The use of trap-nests to support crop pollinators in agricultural areas.

Amy-Marie Gilpin[1*], Laura E. Brettell[1,2], James M. Cook[1] and Sally A. Power[1].

[1]*Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW, 2751, Australia.*

[2]*Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, United Kingdom.*

**Corresponding author.*

Abstract

Supporting and promoting invertebrate diversity within agricultural ecosystems has numerous benefits, including the provision of pollination services. Many insects, including wild pollinators, require floral resources for food and structural habitat for nesting. To support pollinators, research studies and agri-environment schemes have sought to supplement floral resources, but little is known about the value of different types of nesting habitat enhancements (e.g. trap-nests or bee hotels). We deployed eight replicates of each of three types (bamboo reed, hardwood block and sand/cement brick) of trap-nests at five orchards in two apple and cherry growing regions (Bilpin and Orange) in Australia. Both reed and hardwood block trap-nests attracted a diverse array of invertebrates, such as ants, wasps, spiders and bees, including a cleptoparasitic bee species (*Thyreus* sp) not previously recorded in the region. Interestingly, two taxa of native bees (*Megachile* (Megachile) and *Megachile* (Eutricharaea)) used the artificial nests and were also observed visiting apple crops. There were significantly more native bees using trap-nests in Orange (n = 65), where orchards are surrounded by agricultural landscapes, than in Bilpin (n = 2), where orchards are surrounded by native forests. Our findings show that artificial nest enhancements are used by native bees, as well as other non-target invertebrate taxa, some of which can be predators of bees (ants, wasps and spiders). Nesting habitat augmentation thus has potential to be used as a conservation tool, especially in areas where nesting sites are limited. However, future studies should also consider measures to reduce colonisation by non-target taxa.

Keywords: Bee hotel; artificial nesting habitat enhancement; crop pollination; wild pollinators; wildfire.

Introduction

Pollinators provide a crucial ecosystem service for approximately 90% of plant species (Kearns et al., 1998), including many crops, such as apples (*Malus domestica*) and sweet cherry (*Prunus avium* L.), in which insect pollination is associated with high fruit quality (Garratt et al., 2014) and/or yield (Holzschuh et al., 2012). The role of native pollinators in providing pollination services to crops (Kremen et al., 2002 Garibaldi et al., 2013) is increasingly important given widespread threats to honeybees, the dominant managed pollinator worldwide (Gill et al., 2016). However, agricultural intensification generally leads to both habitat fragmentation (Ricketts et al., 2008) and increased use of agrochemicals (Iwasaki & Hogendoorn, 2021; Siviter & Muth, 2020). These stresses, along with the shift from native flowering systems to highly seasonal crop floral resources, have meant that many native pollinators are threatened (Winfree, 2010) and, in some cases, have been lost from their natural ranges (Boyle & Philogène, 1983; Brown & Paxton, 2009; Lima & Marchioro, 2021).

Agricultural landscapes typically have limited native habitat and often suffer from a simplification in terms of both floral diversity and vegetation structure (Landis, 2017). Agri-environment schemes, which seek to address these deficiencies (Kohler et al., 2008), are being increasingly adopted in many areas of Europe (Albrecht et al., 2020; McKerchar et al., 2020), North America (Hannon & Sisk, 2009) and Canada (Gervais et al., 2021). This can have important implications for wild insect distribution and abundance (Evans et al., 2018), considering the otherwise typically reduced availability of food resources and nesting sites (Kim et al., 2006) in agricultural landscapes. The benefits of conserving invertebrate diversity within agricultural systems are several, including biological pest control and improvements to soil health through processes such as soil aeration and decomposition (New, 2005; Saunders, 2018), all of which are likely to lead to more resilient and functional agro-ecosystems.

Crops such as apples and many sweet cherry varieties are heavily dependent on insect pollinators to set fruit, with many growers using honeybee (*Apis mellifera* L) hives during the crop flowering period to achieve pollination (Cunningham et al., 2002; Eeraerts et al., 2020; Pardo & Borges, 2020). However, there is mounting evidence that honeybees are not as effective as some wild pollinators (often native bees) for certain crops (Garibaldi et al., 2013; Rader et al., 2016), including cherry (Eeraerts et al., 2020; Holzschuh et al., 2012) and apple (Malinger & Gratton, 2014). Therefore, developing ways to enhance or retain wild insect pollinator populations within agricultural landscapes is crucial to not only ensuring that agricultural production is secure and not over-reliant on one pollinator species, but also to maintain and conserve pollinator populations more generally within these modified landscapes. This should, in turn, lead to pollination systems that are more resilient to perturbations, as each pollinator species responds differently to environmental fluctuations (Senapathi et al., 2015).

Currently, very little is known about the nesting habitat requirements and nesting preferences of wild native bees (Harmon-Threatt, 2020), especially for solitary bees and in countries outside of Europe and North America (Brown et al., 2020). Understanding the requirements for nest site selection and their availability within agricultural landscapes is important given the extent to which wild insect pollinators (Garibaldi et al., 2013; Rader et al., 2016), including native solitary bees, contribute to crop production (Bänsch et al., 2021; Woodcock et al., 2013). Nesting habitat support, in addition to floral resource availability, is important for not only stabilising pollination services delivered to crops, but also for increasing the functional and taxonomic diversity of pollinators (Evans, et al., 2018; Kim et al., 2006; Kline & Joshi, 2020). In addition, it may support other invertebrate groups within agricultural systems that also assist in ecosystem function (Jankielsohn, 2018; New, 2005). Further, understanding nesting requirements is crucial for conservation initiatives within these highly altered systems, given the extent to which native vegetation is often removed and the floral landscape simplified (Landis, 2017). One way to augment wild pollinator nesting habitat, which has recently grown in popularity, is the installation of trap-nests, otherwise known as bee hotels (MacIvor & Packer, 2015).

Trap-nests are typically artificial nesting structures made from wood, bamboo, reeds, paper straws, rammed earth, sand and cement mix or plastic, designed to attract cavity-nesting semi-social or solitary bees. Studies have shown that trap-nests can also provide a nesting or habitat resource for various other types of insects, including wasps (MacIvor & Packer, 2015; Staab et al., 2018) although knowledge of the degree to which crop pollinators or insects more broadly use trap-nests in agroecosystems is limited. The utilisation of trap-nests by invertebrates is influenced by many factors, including their innate nesting or habitat preferences e.g. for wood, stems or compact sand or soil. The time of year may also affect trap-nest use, with, for example, native bee activity typically more focussed in the warmer months (Dollin et al., 2016), while ants and spiders are active throughout the year. Invertebrate use of trap-nests is also likely to depend on the availability of nesting habitat within the local area.

