not specifically aim to test for difference between cherry cultivars, the overwhelming importance of bees was evident for two different cultivars (Figures 9,10) and also across regions (Figure 10). Only bees – Honey bees, stingless bees, and two genera of solitary native bees (*Lassioglossum* and *Leioproctus*) - consistently carried large amounts of loose cherry pollen on their bodies (Figures 9,10), although which bee carried the most varied between some crop x region x cultivar combinations.

When combining these data with the crop flower visitation rates data (section above) it is clear that bees dominate cherry pollination services and that Honey bees and several native bee species all carry plenty of cherry pollen, but the key native bees species vary by region.

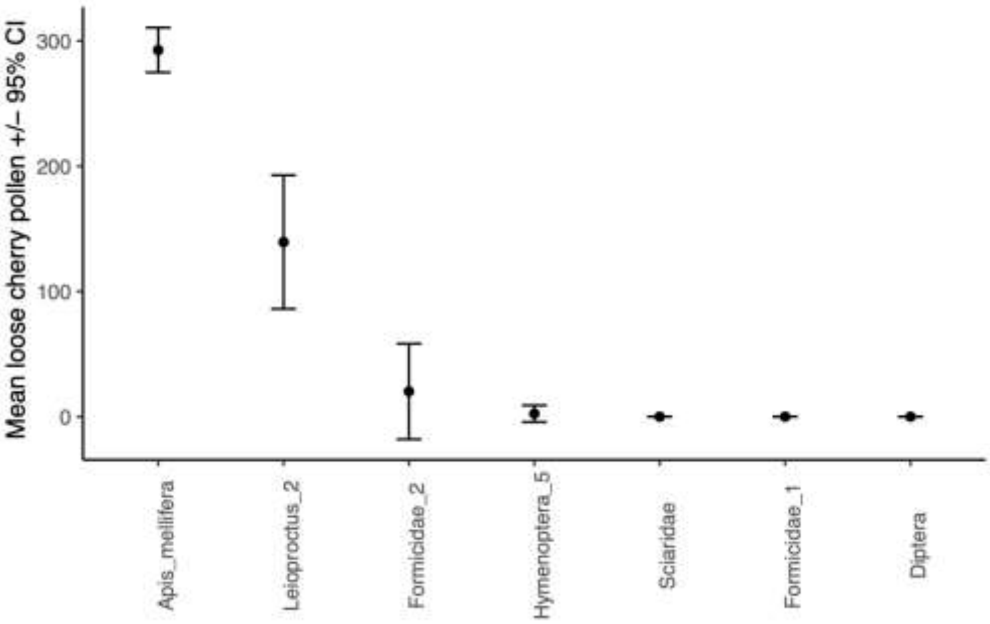


Figure 9 – ***Pollen transport for Ron's seedling cherry flowers***. *Average loose cherry pollen on bodies of insects visiting cherry flowers in Young in 2018. Loose cherry pollen on bodies of insect visitors differed by species (H6 = 72.699, p < 0.00001). Dunn's post-hoc test (with Bonferroni correction) found that A. mellifera had more loose cherry pollen than all other morphospecies. In addition, Honey bees on Ron's seedling and Lapins cherry cultivars did not differ in the amount of loose cherry pollen on their bodies (U = 278, p = 0.9736).*

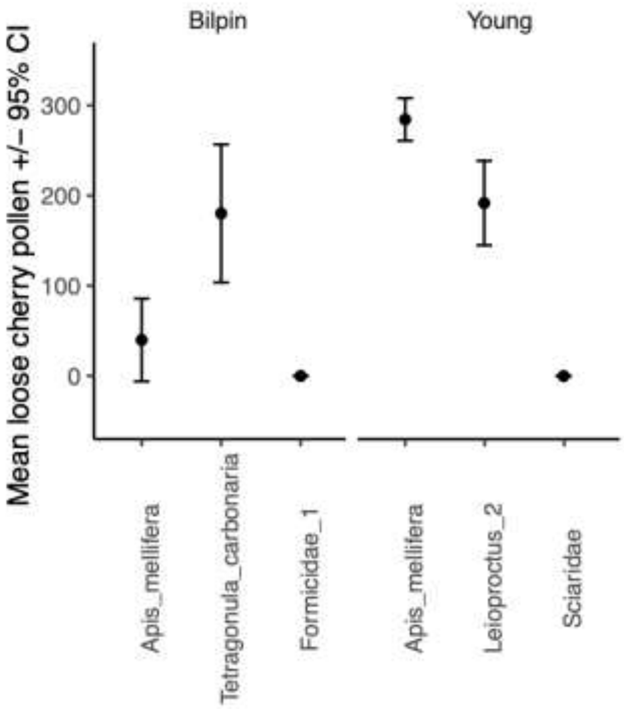


Figure 10 – ***Pollen transport for Lapins cherry flowers***. *Average loose cherry pollen on the bodies of insects visiting flowers in Bilpin (left) and Young (right) in 2018. T. carbonaria had significantly more loose pollen than A. mellifera or Formicidae sp. 1. (an ant). In Young, A. mellifera had significantly more pollen than Leioproctus sp. 2 (solitary bee) and Sciaridae sp. (fly). Meanwhile, A. mellifera in Young had significantly more loose pollen than the same species in Bilpin.*

2a. Database of non-crop floral resources used by pollinators on farm

Survey work in Bilpin, Orange and Young revealed that the non-crop floral resources growing on farms used by pollinators consist largely of common exotic weeds, such as dandelion, fumitory and *Plantago*, as well as clover, which some farmers also grow between crop rows or in patches to support pollinators. Meanwhile, the landscape surrounding focal orchards differed dramatically between Orange, where it was mostly other agricultural land offering similar weedy floral resources, and Bilpin where orchards were often bordered by native bushland. The native habitat offered a wide range of (mostly native plant) floral resources at different times of year. Please see Appendix 1, which details the key native flowering species within apple and cherry orchards in Bilpin. Details of the flowering timing and duration, as well as flower colour and the type of floral resources (nectar, pollen, other) which are used by different insect flower visitors.

2b. Analysis of seasonal floral resources used by bees.

Over a one-year period we quantified the flowering phenology, floral traits and flower visiting insects of 40 plant species from Cumberland Plain Woodland, a threatened ecological community located within the Sydney region. These plants are all grown by Greening Australia to produce seed for planting / restoration projects. We found that flowering diversity, floral traits (such as colour and structure) and insect visitation differed significantly across seasons. Flower colour and structure were the main drivers of flower-visiting insect abundance and species richness and were strongly intercorrelated. Open flower structures facilitating easy access to floral rewards were more commonly visited across insect groups. Meanwhile, we also found a range of unique associations between particular plant species and their specialist insect visitors. Overall, relationships between traits and insect visits differed greatly according to insect group and clear seasonal patterns were apparent for flowering plant richness ($H = 545.37$, $p < 0.001$), floral abundance ($H = 142.91$, $p < 0.001$), flower-visiting insect abundance ($\chi2 = 21.92$, $p<0.001$) and species richness ($\chi2 = 10.72$, $p<0.05$). Peaks in insect abundance and flowering plant species richness showed a trend of decreasing from spring towards summer, autumn and winter (Figures 11, 12).

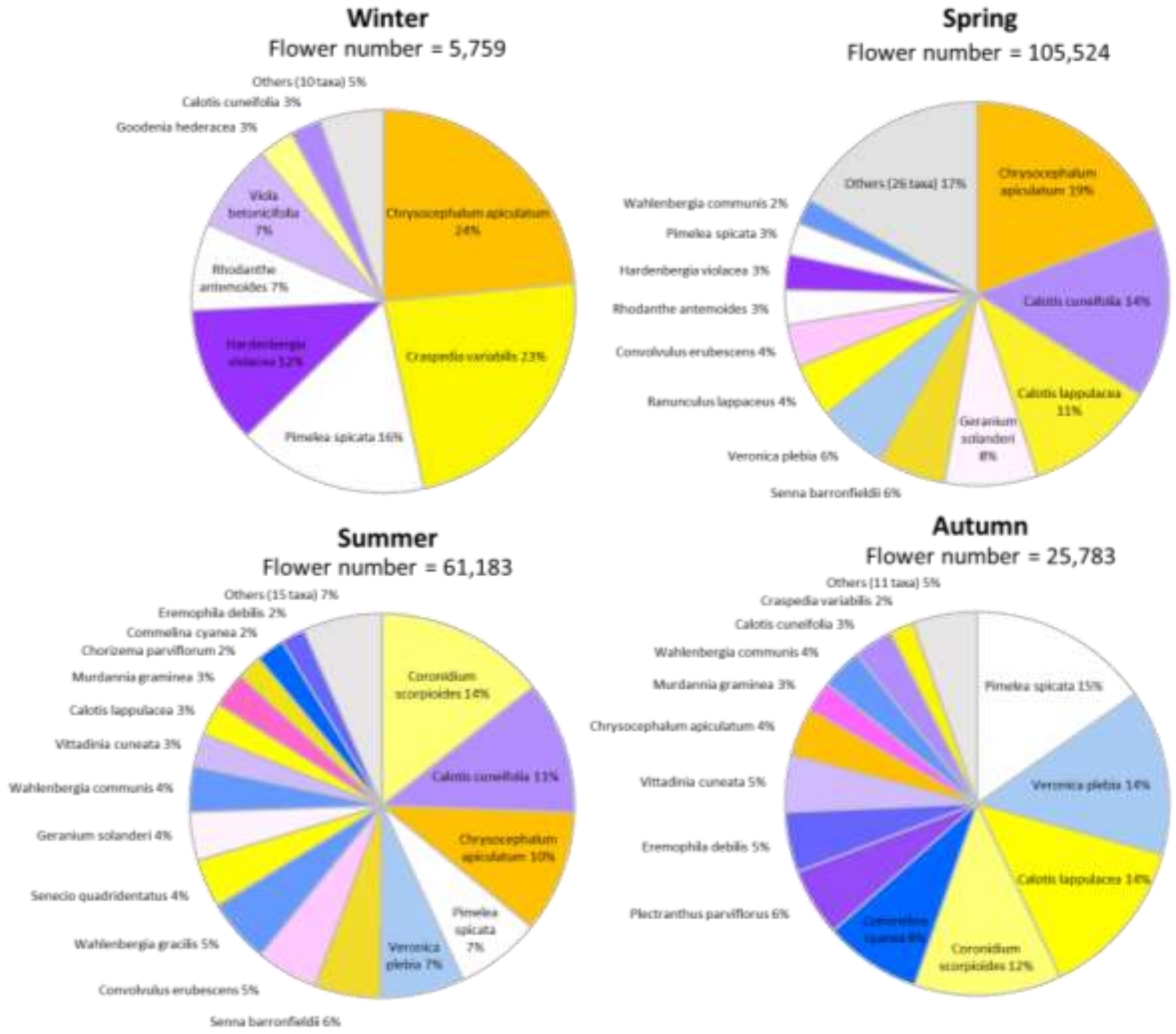


Figure 11. *The contribution of flowering plant taxa to total flower numbers in different seasons. Percentages are scaled for the total number of flowers counted per season. Taxa with > 2% contribution are shown, and all other taxa are grouped as 'Others'. Slice colour represents flower colour for each species. 'Flower number' indicates relative seasonal floral abundance and 'Plant taxa' flowering plant species richness. Lowercase letters indicate pairwise comparisons based on Tukey's Honestly Significant Difference (HSD) Tests, where treatments not sharing a letter are significantly different at α < 0.05.*

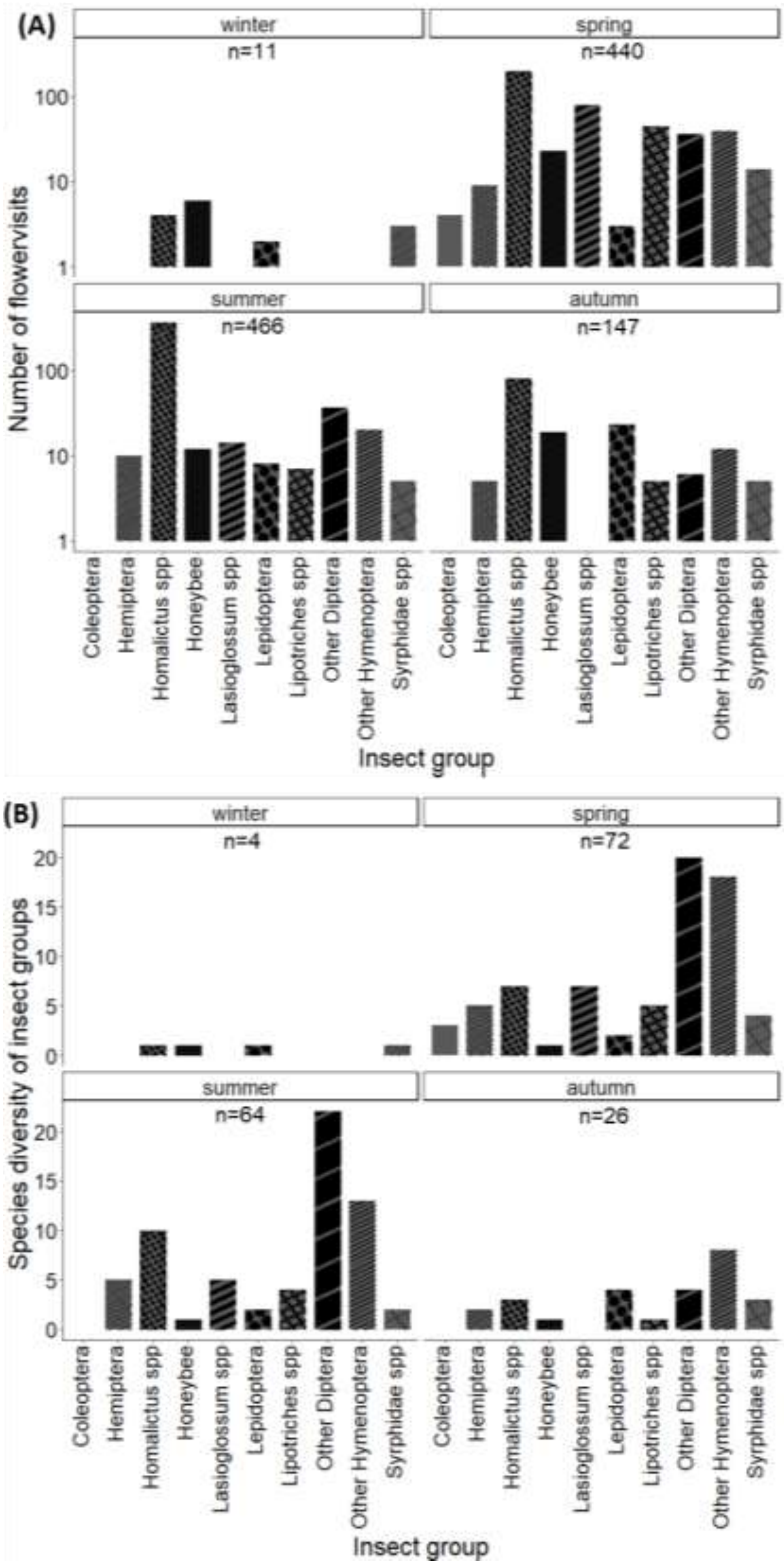


Figure 12. *(A) Number of flower visits made by each insect group (log-scale) to all flowering Cumberland Plain Woodland plant species for each season. (B) Species diversity recorded per insect group. In (A) 'n' indicates the total number of flower visits observed per season and in (B) the total number of insect species recorded per season. Lowercase letters indicate pairwise comparisons based on Tukey's Honestly Significant Difference (HSD) Tests, where treatments not sharing a letter are significantly different at α < 0.05.*

c. Analysis of value to bees of a panel of nursery cultivated native plant species

Through extensive surveys over a one-year period of 40 flowering Cumberland Plain Woodland species we found that a wide variety of insects such as native bees, wasps, flies and beetles rely on the floral resources produced by these plants. In general, we found that as the number of species that were in flower increased, so did flower visitation (Figure 13a) and flower visitor richness (Figure 13b). Likewise, we found a significant positive relationship between the number of flowers available and both the number of flower visits (Figure 13c) and flower visitor richness (Figure 13d). However, we also found a significant negative relationship between the length of the flowering window and the number of flower visits (Figure 13e), as well as flower visitor richness (Figure 13f).

d. Analysis of farm-level plantings to support bees.