Our experiment sought to determine the efficacy of trap-nests as a tool for enhancing nesting habitat and to evaluate nesting habitat preferences of insects within an agricultural setting across two apple and cherry growing regions in Australia. Specifically, we ask: 1) Which invertebrate species use artificial nesting habitat and does this change throughout the year and between differing vegetation landscapes (areas with high and low amounts of native semi-natural vegetation)? 2) Do trap-nests provide a nesting habitat resource for known pollinators of apple and cherry and/or the wider bee community? We also make comment on the colonisation of artificial nests following a major wildfire.

Methods

Experimental design

Three different types of trap-nests (bamboo reed, hardwood block and sand and cement bricks) were introduced to five orchards in each of two apple and cherry growing regions - Bilpin (33.5000° S, 150.5333° E) and Orange (33.2833° S, 149.1000° E) in New South Wales, Australia (Figure 1). The three different types of trap-nests were selected based on the nesting habitat preferences of native bees (according to the Atlas of Living Australia occurrence records 2020 a and b) whose typical ranges overlap the study regions. Orchards were separated from each other by an average of 3 km ±0.6 with a minimum of 700 m in Bilpin and 2 km ±0.3 with a minimum of 860 m in Orange. All orchards in Bilpin grew apples with two also growing cherry, whilst in Orange, three orchards grew both apple and cherry, one grew only apple and one only cherry. These two regions have clear differences in vegetation types surrounding the study orchards, with Orange featuring highly altered agricultural landscapes dominated by pastoral grazing land (Figure 1). In contrast, all study orchards at Bilpin were directly adjacent to vast stands of native bushland within the Blue Mountains and Wollemi National Parks (Figure 1).

Within each orchard, two sites were selected, approximately 500 metres apart, ensuring they were each in a warm, sunny and elevated (so as to prevent flooding) position on the edge of the orchard, to avoid chemical sprays. Four of each trap-nest type (reed, hardwood and sand/cement) were placed in each of the two locations. Trap nests were placed out at each orchard in August 2018 and remained there until May (Bilpin) and June (Orange)

2020. Trap-nests at one orchard in Bilpin were destroyed in a wildfire in January 2020 and, as such, data for this location were not collected past this point. Reed and hardwood block trap-nests were placed off the ground using existing features (e.g. rocks, trees or on top of abandoned farm equipment), in an effort to minimise predation by ants and water logging, while sand and cement brick trap-nests were positioned on the ground adjacent to the reed and hardwood trap nests in an effort to best replicate the nesting habitat of ground-nesting bees (Smith & Heard, 2016).

Trap-nest design

Bamboo reed trap-nests

These comprised of bamboo internode lengths (100-140 mm long) that were closed at one end. Approximately 60 lengths were bundled together using cable ties and placed within a 150 mm length of 90 mm diameter PVC pipe (see Figure 2a), with the pipe providing a protective overhang of at least 10 mm above the ends of the bamboo reeds. Each bundle contained bamboo lengths that ranged in diameter from ~3-12 mm, to ensure that they were attractive to a diverse range of bees (Smith & Heard, 2016).

Hardwood block trap-nest

Each of the hardwood blocks (90 mm x 45 mm x 200 mm) had 9 holes (three each of three sizes of hole - 5.5 mm, 6.5 mm and 8.5 mm), with two holes of each size in one end and one in the other. Each hole was drilled to approximately 150 mm depth, as recommended by Smith & Heard, (2016). Using a blow torch, the entrance to each hole was lightly charred to remove splinters and smooth the entrance (Smith & Heard, 2016). Paper straws (5 mm, 6 mm and 8 mm diameter) were placed into each of the drilled holes to allow for nest removal in the field. Four hardwood blocks were fixed together and fitted with a corrugated plastic (corflute) roof (see Figure 2b) for protection against sun and rain.

Sand and cement brick trap-nest

Brick trap-nests were made from premixed sand and cement mix, and sand (1:2 ratio) following similar methods to those described in Halcroft, (2018). After being combined with water, the mixture was placed into a mould constructed from a 150 mm length of 90 mm PVC pipe, to within approximately 20 mm of the edge of the pipe, with this overhang providing a protective roof. Using an 8 mm drill bit, three starter holes were made into one face on each of the bricks to a depth of 20 mm (see Figure 2c).

Identification of trap-nest inhabitants

Various inhabitants may take up occupancy in trap-nests and the most direct way to identify them precisely is by observing the adults entering or leaving the nest hole, or the subsequent offspring emerging. The former can consume a prohibitively large amount of observer time, while the latter requires a delay of months and is not always successful since nothing may emerge, or the emergent insects may be parasites of the original nest inhabitants. However, fortunately, coarse identification is relatively easy, since invertebrates differ in the type of nest hole covering (e.g. mud, resin, silk) that they produce (Halcroft & Batley, 2014). This provides a straightforward way to make repeat censuses of trap-nest occupancy by different invertebrate groups.

Thus, the number of nests or structures (open and closed) created in each of the trap-nests were counted approximately every 6-8 weeks to allow for colonisation between sampling events (differences were due to inclement weather, bushfires and COVID-19 travel restrictions). Nests and structures created within the trap-nests were categorised by the materials used to construct the nests/structures and were compared to reference identification guides for Australian native bees and invertebrates (Dollin et al., 2016; Halcroft & Batley, 2014; Houston, 2018). Trap-nest occupancy data were then used to compare use of the different trap-nest types between the study regions. Due to the non-normal distribution and upper limit to the occupancy data, analyses were undertaken using non-parametric Mann-Whitney U tests in R version 4.1.0 (R Core Team, 2021).

Additionally, five-minute observations of trap-nest visitors were undertaken on each sampling occasion following nest counts, with any invertebrates that were either inside, or that came into contact with, the artificial trap-nest recorded. After observations had concluded, any bamboo reeds or paper straws (in the case of the hardwood block [see methods above]) that had evidence of occupancy were removed and transported to the laboratory in an ice cooler box where they were stored individually at -20°C for later identification. Where possible, inhabitants were identified in the field or under a dissecting microscope using reference books (Dollin et al., 2016; Halcroft & Batley, 2014).

To determine whether trap-nests provided habitat for insects known to visit apple and cherry crops, or if they are used by other invertebrates, we compared our occupancy/visitation data to previous surveys of visitors to apple (Gilpin et al. *In review*) and cherry flowers (Gilpin et al., 2022) in the same regions and to records found in the Atlas of Living Australia (ALA) (Atlas of Living Australia, 2020 a, b). Our search of the ALA was limited to species within the four native bee families - Apidae, Colletidae, Halictidae and Megachilidae - known to inhabit either region (Bilpin or Orange). We searched for all records of each family of native bee within a 10-kilometre radius encompassing the study orchards in both Bilpin and Orange. For quality control, ALA records were limited to those validated or submitted by the Australian Museum, OZCAM (Online Zoological Collections of Australian Museums) and the Pest and Disease Image Library PaDIL Bee (PaDIL website at http://www.padil.gov.au). Comparisons with previously collected observation data and ALA records were limited to native bees as this could be determined through nesting structure and nesting materials used, whereas other taxonomic groups could only be identified to higher levels, making it unfeasible to compare between datasets.