Floral strips of either native (using species native to Cumberland Plain Woodland in Sydney) or exotic (using a commercially available pollinator friendly seed mix) were established within an apple orchard in Bilpin and within a horticultural context in Richmond, NSW. Diversity and abundance of native pollinators was highest on native strips, with 7700 visits from 270 morphospecies across an 11-month period, compared to 1400 Honey bee visits. Exotic strips recorded 3400 visits from 215 morphospecies, and 5800 Honeybee visits over 9 months. We observed 50% overlap of species between natives and apple flowers, and 40% overlap between natives and exotics. During winter months both native and exotic strips attracted high diversities and abundances of insects, but only exotic strips attracted Honeybees. During apple bloom 70% of Honeybee and 10% of native pollinator visits were to apple flowers. Our results suggest that exotic flower strips do provide attractive, resource-rich flowering patches, but that these are for relatively short durations, which leave a floral resource gap during late winter and most of spring (including during apple bloom). We also found that native perennial species were able to fill these ‘floral resource gaps’, while also providing resources for specialist pollinator species and encouraging a greater level of native pollinator diversity over much longer durations.

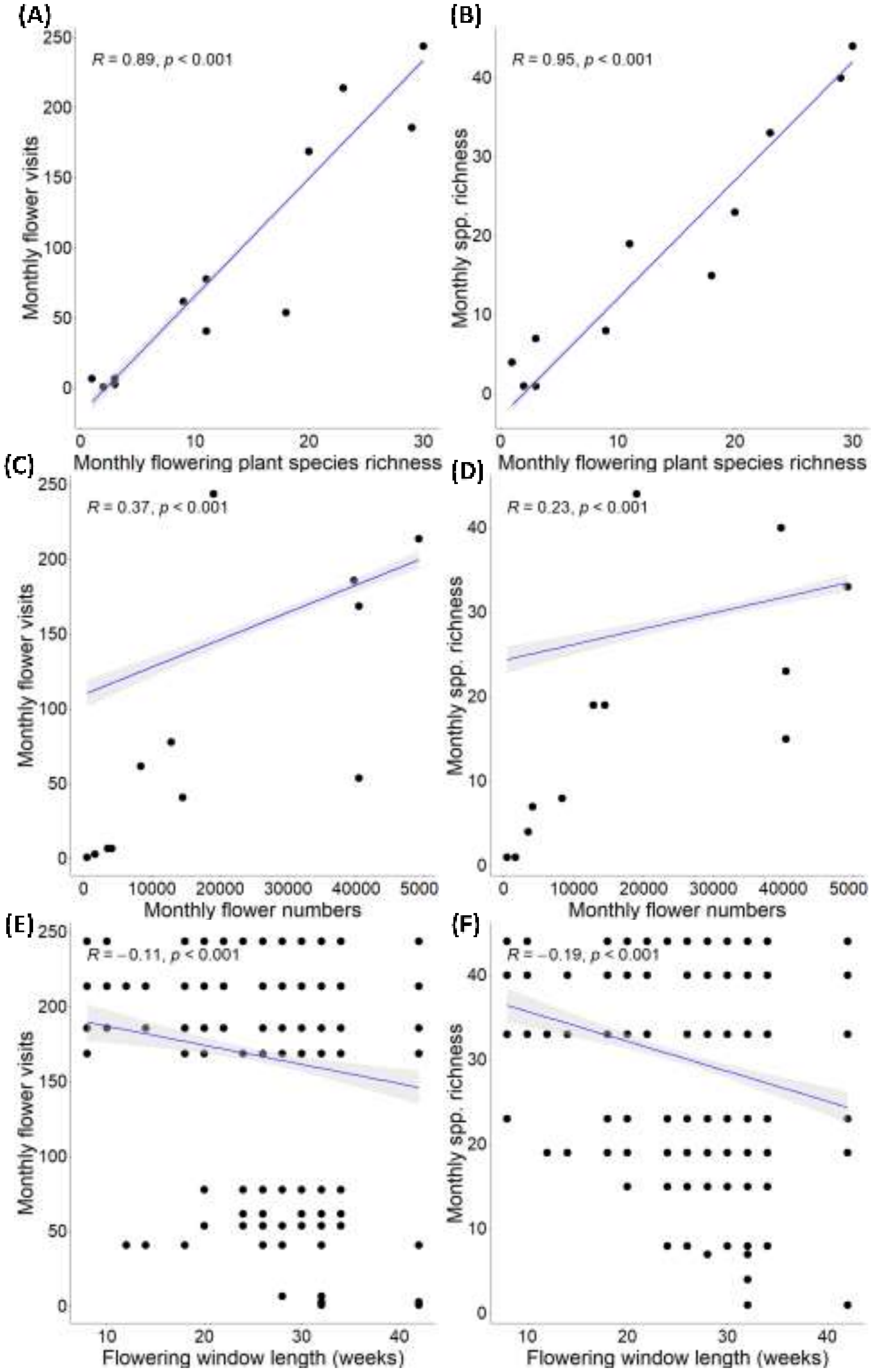


Figure 13. *Relationships between (A) monthly flower visits and monthly flowering spp richness, (B) monthly pollinator richness and monthly flowering plant species richness, (C) monthly flower visits and flower numbers, (D) monthly pollinator richness and total flower numbers produced per month and (E) monthly flower visits and flowering window length, (F) monthly pollinator richness and flowering window length in weeks across the year, of 40 herbaceous plant species native to Cumberland Plain Woodland growing at the Seed Production Area of Greening Australia in Richmond, NSW, Australia.*

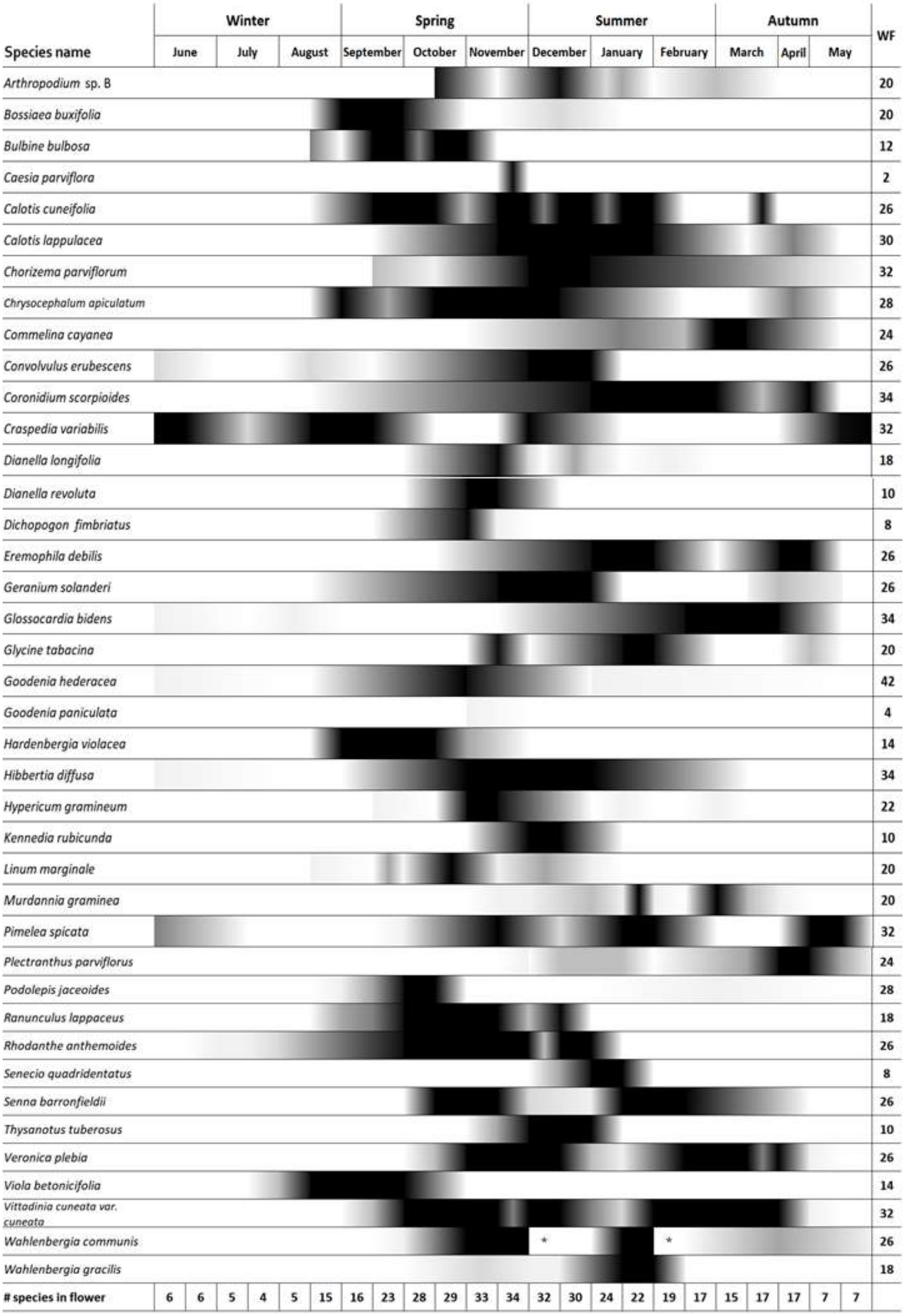


Figure 14. *Floral calendar illustrating flowering phenology for 40 herbaceous plant species native to Cumberland Plain Woodland growing at the Seed Production Area. Data shown were collated from fortnightly flowering phenology surveys. The darker the colour, the higher the flower number produced by individual species, black indicating flowering peaks (where relative flower numbers per plant species exceeded 50% of total flower numbers recorded during the one-year survey period). The last row shows the total number of plant species in flower per fortnightly sampling session. Asterisks indicate that plants within a plot were harvested for seed, with flowers therefore removed. The last column, 'WF', indicates the number of weeks each plant species was in flower. From this calendar, we selected 23 native flowering plant species for a seed mix which was sown into on-farm floral enhancements to contrast native vs exotic floral mixes and how they support wild pollinator populations.*

Work Package 3.

3a. Database of flowering windows for native species.

To assess the flowering window length of 40 Cumberland Plain Woodland species and to develop an accurate floral calendar, flowering phenology surveys were undertaken fortnightly by assessing flower number per plant, per species, and per growing cell (i.e. experimental plot) at the Greening Australia Seed Production Area at WSU for a full year (Figure 14).

3b. Evaluation of the impact of experimental climate manipulations on plant phenology and quality and quantity of nectar and pollen. Please see outcomes in next major section.

Work Package 4.

4a. Database of sequence data and host records for viruses of Australian bees. Sequence data and host records for viruses as reported in Brettell et al. (2020) have been deposited in Genbank, the open global repository for such molecular data. Host records are also summarised in Figure X below.

4b. Analysis of which viruses are shared and transmitted across coexisting bee species and which strains are specific to particular bee species. Common Honey bee-associated viruses are also found in co-occurring native pollinators in orchards, with BQCV by far the most common virus in both Honey bees and other insects. Black Queen Cell Virus was present in almost all individual Honey bees tested and in smaller proportions in all native pollinators (Figure 15). Sacbrood virus was moderately common in Honeybees and also appeared in small proportions of native pollinators. Interestingly, we also found the *Nosema ceranae* fungal pathogen in ground nesting bees as well as Honey bees.

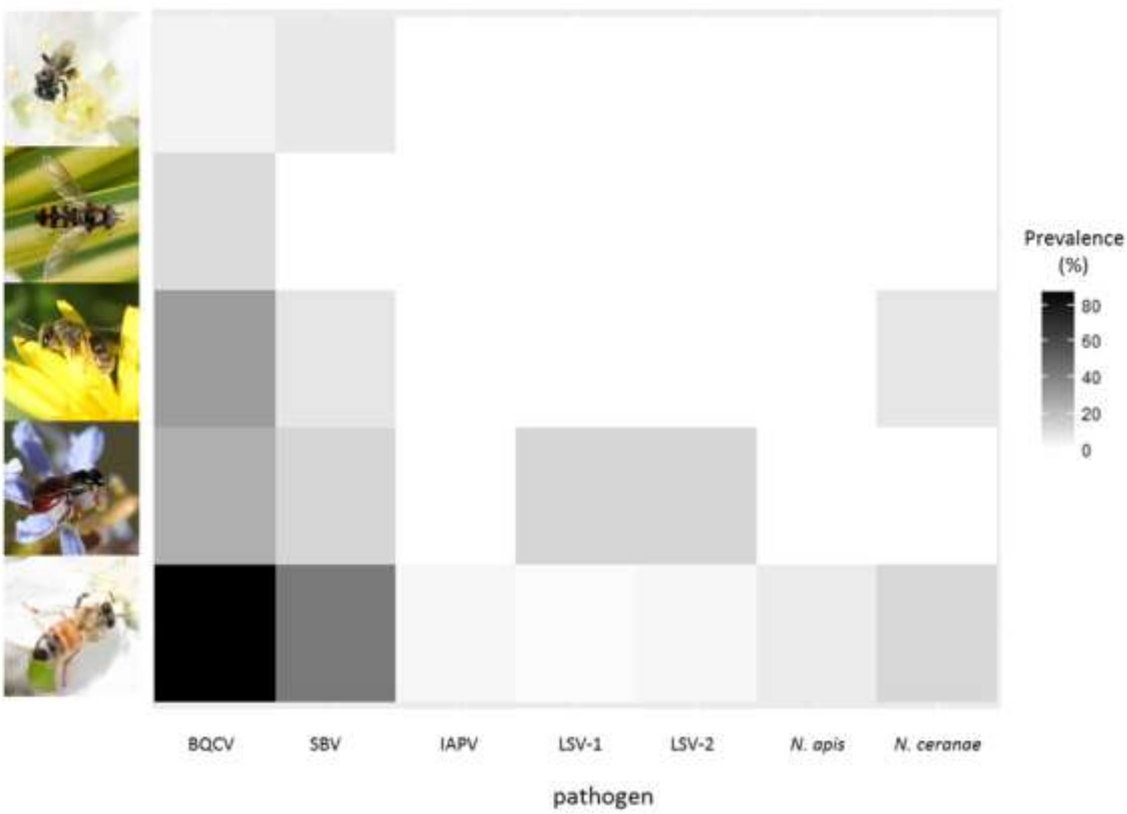


Figure 15. *Viruses in pollinators in Bilpin and Orange fruit orchards. Heat map showing the proportion of individuals of each insect group tested (Top to bottom: stingless bees, hoverflies, ground nesting bees, stem-nesting bees and Honey bees) that tested positive for the five viruses BQCV, SBV, IAPV, LSV1, LSV2 and the two microsporidian pathogens N. apis and N. ceranae. Darker represents higher levels of disease incidence.*

4c. Evaluation of disease incidence in co-located managed Honeybees and stingless bees versus wild-caught bees – stingless bees have very low disease incidence. Our studies of both wild stingless bees on orchard crop flowers (Brettell et al. 2020) and of managed stingless bee hives co-located with Honeybee hives (Roy 2021) showed that stingless bees in general have very low prevalence of known bee diseases. The only Honeybee-associated diseases we detected in stingless bees were BQCV and SBV, the commonest ones in Honey bees. It seems that native pollinators in general have very low incidences of known Honey bee-associated viruses, but most were detected in at least one native pollinator species (Figure 15).

Work Package Five:

a. Online web information for growers providing information on pollinators and recommendations on optimising farm-level practices to sustain pollinators

b. Printed materials for growers providing information on pollinators and recommendations on optimising farm-level practices to sustain pollinators.

(a and b) Throughout the project we generated information and materials targeted primarily at horticultural growers, but also beekeepers and the wider public. This involved using a wide range of media, including online fact sheets, videos and podcasts, in person workshops and field visits, as well as online Q&A sessions, printed grower magazine articles, and radio and TV appearances to discuss key issues. The full range of activities is listed in **Appendix 2**, with printed grower articles provided in **Appendix 3**.