Invertebrate colonisation of trap-nests following a major bushfire

In December 2019 a large out-of-control wildfire swept through Bilpin, severely burning the native vegetation surrounding each of the five study orchards and, in some cases, also burning parts of the orchards (with all of the artificial nests at one site burnt). Subsequently, this provided us with a unique opportunity to observe invertebrate colonisation directly after the fire, although colonisation was only observed for 4 months post-fire due to the introduction of COVID-19 travel restrictions.

Results

Identification of nest inhabitants.

Nests were classified into nine categories, based upon the materials used and the colour of the nest. The categories were: red resin, masticated leaf tissue, resin/sticks, leaf, mud, sand/plant material, soil/sand, web and unknown burrow (Figure S1). Therefore the most likely inhabitants of the nests were determined to be:

1. Megachilid resin bees (subgenus *Megachile*) in the red resin, masticated leaf and the resin/stick nests,
2. Megachilid leaf-cutter bees (subgenus *Eutricharaea*) in the leaf nests,
3. Wasps (*Abispa ephippium*) in the mud nests,
4. Ants in the sand/plant material nests,
5. Spiders in the web structures

6. Termites in the soil/sand nests.

Trap-nest use, by type

Bamboo reed trap-nest

In Bilpin, nest and structure counts revealed that bamboo trap-nests were most frequently used by ants (37 nests observed) followed by spiders (11) (Table 1). Both groups primarily created nests and structures between the reeds, rather than using the reeds themselves, commonly colonising the entire trap-nest structure. Within the reeds, we recorded wasp nests (8) and red resin megachilid bee nests (1) (Table 1). In Orange, bamboo reed nests harboured wasp nests (26), along with ants (12), red resin nests (12), masticated leaf nests (5), spider webs (8) and leaf-cutter bee nests (2) (Table 1). During the five-minute trap-nest observations conducted throughout the study period, in both Bilpin and Orange, we also observed spiders using the trap-nests. Only one megachilid bee was observed making a nest; this was in summer 2019 at Orange.

Hardwood block trap-nest

Nest and structure counts revealed that hardwood block trap-nests located in Bilpin were most frequently used by ants (101), followed by spiders (41), wasps (31), termites (12) and a single red resin megachilid bee (Table 1). In Orange, hardwood block trap-nests were again most frequently used by wasps (127), ants (113), spiders (20) and megachilid bees, including those that made nests from red resin (23) and masticated leaf (20), as well as a combination of stick and resin (3) (Table 1).

During trap-nest observations, ants and spiders were frequently observed within hardwood block trap-nests at both Bilpin and Orange. Native bees were only observed visiting hardwood trap-nests during summer 2019 at Orange; this included a distinctive cuckoo bee (*Thyreus* sp), not previously recorded on the ALA database within this area. At two orchards in Orange, megachilid bees were observed making red resin nests.

Sand and cement brick trap-nests

Nest and structure counts revealed that sand and cement brick trap-nests in Bilpin were mostly used by spiders (26) and wasps (3) (Table 1). There was some evidence that inhabitants had been digging entrance holes, with 8 separate tunnels observed. Only a few invertebrates were associated with the sand and cement brick trap-nests in Orange, with comparatively few spider webs (3) and evidence of only two burrows (Table 1). Only the occasional spider was seen using the sand and cement brick trap-nests during the five-minute observations.

How does nest occupancy change throughout the year and following fire?

Bamboo reed trap-nests

In Bilpin, ant nests and spider webs were recorded during observations throughout summer, winter and spring. Wasp nests were only recorded twice - once in spring and again in summer - and red resin nests were observed once in summer (February) (Figure 3a). In Orange, red resin nests were observed in spring (September), summer (January and February) and at the start of winter (May) during the 16-month survey period (Figure 3b). A similar pattern of occupancy was seen for wasp nests. Masticated leaf nests were observed during summer, autumn and at the start of winter while ant nests were recorded during late summer (February) and late spring (May; Figure 3b).

Hardwood block trap-nests

Ant and wasp nests were generally observed year-round in the hardwood blocks at both Bilpin (Figure 3a) and Orange (Figure 3b). Red resin bee nests were recorded during summer in both Bilpin and Orange and also at the beginning of winter and spring in Orange. Masticated leaf bee nests were recorded during summer, autumn and at the beginning of winter in Orange (Figure 3b). Stick and resin bee nests were observed at the start of winter and again in summer at Orange (Figure 3b), while spider webs were observed across all seasons in both regions.

Sand and cement brick trap-nests

Very few invertebrates used the sand and cement bricks compared to the reed and hardwood block trap-nests. Spider webs were generally seen in the sand and cement bricks throughout the year (8 of 10 observations) in Bilpin (Figure 3a) and in summer, winter and spring in Orange (Figure 3b). Wasp nests were observed in March 2019 and January 2020 in Bilpin (Figure 3a). Both wasp nests and spider webs were restricted to the pre-drilled holes made into the bricks, with no evidence of fresh excavations.

Invertebrate colonisation of trap-nests postfire in Bilpin

Between 14/1/2020 (first observation after the fire on 21/12/2019) and the last observation of the trap-nests (1/5/2020), there were no new nests made by native bees in any of the three trap-nest types in Bilpin. However, less than a month after the fires we observed, ant nests (14), a wasp nest (1), spider webs (13) and approximately four months later we observed structures made by termites (12) in the hardwood blocks. Four months after the fire spider webs (2) were observed in the bamboo reed trap-nests and the sand/cement blocks (1) as well as burrows made by an unidentified species (8) (Figure 3a).

Differences in trap-nest use between regions

We found that significantly more native bees (both subgenera - *Megachile* and *Eutricharaea* combined) used both the reed (W=60542, p = 0.01) and hardwood block trap-nests (W=3538, p = 0.0001) in Orange compared to Bilpin (Table 1 and 2). In addition, we found significantly more wasps used the reed (W=60196, p = 0.02) and hardwood block trap-nests in Orange compared to Bilpin (W=3125, p = 0.0001), while significantly more spiders used the sand and cement trap-nests in Bilpin than Orange (W=64213, p = 0.0001) (Table 1 and 2).

Are trap-nests occupied by species that are known visitors of apple and cherry crops or a wider diversity of native bees within each region?

Comparing the trap-nest occupancy and observation data to our previously collected cherry (Gilpin et al., 2022) and apple (Gilpin et al., *In review*) (Table 3) flower visitor data within Bilpin, we found that megachilid bees were the only taxon to both visit apple flowers and occupy the trap-nests (both bamboo reed and hardwood block trap-nest types). Meanwhile, there was no overlap recorded in the native bee visitors to cherry and nest occupants in Bilpin. Occurrence records from the Atlas of Living Australia show that there is a wide variety of cavity-, wood- and ground-nesting native bees in the Bilpin area (Table S1), including two species of megachilid bees (*Megachile hackeri* and *M. heliophila*) (Table S1), which may have been the species nesting within the reed and hardwood trap-nests.