India study visit (contract variation) and research links. The Study Tour was undertaken in April 2017 when 12 horticultural growers and HIA representatives, along with WSU researchers, to investigate how both stingless bees and crop pollination are managed in India. The Indian study tour involved two components – a south Indian tour focused on stingless bees and tropical crops, and a north Indian tour focused on apple and pear pollination. Growers and HIA representatives who attended the Study Tour in Kerala, India, April 2017 included:

Name	Organisation	E-mail address
William Kenneth Shields	Shields Orchard (apple)	wshields2@bigpond.com
Robert Wheatley	OLAM (almond)	Robert.wheatley@olamnet.com
Chris Searle	Macadamia grower	c.w.searle@bigpond.com
Ed Fagan	Mulyan Farms (vegetable)	ed@mulyan.com.au
Jill Houser	Mooloolah Valley Orchard (lychee)	algaeo@australianlychee.com.au
Tyson Milne	Seven Fields (mango)	Richard.Byllaardt@sevenfields.com.au
Lachlan Donovan	Avodon Bundaberg	lachlan@avodon.com.au
Mark Maclaughlan	Skybury (papaya)	mark@skybury.com.au
Paul McLaughlin	Desert Farms Pty Ltd. (melon)	Paul@pmgagriculture.com.au
Tim Heard	Sugarbag (stingless bees)	tim@sugarbag.net
Alok Kumar	Horticulture Innovation Australia	Alok.Kumar@horticulture.com.au
Ashley Zamek	Horticulture Innovation Australia	Ashley.Zamek@horticulture.com.au

The study tour (for full report see **Appendix 5 and 6**) also provided opportunities for presentations and roundtable discussions of existing Australian and Indian pollination research programs and stingless bees. In addition, there were both formal and informal discussions between WSU researchers, Australian growers / beekeeper and HIA staff on the potential of stingless bees in Australian crop pollination, and the need for targeted research to address open questions in this area.

Significant outputs stemming from this study tour include the subsequent project PH16000 “Stingless bees as effective managed pollinators for Australian horticulture” and the establishment of two ongoing dual PhDs between WSU and different Indian Universities. These are focused on almond pollination in Kashmir (Kashmir University of Agriculture, Science & Technology) and the role of non-crop floral resources in cropping landscapes (Haryana Agricultural University).

Outcomes

1. We have generated new knowledge on the diversity of insect pollinators visiting selected orchard crops in horticultural production areas in NSW, and the role of these various insect in crop pollination. Specifically, we have found that a combination of Honey bees and native bees are the key species providing pollination services to apple and sweet cherry, while almond relies almost entirely on Honey bees due to its late winter flowering. The key native bee species vary between regions. Stingless bees can be highly abundant crop visitors where they are present (e.g. Bilpin) but are not found in many apple and cherry growing regions. Solitary bees such as *Exoneura* and *Lasioglossum* native bee species both carry and deposit a lot of crop pollen, making them very efficient at the individual level. They are not currently as abundant as Honey bees but can play complementary roles and provide resilience in pollination services. Hoverflies are found very widely but usually made up only a few percent of all insect visitors to crop flowers (Robertson 2020). Nevertheless, they may contribute to the resilience of pollination services, especially during cold weather when some bees do not forage. In addition, the larvae of several species are predators of pests such as aphids.

Figure 16. *Apple pollinators in Bilpin. Clockwise from top left: Honey bee from managed hive marked with green dust; Honey bee from managed hive marked with red dust; native stingless bee (Tetragonula carbonaria); native fly. Photos – James Cook.*

2. We developed a method to assess the contribution of rented bee hives to pollination services on a farm. A question many growers ask themselves is whether it is worthwhile to rent Honey bee hives for pollination of their crops. While most of our (and many other) studies have made good assessments of the role of Honey bees versus other pollinators in a given situation, the relative role of wild bees versus those from rented hives is almost never known. We developed a technique that involves fitting on-farm hives with marking blocks that deposit coloured dusts on bees leaving the hive and then conducting surveys of marked (from farm hives) versus unmarked bees (not from farm hives) visiting crop flowers (Figure 16). This allows a test of the relative contribution of the rented hives and we will use this approach in the new project PH20002 "Loss of horticultural pollination services from wild insects following bushfires".

3. Evaluation of a range of plant species for use as floral resource enhancements at the farm scale. We conducted a series of surveys and experiments investigating the suitability of a large number of native floral species for supporting pollinators. The data collected provide the evidence for selecting sets of flowering plants that can support particular types of pollinators, and provide floral resources at particular times of year, including times that may represent floral resource shortages for resident bees and other pollinators.

4. Native plant on-farm floral enhancements support a wider range of wild pollinators compared to commercially available exotic seed mixes. Our study shows that low-maintenance long-lived perennial native floral strips can provide diverse and persistent floral resources for a wide range of insect groups, other than just bees. Overall, the attractiveness of both exotic and native strips differed across seasons and insect species, but also within insect groups in relation to the abundance and diversity of insect visitors. Exotic and native plant mixes can provide complementary benefits (Figure 17), with exotics boosting Honey bee numbers during crop flowering, while natives act to support pollinators throughout the year.

Figure 17. *Native (left) and exotic (right) floral strips planted within horticultural areas to provide alternative floral resources for pollinators. Photo – Lena Schmidt.*

5. Demonstration that non-crop floral diversity around farms can increase pollinator diversity and/or crop flower visitation by pollinators. We found that co-flowering floral resources available within and surrounding apple and cherry orchards support pollinators with vital nutrition during and outside the crop flowering period and can enhance pollinator visitation to apple (Figure 18) and cherry flowers (Figure 19) during crop flowering.

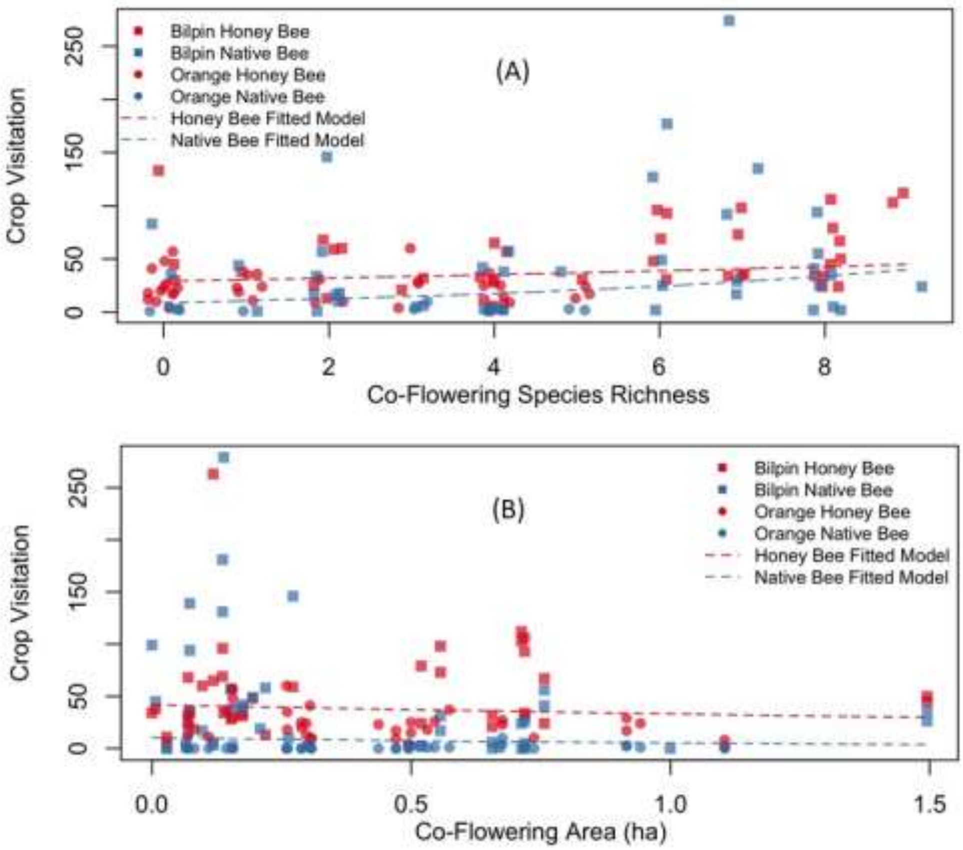


Figure 18. *(A) Apple crop visitation increases with combined plant species richness within the orchard and surrounding matrix for each species group (Honey bee and Native bees) for Bilpin and Orange. (B) But did not appear to increase with floral area (ha) within the orchard alone.*