In Orange, there was no overlap in the pollinator assemblage observed visiting apple or cherry and the invertebrates using the trap-nests. However, ALA occurrence records show that, in the local area, there is a wide variety of native bee species with potential to use these three trap-nest types given their preference for nesting within cavities made from wood and sand/soil (Table S1). Based upon ALA records of known bees in the area and the construction of red resin and masticated leaf nests that we observed within the bamboo and hardwood block trap-nests, we conclude that it is likely that one or more of three species of *Megachile* bees (*M. apicata, M. ordinaria, M. sequior)* created these nests.

Discussion

We investigated the uptake and use of three types of popular trap-nests by flower visitors of apple and cherry crops and the wider assemblage of invertebrates within agroecosystems in NSW, Australia. Reed and hardwood block trap-nests had greater invertebrate activity than sand and cement brick trap-nests in both crop growing regions (Bilpin and Orange). Overall, significantly more native bees used the reed and hardwood block trap-nests in Orange than in Bilpin. Although known colloquially as bee hotels, in addition to attracting Megachilid resin and leaf-cutter bees, the reed and hardwood trap-nests also attracted ants, spiders and wasps. There was some overlap in the native bees using the trap-nests in Bilpin and those observed visiting apple flowers, but no overlap in the nest occupants and flower visitors of cherry in either Bilpin or Orange or for apple in Orange. However, ALA occurrence records (Atlas of Living Australia, 2020 a and b) did show that, in both Bilpin and Orange, there are a variety of native bee species that could potentially use the trap-nests.

Our finding that hardwood and reed trap-nests had greater numbers of both wild bee and other invertebrate inhabitants than sand and cement trap-nests may be due to: (1) low abundance of ground-nesting bees that are commonly associated with sand and cement nest types (Halcroft & Batley, 2014; Halcroft, 2018); (2) potentially a longer time is required for these bees to become aware of the nesting opportunity; (3) less demand for new nest sites within these areas due to the relative abundance of such micro-sites. Indeed, Brown et al., (2020) documented a positive association between ground-nesting bee abundance and the amount of open agricultural pastureland in south-east Australia, therefore it is possible that in our study locations there may already be sufficient nesting habitat and thus low demand for additional sites.

Our study also found significant differences between Bilpin and Orange regions in the total number of native bees and wasps using the trap-nests, with far more recorded at Orange. This may reflect a lack of pre-existing nesting opportunities within the highly altered agricultural environments of Orange in contrast to the diverse, habitat rich, native plant communities found at Bilpin. Our findings are, however, confounded by differences between the regions in terms of their invertebrate and plant community compositions, so it would require additional study of paired sites to determine the implications of these differences.

According to ALA records, numerous native bee species have been observed in both Bilpin and Orange that have the potential to use artificial nest enhancements. In Bilpin, there are records of *Xylocopa aeratus*, *Amegilla asserta* and *Amegilla pulchra,* all bees that can nest in sandy, compacted soil similar to that mimicked by the sand and cement bricks. Similarly, *Exoneura bicolour*, *Megachile hackeri* and *Megachile heliophila,* as well as numerous colletid bees, have also been recorded in the Bilpin region and may use hardwood block and artificial reed trap-nest enhancements (Halcroft & Batley, 2014). However, the use of artificial nests is potentially more likely in areas that are lacking suitable habitat following disturbances, including anthropogenic land-use changes and fire, although studies which monitor occupancy over a longer period post-disturbance are needed to determine their potential use.

In Orange, blue-banded bees, *Amegilla chlorocyanea* and *A. asserta* (both potential sand and cement brick trap-nest occupants) have been recorded on the ALA database. While these were not observed during the apple or cherry flower-visitor observations, they have been recorded using sand and cement brick trap-nests (Halcroft & Batley, 2014; Smith & Heard, 2016). In contrast, ground-nesting *Lassioglossum* spp. and *Homalictus* spp. native bees were observed visiting apple and cherry flowers but were not recorded in sand and cement trap-nests. It is likely that the firmness of these trap-nests does not suit the nesting preferences of these bees as they typically prefer sandy loam soil (Smith & Heard, 2016). ALA records (Atlas of Living Australia, 2020 a and b) also showed that *Megachile apicata*, *M, sequuior* and *M. ordinaria,* as well as numerous colletid bees, had been previously observed in Orange and these could potentially use the hardwood/reed trap-nests. The discrepancy between the species documented in the ALA records and those observed using the trap-nests may be due to generally low abundance of these bee species in the study area, competition or predation between invertebrate species within the trap-nests, location of the trap-nests in terms of the ideal amount of sun/shade or heat/cold exposure or the time frame of nest deployment being too short for the bees to recognise the nesting opportunity. The diversity of native bees and their intrinsic nesting preferences observed during this study and on the ALA database highlight the need to consider a range of different artificial nest enhancements in order to cater for resident communities of native bees.

The reed and hardwood block trap-nests not only attracted a range of native bees, but also provided nesting habitat for a wide range of other invertebrate functional groups, including scavengers and predators of bees, such as ants, spiders, wasps and a cleptoparasitic cuckoo bee. Diverse invertebrate assemblages can provide a wide variety of ecosystem services in agro-ecosystems, from pollination through to pest-control and nutrient cycling (Saunders 2018), with the latter often being overlooked but no less crucial elements of healthy, functional ecosystems. The capacity of trap-nests to support the diversity and fecundity of solitary, cavity-nesting bees in trap nests located in natural environments and apple orchards in Nova Scotia, Canada was demonstrated by Sheffield et al., (2008). Their study documented similar uptake in trap-nests deployed in paired locations (natural and apple orchards), and they concluded that trap-nests can be used as a tool to increase and maintain cavity-nesting bee populations within apple orchards. For these findings to be tested within an Australian context, further research should consider locally paired (natural and agricultural) comparisons to test the degree of overlap in the assemblage of invertebrates that use the trap-nests.

The capacity for trap-nests to support a diverse invertebrate assemblage has also been shown in a study of 600 trap-nests over three years in Toronto, Canada, by McIvor & Packer, (2015). These authors found that native wasps were the most abundant insect group using the trap-nests, occupying 75% of these each year and far outnumbering native and introduced bees. McIvor & Packer, (2015) also found that native bees were parasitised significantly more than introduced bees. Trap-nests may therefore increase the risk of parasitism and potentially even disease, as well as creating an environment for bee predators and competitors due to the aggregational nature and potentially high density and proximity of nests (McIvor & Packer, 2015). However, more research is needed to understand whether the incidences of disease, predation and competition are higher for native bees that use these artificial nests compared to more natural alternatives.