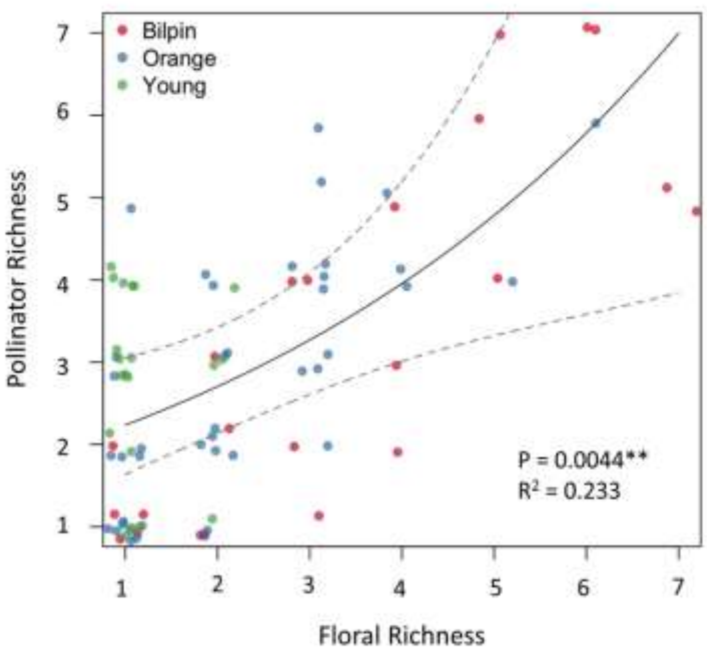


Figure 19*. In Cherry orchards, pollinator species diversity increases with the co-flowering floral species diversity (within cherry orchards and wider matrix combined) in Bilpin (red), Orange (Blue) and Young (green). Lines indicate the 95% confidence interval.*

6. Determination of the effects of elevated CO_2 on the phenology, quality and quantity of pollen and nectar produced by *Eucalyptus* trees. Eucalypts are one of the most important floral resources for Australian pollinators, and especially for Honey bees. We showed that elevated CO_2 does not decrease nectar quality in *Eucalyptus tereticornis* flowers. HPLC analysis of nectar found that nectar concentrations of the three sugars (Glucose, Sucrose and Fructose) did not differ between flowers grown under ambient or elevated CO_2 conditions. Additionally, elevated CO_2 did not change which insects visited *Eucalyptus* flowers, but reduced the number of Honey bees visiting (Figure 19).

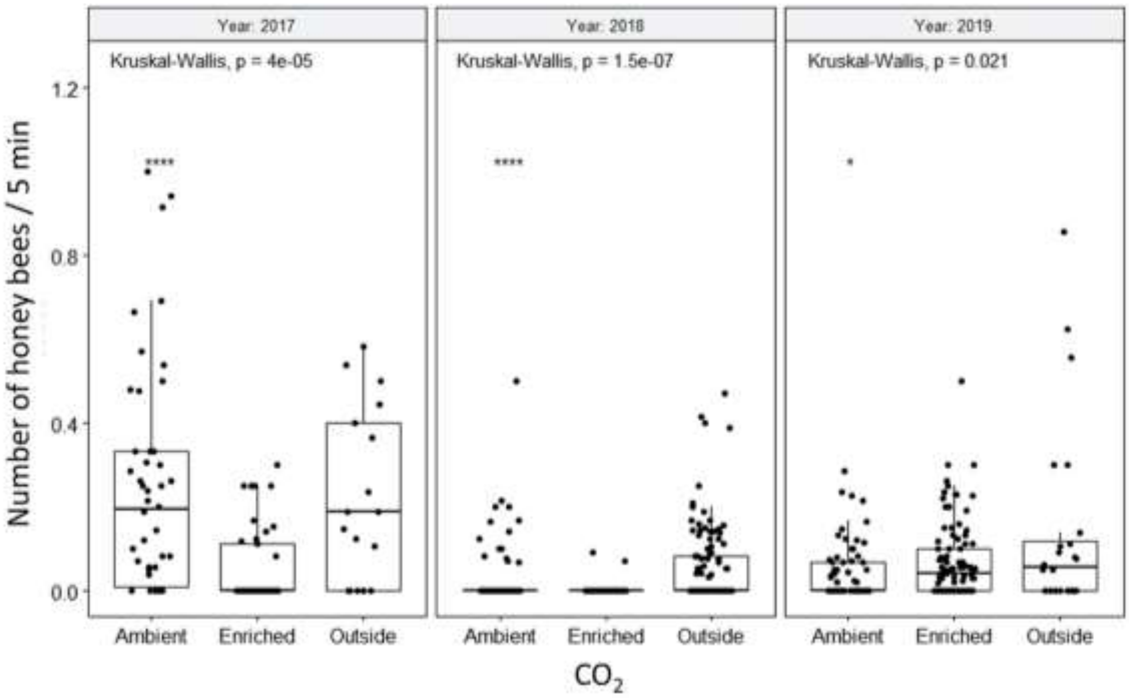


Figure 20. *The number of Honeybees (Apis mellifera) observed visiting Eucalyptus tereticornis flowers grown under ambient and enriched CO_2 in treatment rings, as well as outside of the treatment rings in 2017, 2018, 2019. A Non-parametric Kruskal Wallis analysis with a post-hoc Dunn's test (+holm correction) shows a significant difference across all treatments across all 3 years.*

7. Determination that the effects of elevated temperatures on plant phenology and nectar production can be strong but vary greatly between plant species. Responses of 10 native floral species to elevated temperatures were tested in a glasshouse experiment. Overall, responses varied greatly between species (Figure 20), but there was a general trend towards higher concentrations of most sugar constituents under elevated temperature (Figure 20).

Plant species	Nectar-producing flowers	Nectar quantity	Nectar quality	Reproductive biomass	Total biomass
Calotis cuneifolia	–	–	–	⇔	↓
Convolvulus erubescens	⇔	↑	↑	⇔	⇔
Craspedia variabilis	–	–	–	↓	↓
Hardenbergia violacea	–	–	–	–	↑
Geranium solanderi	↑	↑	⇔	↑	↑
Linum marginale	–	–	–	⇔	⇔
Plectranthus parviflorus	↑	↑	↑	↓	↓
Podolepis jaceoides	–	–	–	↑	↓
Ranunculus lappaceus	–	–	–	↑	↓
Senecio quadridentatus	–	–	–	↓	↓
Viola betonicifolia	–	–	–	↑	↑
Wahlenbergia gracilis	–	–	–	↑	↑

Figure 21: *Summary of individual species responses (nectar-flower production, nectar quantity & quality, reproductive biomass, total biomass) to elevated temperatures (+4°C) during the glasshouse experiment.*

8. Common Honeybee-associated viruses are also found in co-occurring native pollinators in orchards, with BQCV by far the most common virus in both Honeybees and other insects. Black Queen Cell Virus was present in almost all Honeybees tested and in much smaller proportions in all native pollinators (Figure 15). Sacbrood virus was moderately common in Honey bees and also appeared in small proportions of native pollinators. Interestingly, we also found the *Nosema ceranae* fungal pathogen in ground-nesting bees, as well as Honey bees.