Although not an initial goal of the study, the wildfire that affected Bilpin in December 2019 provided an opportunity to gain insight into colonisation of trap-nests by invertebrates before and, albeit for a limited time, after the fire. The wildfire dramatically impacted the native vegetation surrounding the orchards of the region, although the majority of the orchards were successfully defended by fire-fighting operations. Perhaps unsurprisingly, invertebrates that inhabit the orchard, such as ants, spiders and termites, and which therefore presumably were not impacted directly by the fire, were found to recolonise the trap-nests in the early months post-fire. Although we did not observe any native bee nests after the wildfire, this may be due to the limited time (4 months) that the study continued post-fire before being impacted by COVID-19 travel restrictions, as well as the generally low occupancy by bees in the region overall. The study of bee responses to fire regimes by Lazarina et al., (2016) found an increase in ground-nesting bee abundance post-fire, which the authors attribute to an increase in the availability of bare earth habitat. Importantly, the temporal scale of their study was greater than ours, with “recently burnt’ sites categorised as those impacted by fire within a 4-year period. Future studies should be conducted over the longest practical time-frame to gain a true indication of the colonisation of trap-nests by native bees post-fire.

Conclusions

Our research highlights the potential of trap-nests to support crop-pollinating native bees and other invertebrates in agricultural landscapes. Trap-nests may therefore have the capacity to be an important conservation tool in areas with low nest site availability or following disturbance such as bushfire.

Authors’ contributions

A-MG, and LEB conceived the ideas and designed the methodology, A-MG and LEB collected the data, A-MG and LEB analysed the data; A-MG, LEB, JMC and SAP wrote the manuscript. This work was supported by the “Healthy bee populations for sustainable pollination in horticulture” and is funded by the Horticulture Frontiers Pollination Fund, part of the Horticulture Frontiers strategic partnership initiative developed by Horticulture Innovation Australia, with co-investment from Western Sydney University, Bayer CropScience, Syngenta Asia-Pacific and Greening Australia, and contributions from the Australian Government.

Acknowledgments

We would like to sincerely thank all of the farmers and orchard owners, Bill Shields, Joe Saliba, Sean Lonergan, John Galbraith, Cedric Leathbridge, Tim Hall, Graeme Eastwood, Tim West, Frank Rosetta, Gabriel and Robert Cunial for allowing us to use their property to establish and monitor the bee hotels. We would also like to thank Steve Leake for helping to design and make the bee hotels, Jasmine Grinyer for reviewing the manuscript, Stephanie Gilpin for fieldwork assistance and Laura Lopresti for creating the aerial map of our study locations. We would also like to acknowledge the traditional owners of the land on which our research took place, the Wiradjuri, Dharug and Darkinjung peoples.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Albrecht, M., Kleijn, D., Williams, N.M., Tschumi, M., Blaauw, B.R., Bommarco, R., Campbell, A.J., Dainese, M., Drummond, F.A., Entling, M.H. & Ganser, D., et al. (2020). The effectiveness of flower strips and hedgerows on pest control, pollination services and crop yield: a quantitative synthesis. *Ecology Letters*. doi: 10.1111/ele.13576

Asare, E., Hoshide, A.K., Drummond, F.A., Criner, G.K. and Chen, X. (2017). Economic risk of bee pollination in Maine wild blueberry, *Vaccinium angustifolium*. *Journal of Economic Entomology, 110*(5), 1980-1992.

Atlas of Living Australia (a) occurrence download at https://doi.org/10.26197/ala.bb675f48-97da-4203-8061-2b06836f5a8b Accessed 29 April 2020.

Atlas of Living Australia (b) occurrence download at https://doi.org/10.26197/ala.f46d358e-6b9e-4d23-b566-cf05787834d4 Accessed 29 April 2020.

Bänsch, S., Tscharntke, T., Gabriel, D. & Westphal, C. (2021). Crop pollination services: complementary resource use by social vs solitary bees facing crops with contrasting flower supply. *Journal of Applied Ecology*, *58*(3), 476-485.

Benuszak, J., Laurent, M. & Chauzat, M.P. (2017). The exposure of honey bees (Apis mellifera; Hymenoptera: Apidae) to pesticides: room for improvement in research. Sci. Total Environ. 587–588, 423–438. https://doi.org/10.1016/j. scitotenv.2017.02.062.

Boyle, R.M.D. & Philogène, B.J.R. (1983). The native pollinators of an apple orchard: variations and significance. *Journal of Horticultural Science*. *58*: 355- 363.

Brown, J., Barton, P.S. & Cunningham, S.A. (2020). Flower visitation and land cover associations of above ground-and below ground-nesting native bees in an agricultural region of south-east Australia. *Agriculture, Ecosystems & Environment*, *295*, 106895.

Brown, M.J. & Paxton, R.J. (2009). The conservation of bees: a global perspective. *Apidologie*, *40*(3), 410-416.

Campbell, J.W., Smithers, C., Irvin, A., Kimmel, C.B., Stanley-Stahr, C., Daniels, J.C. & Ellis, J.D. (2017). Trap nesting wasps and bees in agriculture: a comparison of sown wildflower and fallow plots in Florida. *Insects*, *8*(4), 107.

Cunningham, S.A., FitzGibbon, F. & Heard, T.A. (2002). The future of pollinators for Australian agriculture. *Australian Journal of Agricultural Research*, *53*(8), 893-900.

Dollin, A., Hogendoorn, K., Heard, T., Cunningham, S., Rader, R., Saunders, M., Latty, T., Threlfall, C., Smith, T., Halcroft, M., & Lloyd-Pritchard, D. (2016). Australian Native Bees. NSW Department of Primary Industries.

Eeraerts, M., Vanderhaegen, R., Smagghe, G. & Meeus, I. (2020). Pollination efficiency and foraging behaviour of honey bees and non-Apis bees to sweet cherry. *Agricultural and Forest Entomology*, *22*(1), 75-82.

ESRI. “World Imagery” [basemap]. 7th February, 2022 http://server.arcgisonline.com/arcgis/rest/services/ESRI_Imagery_World2D?mapServer?f=json&pretty=true

Evans, E., Smart, M., Cariveau, D. & Spivak, M. (2018). Wild, native bees and managed honey bees benefit from similar agricultural land uses. *Agriculture, Ecosystems & Environment*, *268*, 162-170.

Garratt, M.P., Breeze, T.D., Jenner, N., Polce, C., Biesmeijer, J.C. & Potts, S.G. (2014). Avoiding a bad apple: Insect pollination enhances fruit quality and economic value. *Agriculture, Ecosystems & Environment*, *184*, 34-40.

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., Carvalheiro, L.G., Harder, L.D., Afik, O. & Bartomeus, I., (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, *339*(6127), 1608-1611.

Gervais, A., Bélisle, M., Mazerolle, M.J. & Fournier, V. (2021). Landscape enhancements in apple orchards: Higher bumble bee queen species richness, but no effect on apple quality. *Insects*, *12*(5), 421.

Gill, R.J., Baldock, K.C., Brown, M.J., Cresswell, J.E., Dicks, L.V., Fountain, M.T., Garratt, M.P., Gough, L.A., Heard, M.S., Holland, J.M. & Ollerton, J. (2016). Protecting an ecosystem service: approaches to understanding and mitigating threats to wild insect pollinators. In *Advances in Ecological Research* (Vol. 54, 135-206). Academic Press.

Gilpin, A-M., Brettell, L.E., O’Brien, C., Cook, J.M., & Power, S.A. High co-flowering species richness increases pollinator visitation to apple flowers. *Under review*

Gilpin, A-M., O'Brien, C., Kobel, C., Brettell, L.E., Cook, J.M., & Power, S.A. (2022). Co-flowering plants support diverse pollinator populations and facilitate pollinator visitation to sweet cherry crops. *Basic and Applied Ecology,* 63, 36-48.

Halcroft, M. T. & Batley, M. (2014). The Bee Hotel ID Guide. Bee Aware of Your Native Bees, a Community Based Project. Sydney, Australia, Office of Sustainability, University of Western Sydney**.** 60.

Halcroft, M. T. (2018). How to make rammed earth blocks for blue banded bees. Retrieved June 2018, available from www.beesbusiness.com.au/garden.php.

Harmon-Threatt, A. (2020). Influence of Nesting Characteristics on Health of Wild Bee Communities. *Annual Review of Entomology*, *65*, 39-56.

Hannon, L.E. & Sisk, T.D. (2009). Hedgerows in an agri-natural landscape: potential habitat value for native bees. *Biological conservation*, *142*(10), 2140-2154.

Hebert, P.D., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(41), 14812-14817.

Holzschuh, A., Dudenhöffer, J.H. & Tscharntke, T. (2012). Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. *Biological Conservation*, *153*, 101-107.

Houston, T. (2018). *A guide to native bees of Australia*. CSIRO PUBLISHING.

Iwasaki, J.M. & Hogendoorn, K. (2021). Non-insecticide pesticide impacts on bees: A review of methods and reported outcomes. *Agriculture, Ecosystems & Environment*, *314*, 107423.

Jankielsohn, A. (2018). The importance of insects in agricultural ecosystems. *Advances in Entomology*, *6*(2), 62-73.

Kearns, C.A., Inouye, D.W. & Waser, N.M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions, *Annual Review of Ecology and Systematics*, 29, 83-112.

Kim, J., Williams, N. & Kremen, C. (2006). Effects of cultivation and proximity to natural habitat on ground-nesting native bees in California sunflower fields. *Journal of the Kansas Entomological Society*, *79*(4), 309-320.

Klein, A.M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: biological sciences*, *274*(1608), 303-313.

Kline, O, & Joshi, N.K. (2020). Mitigating the effects of habitat loss on solitary bees in agricultural ecosystems. *Agriculture*, *10*(4), 115.

Kohler, F., Verhulst, J., Van Klink, R. & Kleijn, D. (2008). At what spatial scale do high-quality habitats enhance the diversity of forbs and pollinators in intensively farmed landscapes?. *Journal of Applied Ecology*, *45*(3), 753-762.

Kremen, C., Williams, N.M. & Thorp, R.W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences*, *99*(26), 16812-16816.

Landis, D.A. (2017). Designing agricultural landscapes for biodiversity-based ecosystem services. *Basic and Applied Ecology*, *18*, 1-12.

Lazarina, M., Sgardelis, S.P., Tscheulin, T., Kallimanis, A.S., Devalez, J. and Petanidou, T. (2016). Bee response to fire regimes in Mediterranean pine forests: the role of nesting preference, trophic specialization, and body size. *Basic and Applied Ecology*, *17*(4), 308-320.

Lima, V.P. and Marchioro, C.A. (2021). Brazilian stingless bees are threatened by habitat conversion and climate change. *Regional Environmental Change*, *21*(1), 1-12.

MacIvor, J.S. & Packer, L. (2015). 'Bee hotels' as tools for native pollinator conservation: a premature verdict?. *PloS One*, *10*(3), e0122126.

Mallinger, R.E. & Gratton, C. (2015). Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. *Journal of Applied Ecology*, *52*(2), 323-330.

McKerchar, M., Potts, S.G., Fountain, M.T., Garratt, M.P. & Westbury, D.B. (2020). The potential for wildflower interventions to enhance natural enemies and pollinators in commercial apple orchards is limited by other management practices. *Agriculture, Ecosystems & Environment*, *301*, 107034.

Meiklejohn, K.A., Damaso, N. & Robertson, J.M. (2019). Assessment of BOLD and GenBank–Their accuracy and reliability for the identification of biological materials. *PloS One*, *14*(6), e0217084.

New, T.R. (2005). *Invertebrate conservation and agricultural ecosystems*. Cambridge University Press.

OZCAM (Online Zoological Collections of Australian Museums) Provider, accessed through Atlas of Living Australia website April 2021.

Pardo, A. & Borges, P.A. (2020). Worldwide importance of insect pollination in apple orchards: A review. *Agriculture, Ecosystems & Environment*, *293*, 106839.

Paton, D.C. (1993). Honeybees in the Australian environment. *Bioscience*, *43*(2), 95-103.

Pest and Disease Image Library PaDIL Bee (PaDIL website at http://www.padil.gov.au) accessed through the Atlas of Living Australia website April 2021.

Potts, S.G., Roberts, S.P., Dean, R., Marris, G., Brown, M.A., Jones, R., Neumann, P. & Settele, J. (2010). Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research*, *49*(1), 15-22.

Rader, R., Bartomeus, I., Garibaldi, L.A., Garratt, M.P., Howlett, B.G., Winfree, R., Cunningham, S.A., Mayfield, M.M., Arthur, A.D., Andersson, G.K. & Bommarco, R., (2016). Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences*, *113*(1), 146-151.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Ricketts, T.H., Regetz, J., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S.S., Klein, A.M., Mayfield, M.M. & Morandin, L.A., (2008). Landscape effects on crop pollination services: are there general patterns? *Ecology Letters*, *11*(5), 499-515.

Senapathi, D., Biesmeijer, J.C., Breeze, T.D., Kleijn, D., Potts, S.G. & Carvalheiro, L.G., (2015). Pollinator conservation—the difference between managing for pollination services and preserving pollinator diversity. *Current Opinion in Insect Science*, *12*, 93-101.

Sheffield, C.S., Kevan, P.G., Westby, S.M. & Smith, R.F. (2008). Diversity of cavity-nesting bees (Hymenoptera: Apoidea) within apple orchards and wild habitats in the Annapolis Valley, Nova Scotia, Canada. *The Canadian Entomologist*, *140*(2), 235-249.