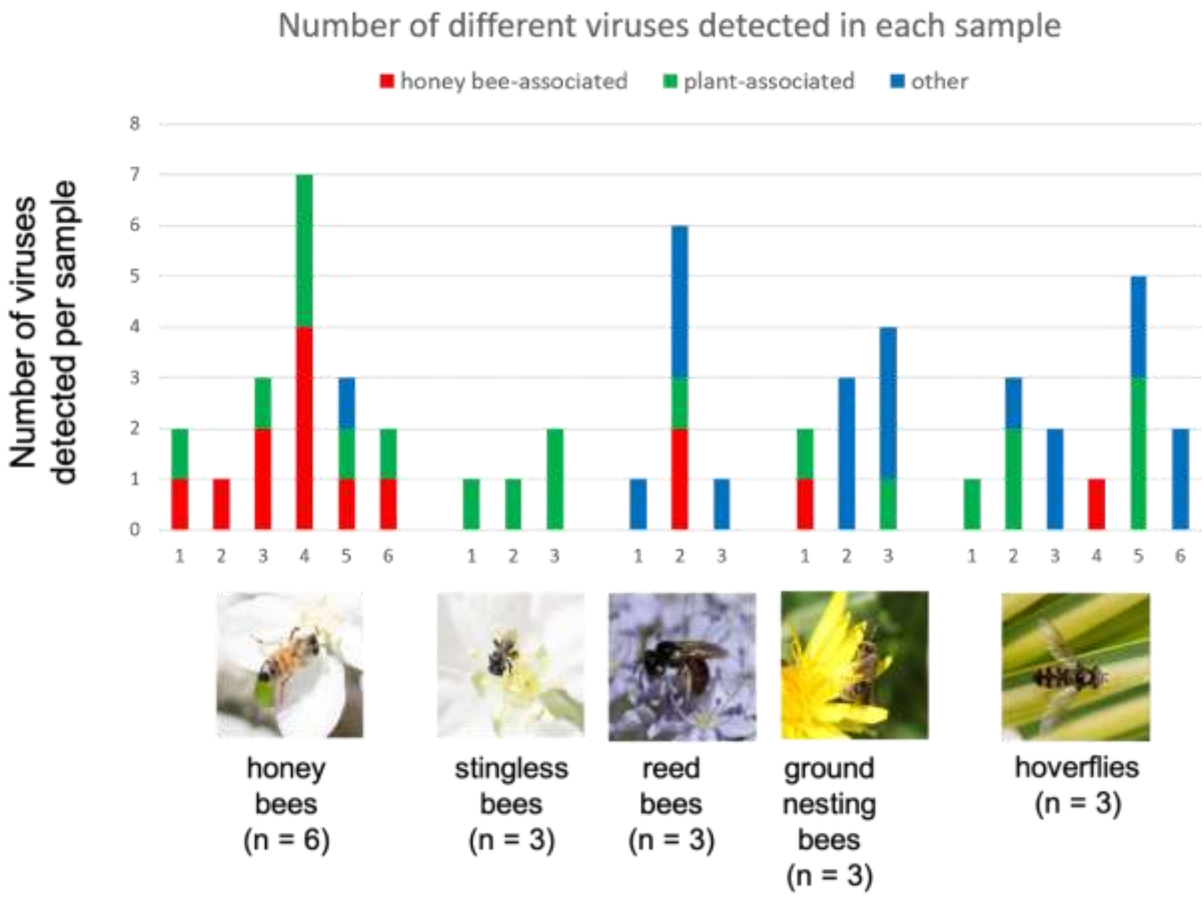
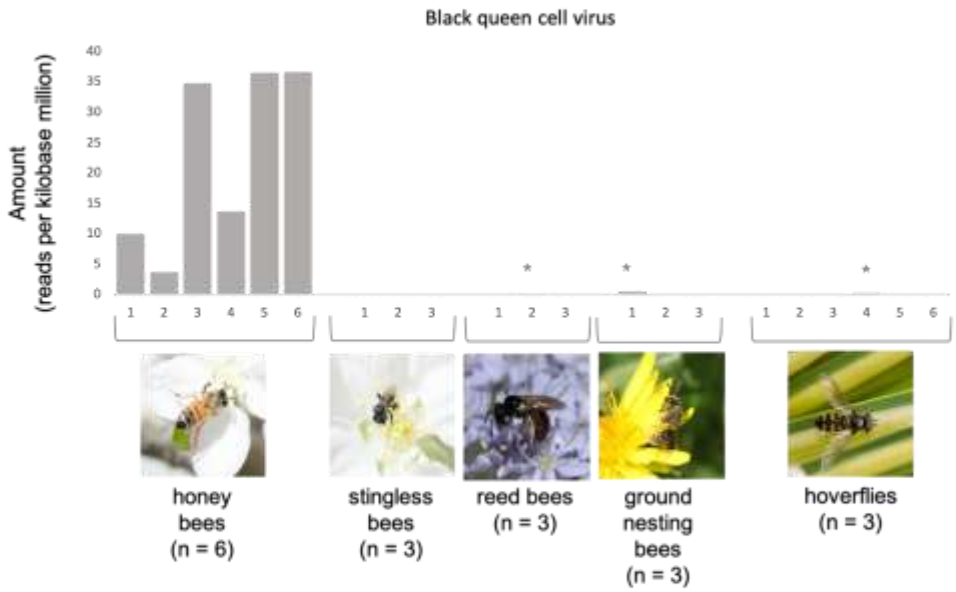


Figure 22: *The number of co-infecting viruses present in each individual (grouped by taxon). Histograms are coloured according to whether viruses are known Honey bee-infecting (red) or plant infecting viruses (green), or other novel virus fragments (blue).*

9. Identification of novel viruses infecting native Australian bees and preliminary evaluation of their effects. We did not detect any complete new viruses from Honey bees, native bees or hoverflies despite extensive and successful next generation sequencing and transcriptomics studies. However, we did find fragments of a number of undescribed viruses, as well as known Honey bee- and, interestingly, plant- associated viruses, often with multiple viruses per individual insect (Figure 21). Most viruses were present at very low levels, suggesting they were not active infections, but we saw larger amounts of BQCV and apple stem grooving virus (Figure 22), suggesting that these may actively replicate in native pollinators and require further study.



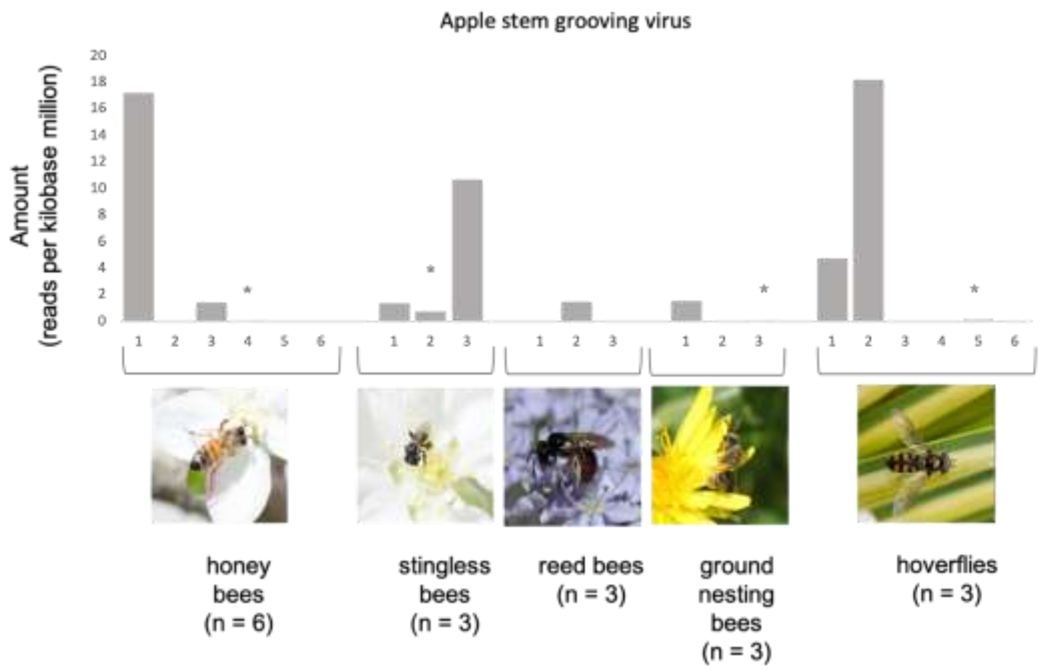


Figure 23. *The number of (a) black queen cell virus and (b) apple stem grooving virus (reads per kilobase million) in each individual sample of Honeybees, stingless bees, reed bees, ground nesting bees and hoverflies. Asterisks denote negligible virus levels. It was only Honey bees that harboured any level of BQCV that could suggest a true infection, whereas samples with higher levels of ASGV were spread across all taxa. However, we cannot yet say whether the virus was infecting the insects themselves or carried on their bodies.*

10. Increase in grower understanding of the importance and value of diverse pollinator species - beyond the well-known Honeybee - and key aspects of bee biology. This was achieved through a wide range of activities as detailed in **Appendix 2** and **Appendix 5**. Grower fact sheets are compiled in **Appendix 3** and grower magazine articles are compiled in **Appendix 4.**

11. Increased understanding by growers of current best practices for floral plantings, pest management and chemical use, to encourage healthy pollinator populations at a local level. This was achieved through a wide range of activities as detailed in Appendix 2 and Appendix 5. Grower fact sheets are compiled in **Appendix 3** and grower magazine articles are compiled in **Appendix 4.**

Monitoring and evaluation

Overall project performance was well aligned with the original aims and deliverables of this broad project:

Project deliverable	End results	Comments
Work Package One: a. Database on pollinator species visiting a range of horticultural crops under field conditions. b. Assessment of which native pollinator species transfer pollen between plants of selected key crop species, and the impact of their services on crop yield / quality.	Database established and analysed Pollen transfer tested for many insect species on apple and cherry and impact tested further on apple	Large data sets generated for these activities have already revealed results described in outputs section. They are being further analysed statistically to complete scientific publications (probably four in total) in progress This study highlights a key knowledge gap about relative role of wild versus managed Honey bees in field crop pollination
Work Package Two: a. Database of non-crop floral resources used by pollinator species on farms b. Analysis of seasonal floral resources by key bee species c. Analysis of value to bees of a panel of nursery-cultivated floral species d. Analysis of impacts of farm-level floral plantings to support bees	Database established and analysed Floral species value assessed by surveys and experiments Native and exotic plantings assessed	Large data sets generated for these activities have already revealed results described in outputs section. They are being further analysed statistically to complete scientific publications (probably four in total) in progress
Work Package Three: a. Database of flowering periods for key native floral species b. Evaluation of the impact of	Database established and analysed Large field experiment (EucFACE) on elevated CO_2 effects competed;	Large data sets generated for these activities have already revealed results described in outputs section. They are being further analysed statistically to complete scientific publications

experimental climate manipulations on plant flowering phenology and the quantity and quality of nectar and pollen produced for selected key plant species	glasshouse experiments on elevated temperature completed.	(probably three in total) in progress
Work Package Four: a. Database of sequence data and host records for viruses of Australian bees b. Analysis of which viruses are shared and transmitted across coexisting bee species and which strains are specific to particular bee species c. Evaluation of disease incidence in co-located managed Honeybees and stingless bees versus wild-caught bees	Database established and analysed Virus / host relationships and sharing analyzed and published Disease incidence data collected and analyzed	Large data sets generated for these activities have already revealed results described in outputs section. Two scientific papers have already been published (see publications section) with another 1 or 2 to follow
Work Package Five: a. Online web information for growers providing information on pollinators and recommendations on optimising farm-level practices to sustain pollinators. b. Printed materials for growers providing information on pollinators and recommendations on optimising farm-level practices to sustain pollinators.	Extension materials developed and made available via fact sheets, workshops, printed grower magazine articles and other media.	See Appendices 2-4.
India study visit contract variation	Successful study visit conducted with 9 Australian growers (across different industries) participating.	See Appendices 5 and 6

Recommendations

1. There are substantial differences between crop growing areas in the set of insect species providing pollination services to crops such as apple and cherry. Consequently, it is important to assess crop pollination services at regional level to better understand and manage local cropping situations.

2. For apples and cherries, pollination currently depends mainly on bees – both Honey bees and native bees. At the individual insect level, some solitary native bees are the most efficient pollinators, but highly social bees like Honey bees and stingless bees are the most effective overall, due to a combination of good efficiency at the individual level and very large numbers. The services of resident wild bees (both native bees and Honey bees) can be supported by retaining or enhancing non-crop floral resource diversity, both during, and especially outside of the main crop flowering period.

3. Honey bees are important pollinators of many crops, and many growers rent Honeybee hives for the crop pollination season. However, there are major knowledge gaps about the contribution of rented hives (versus wild Honey bees) to pollination services on farms. Both the return on rented hives and the risk of potential loss of wild Honey bee services therefore remain highly uncertain for most field cropping situations in Australia and should be priorities for future research.

4. Planting or retaining co-flowering non-crop floral species during, as well as outside of, the crop flowering period leads to a greater diversity and abundance of resident pollinators and increases their visitation rates to cherry and apple flowers. Consequently, to support and attract wild pollinators within horticultural areas, we recommend floral enhancement plantings that use a diverse range of native, locally sourced flowering species. Ideally, these should include a wide array of morphologies, colours and offer a variety of nectar and pollen resources year-round. Measures to benefit native pollinators are also likely to be beneficial to both wild and managed Honey bees.

5. Diverse co-flowering plant species can benefit local pollinator diversity and crop flower visitation by bees. We recommend floral enhancements /restorations that use locally sourced native species specific to the native habitat in the growing region. However, given the threat of climate change, a next step could be to develop more seed mixes to withstand predicted climate change across broader horticultural regions.

6. Our research has highlighted the need for diverse co-flowering species to help sustain and conserve pollinators. However, future research should further consider the availability of nesting opportunities in the habitat surrounding growing areas as this can also limit resident pollinator populations. Existing evidence from this and other projects suggests that some ground-nesting bees may increase in disturbed areas, due to greater accessibility of sunny areas of exposed soil for nesting. In contrast, stem and cavity nesting bees may be more reliant on diverse woody and non-woody (and generally perennial) plant species. For example, allodapine bees often nest in old fronds of tree ferns or stems of grass trees.

7. Native bees, and some other native pollinators, carry some known Honeybee viruses. These appear to be “spilling over” from the Honeybees to the native bees and were generally at much lower prevalence in the native insects than in the Honeybees. Future research should assess whether these pathogens cause symptomatic disease in the native insects, as they do in Honeybees.

8. Our virus screening revealed surprisingly high levels of Apple Stem Grooving Virus (ASGV) in Honeybees and some native pollinators. Such high levels of virus could indicate that ASGV replicates in insects as well as in plants and this should be investigated. Even if this is not the case, the high levels detected raise the possibility that pollinators may vector ASGV between trees and this deserves further study.

Refereed scientific publications

Published Journal Articles

Bigot, D., Dalmon A., Roy B., Hou C., Germain M., Romary M., Deng, S., Diao Q., Weinert L., Cook J.M., Herniou E.A., Gayral P. 2017. The Discovery of Halictivirus Resolves the Sinaivirus Phylogeny. *The Journal of General Virology* **98**, 2864–75. https://doi.org/10.1099/jgv.0.000957

Brettell L. E., Riegler, M., O'Brien C., Cook, J. M. 2020. Occurrence of Honey bee-associated pathogens in Varroa-free pollinator communities. *Journal of Invertebrate Pathology* **171**, 107344. https://doi.org/10.1016/j.jip.2020.107344

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Note: **Appendix 7** contains the combined pdf files of research articles that have already been published. Those currently in preparation or under peer review are not yet finalized, but will be forwarded to Hort Innovation when published in final form.

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Intellectual property, commercialisation and confidentiality

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Appendices

Appendix 1. Database of native flowering species used by pollinators

Appendix 2. List of outreach and extension efforts

Appendix 3. Grower fact sheets

*Appendix 4. Industry and outreach magazine articles combined

Appendix 5. Report on India study tour

*Appendix 6. Photos from India study tour

*Appendix 7. Combined pdf file of articles already published in research journals

*Material in appendices 4,6 and 7 may be subject to copyright and/or talent release restrictions and not currently available to publish as part of a public version of this report. Appendices 4 and 7 include several articles published in magazines and journals. Copyright for at least some of these lies with their respective publishers. Appendix 6 includes photos from the India study tour for internal use that include many people from whom we do not have talent release permissions for use in wider publications.