Siviter, H. & Muth, F. (2020). Do novel insecticides pose a threat to beneficial insects? *Proceedings of the Royal Society B*, *287*(1935), 20201265.

Smith, T & Heard, T. (2016). 'Creating artificial nest sites for Australian solitary and semi-social bees' in Australian native bees. AgGuide, A practical Handbook. WHO Printing, Newcastle, Australia. 57-71.

Staab, M., Pufal, G., Tscharntke, T. & Klein, A.M., (2018). Trap nests for bees and wasps to analyse trophic interactions in changing environments—A systematic overview and user guide. *Methods in Ecology and Evolution*, *9*(11), 2226-2239.

Williams, G.R., Tarpy, D.R., VanEngelsdorp, D., Chauzat, M., Cox-Foster, D.L., Delaplane, K.S., Neumann, P., Pettis, J.S., Rogers, R.E.L. & Shutler, D. (2010). Colony collapse disorder in context. BioEssays 32, 845–846. https://doi.org/10.1002/bies.201000075.

Winfree, R. (2010). The conservation and restoration of wild bees. *Annals of the New York Academy of Sciences*, *1195*(1), 169-197.

Woodcock, B.A., Edwards, M., Redhead, J., Meek, W.R., Nuttall, P., Falk, S., Nowakowski, M. & Pywell, R.F. (2013). Crop flower visitation by honeybees, bumblebees and solitary bees: Behavioural differences and diversity responses to landscape. *Agriculture, Ecosystems & Environment*, *171*, 1-8.

Tables.

Table 1. Nesting material used and associated insect presumed to have made the nest, based upon reference identification (Halcroft and Batley 2014; Dollin et al., 2016; Houston 2018), for three different trap-nest types (bamboo reed, hardwood block and sand and cement brick)

placed into apple and or cherry orchards in Bilpin (August 2018-April 2020) and Orange (August 2018- June 2020), NSW.

Table 2. Mann-Whitney U test results comparing the total number of native bees (Megachile subgenera and Megachile (Eutricharaea)), wasps, ants and spiders that used each of the three trap-nests (bamboo reed, hardwood block and sand and cement brick) at Bilpin and Orange.

Table 3. Observations of native bees, identified to family, genus or species level, visiting apple and cherry during early/king, peak and late bloom in 2017 and 2018 in the same study orchards as the trap-nest experiment, in Bilpin and Orange, NSW.

Figures.

Figure 1. Map of the two study locations, Bilpin and Orange located in NSW, Australia. Map created by Laura Lopresti, source (ESRI World Imagery basemap, 2022).

Figure 2. The three different types of trap-nests deployed in apple and cherry orchards in Bilpin and Orange - bamboo reed trap-nest (a), hardwood block trap-nest (b), sand and cement brick trap-nest (c). (d) Laura Brettell inspects a hardwood block trap-nest and extracts a paper straw in which insects are nesting, in an apple orchard in Orange.

Figure 3. Heatmap showing the number of nests/structures made by each insect group observed in three types of trap-nests (bamboo reed, hardwood block and sand and cement) throughout 2019 to 2020 in apple and cherry orchards in Bilpin, (a) and Orange, NSW, Australia (b). Warmer colours represent higher numbers of nests/structures observed. Abbreviated insect groupings are based on nest material or the observed insect known to inhabit the nest, abbreviations include; Leaf = Leaf-cutter bee, Mast leaf = Resin bee (Masticated leaf), Resin (R) = Resin bee (Red Resin), Resin (S) = Resin bee (Resin and

sticks), Misc = burrow holes made by unknown insect. Note the sampling dates differ between Bilpin and Orange, this is due to the geographic distance between the locations, meaning sampling on the same day was not possible.

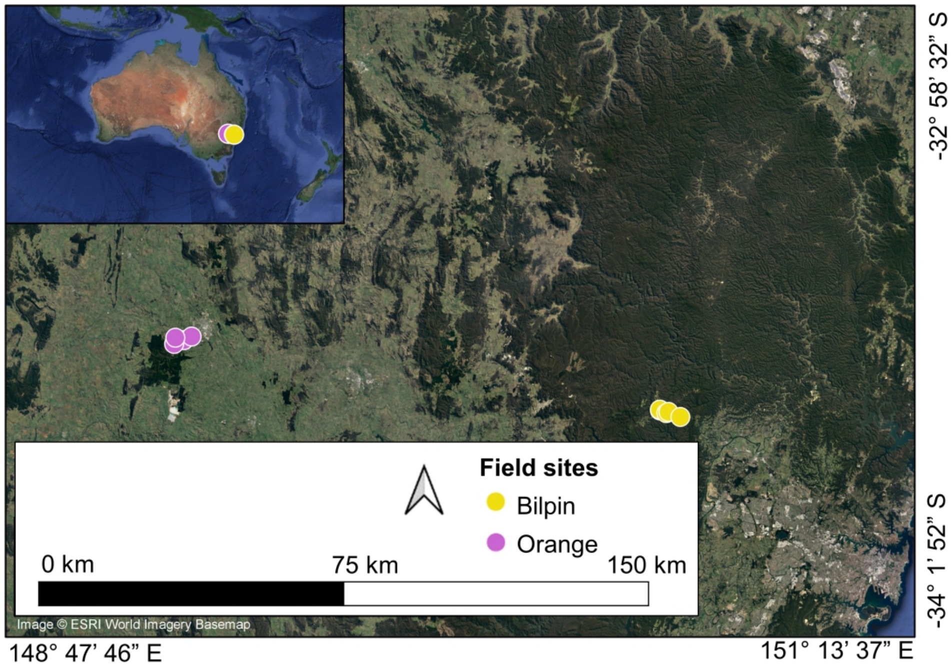

Figure 1. Map of the two study locations, Bilpin and Orange located in NSW, Australia. Map created by Laura Lopresti, source (ESRI World Imagery basemap, 2022).

287x190mm (300 x 300 DPI)

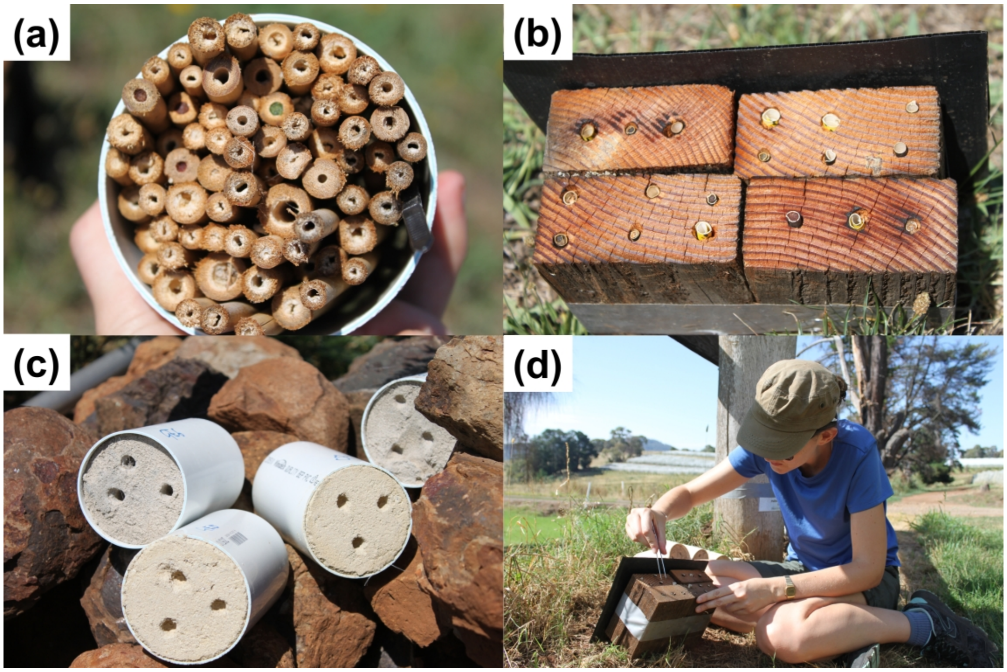

Figure 2. The three different types of trap-nests deployed in apple and cherry orchards in Bilpin and Orange - bamboo reed trap-nest (a), hardwood block trap-nest (b), sand and cement brick trap-nest (c). (d) Laura Brettell inspects a hardwood block trap-nest and extracts a paper straw in which insects are nesting, in an apple orchard in Orange.

285x190mm (300 x 300 DPI)

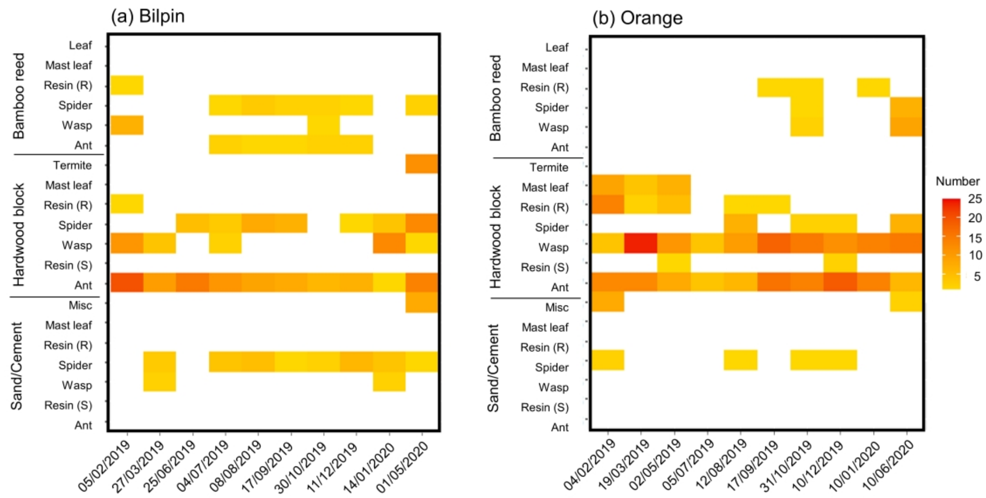

Figure 3. Heatmap showing the number of nests/structures made by each insect group observed in three types of trap-nests (bamboo reed, hardwood block and sand and cement) throughout 2019 to 2020 in apple and cherry orchards in Bilpin, (a) and Orange, NSW, Australia (b). Warmer colours represent higher numbers of nests/structures observed. Abbreviated insect groupings are based on nest material or the observed insect known to inhabit the nest, abbreviations include; Leaf = Leaf-cutter bee, Mast leaf = Resin bee (Masticated leaf), Resin (R) = Resin bee (Red Resin), Resin (S) = Resin bee (Resin and sticks), Misc = burrow holes made by unknown insect. Note the sampling dates differ between Bilpin and Orange, this is due to the geographic distance between the locations, meaning sampling on the same day was not possible.

338x190mm (300 x 300 DPI)

Table 1. Nesting material used and associated insect presumed to have made the nest, based upon reference identification (Halcroft and Batley 2014; Dollin et al., 2016; Houston 2018), for three different trap-nest types (bamboo reed, hardwood block and sand and cement brick) placed into apple and or cherry orchards in Bilpin (August 2018-April 2020) and Orange (August 2018- June 2020), NSW.

		Nest structure material / insect								
		Megachile (Megachile) Resin bee			*Megachile* (Eutricharaea) Leaf-cutter bee	Wasp spp	Ant spp	Spider spp	Termite spp	Miscellaneous
Trap-nest type	Region	Red resin	Resin and stick	Green masticated leaf tissue	Leaf nest	Mud	Plant debris and soil	Web	Soil/sand	Unknown insect burrow
Bamboo reed	Bilpin	1	0	0	0	8	37	11	0	0
	Orange	12	0	5	2	26	12	8	0	0
Hardwood block	Bilpin	1	0	0	0	31	101	41	12	0
	Orange	23	3	20	0	127	113	20	0	0
Sand and cement brick	Bilpin	0	0	0	0	4	0	26	0	8
	Orange	0	0	0	0	0	0	3	0	2

Table 2. Mann-Whitney U test results comparing the number of native bees (Megachile subgenera and Megachile (Eutricharaea)), wasps, ants and spiders that used each of the three trap-nests (bamboo reed, hardwood block and sand and cement brick) at Bilpin and Orange.

	Test statistic			
Trap-nest type	**Total native bees**	**Wasp**	**Ant**	**Spider**
Bamboo Reed	W=60542, p = 0.01	W=60196, p = 0.02	W= 60897, p =0.08	W=60905, p = 0.11
Hardwood block	W=3538, p = 0.0001	W=3125, p = 0.0001	W=4078, p = 0.63	W=3768, p = 0.05
Sand and cement brick	na	W= 67344, p = 0.16	-	W=64213, p = 0.0001

na = indicates no insects were observed. - indicates insufficient data to perform analysis.

Table 3. Observations of native bees, identified to family, genus or species level, visiting apple and cherry during early/king, peak and late bloom in 2017 and 2018 in the same study orchards as the trap-nest experiment, in Bilpin and Orange, NSW.

	Bilpin		**Orange**	
Family/Genus/ Species	**Native bees visiting apple**	**Native bees visiting cherry**	**Native bees visiting apple**	**Native bees visiting cherry**
Megachilidae spp	•			
Lassioglossum spp	•		•	•
Exoneura spp	•			
Homalictus spp	•		•	•
Meroglossa spp	•			
Tetragonula carbonaria	•	•		•

- = The family/genus or species was observed visiting either the apple or cherry flowers during observations conducted throughout the early (or king for apple), peak or late crop flowering period in 2017 or 2018 (Data sources from Gilpin et al., In review a, b).