

Final Report

Honey bee health and pollination under protected and contained environments

Project leader:

Associate Professor Geoff Allen

Delivery partner:

Tasmanian Institute of Agriculture

Project code:

LP15007

Project:

Honey bee health and pollination under protected and contained environments (LP15007)

Disclaimer:

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

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

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

Funding statement:

Honey bee health and pollination under protected and contained environments (LP15007) is funded by the Hort Frontiers Leadership Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from the University of Tasmania and contributions from the Australian Government.

Publishing details:

ISBN 978-0-7341-4839-1

Published and distributed by: Hort Innovation

Level 7

141 Walker Street

North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

© Copyright 2022 Horticulture Innovation Australia Limited

Contents

| | |
|---|-----------|
| Contents | 3 |
| Public summary | 4 |
| Keywords | 4 |
| Introduction | 4 |
| Methodology | 6 |
| Photos/images/other audio-visual material | 6 |
| Results and discussion | 6 |
| Photos/images/other audio-visual material | 9 |
| Outputs | 9 |
| Photos/images/other audio-visual material | 10 |
| Outcomes | 10 |
| Monitoring and evaluation | 12 |
| Recommendations | 12 |
| Refereed scientific publications | 14 |
| Journal article | 14 |
| Intellectual property | 14 |
| Acknowledgements | 14 |
| Appendices | 14 |
| References | 14 |

Public summary

The western honey bee, *Apis mellifera* L. is critical for the pollination of agricultural crops globally; however, honey bee health is currently in decline. This is thought to be caused by interactions between parasites, pathogens, pesticides, environmental change and commercial pollination practices. This pollinator decline has been exacerbated in many modified agricultural production systems, which have generally been developed for optimum plant growth rather than bee health. It is therefore critical that more research is conducted in these modified environments to ensure sustainable horticultural production in the future.

One of the most effective strategies to improve agricultural system design and pollination, is to monitor variation in individual bee behaviour. This can be achieved using radio frequency identification (RFID) systems to tag and monitor bees. This PhD research project focused on developing an RFID system capable of autonomously monitoring individual bees from full strength hives in field locations. Once designed, the potential of this unique system was subsequently demonstrated through the investigation of bee longevity and behaviour in two important horticultural crops: sweet cherry and hybrid carrot seed.

This research provides the first insight into individual honey bee behaviour under a range of polythene and netted crop coverings, whilst also investigating the impact of isolated crops. Overall, the practices utilised in Australian cherry and hybrid carrot seed crops were not found to negatively impact individual honey bee foraging behaviour. The extensive findings of this work have relevance for horticultural producers, beekeepers, and researchers alike. As was the aim under the National Leaders of Horticulture program this project resulted in a formally trained research higher degree graduate, capable of advising industry on honey bee health and crop pollination. The submitted PhD thesis, encompassing the full RFID methodology and findings from two seasons of field trials is presented as an appendix to this report.

Keywords

Protected cropping, *Apis mellifera*, crop pollination, RFID, sweet cherry, hybrid carrot seed

Introduction

The western honey bee, *Apis mellifera* L. is vital for global agricultural production and ecological stability in many regions. It is currently estimated that 35% of food production is reliant on honey bees, with pollination services worth in excess of A\$200 billion per year (Klein *et al.*, 2007; Khalifa *et al.*, 2021). This reliance on honey bees is especially high in horticultural crops (fruit and vegetables), with pollination critical for yield and produce quality (Klein *et al.*, 2007; Hung *et al.*, 2018; Khalifa *et al.*, 2021).

Although honey bees are critical for agricultural production, populations are currently under threat, with declines in colony health observed globally (Goulson *et al.*, 2015; Kulhanek *et al.*, 2017; St. Clair *et al.*, 2020). The most substantial impacts have occurred across Europe and North America, with annual colony losses exceeding 40% (Kulhanek *et al.*, 2017). The decline in bee numbers and health are thought to be caused by complex interactions between commercial pollination practices, parasites, pathogens, pesticides and environmental change (Goulson *et al.*, 2015; Perry *et al.*, 2015; Simone-Finstrom *et al.*, 2016; Kulhanek *et al.*, 2017; St. Clair *et al.*, 2020; Khalifa *et al.*, 2021). Unfortunately, many of these factors that contribute to prolonged stress are associated with modern agriculture.

Despite agricultural environments being known to impact bee behaviour and mortality, the design and management of production systems is still predominantly based on optimised crop growth rather than bee health (Evans *et al.*, 2019; Hall *et al.*, 2020; Kendall *et al.*, 2021). A focus on increasing crop production has led to the rapid adoption of protected cropping systems, such as polytunnels, bird netting and glasshouses. Crops previously grown in open fields are now increasingly being cultivated in covered systems due to key advantages such as increased yield, improved input efficiency, optimised climate and reduced pest damage (Evans *et al.*, 2019; Hall *et al.*, 2020; Kendall *et al.*, 2021). These systems represent the forefront of agricultural technology, with over 30 key crops commonly grown under protective conditions, ranging from apples, tomatoes and berries to lucerne and clover (Kendall *et al.*, 2021).

Although protected cropping represents immense potential, systems may negatively impact on pollinator health (Dag, 2008; Evans *et al.*, 2019; Hall *et al.*, 2020; Kendall *et al.*, 2021). It is estimated that 90% of protected crops are pollinated

by bees (Kendall *et al.*, 2021). However, there is very little understanding or quantification of how these conditions impact bee health and behaviour. Protective covers can potentially disrupt navigation, restrict nutritional collection, amplify pesticide exposure and subject bees to extreme climates (Dag, 2008; Evans *et al.*, 2019; Hall *et al.*, 2020; Kendall *et al.*, 2021). It is therefore essential that understanding is increased, to ensure both bee health and crop pollination is optimised in the future.

In Australia two important crops utilising protective or modified cropping conditions are sweet cherry and hybrid carrot seed. Market demand and price for cherries is driven by fruit size, sugar content and firmness, with the most desirable fruit being large, firm and sweet (Kurlus *et al.*, 2020). As a result, substantial investments into crop protection and growing systems are common (Lang, 2014; Lang *et al.*, 2016). In order to protect the crop, cherry trees are increasingly being grown under protective nets or plastic rain covers, which limit rain exposure, whilst also providing protection from birds and hail (Rubauskis *et al.*, 2013; Lang *et al.*, 2016; Ruisa *et al.*, 2017; Mika *et al.*, 2019; Vávra, 2020). Although protected cropping offers significant advantages for cherry production, there is little understanding of how covers impact the health and behaviour of honey bees (Lang, 2014).

Hybrid carrot seed represents another challenging crop for pollination. Carrot seed is commonly grown in barren locations, which are isolated from other flowering plants, to encourage increased visitation to the poorly nutritious carrot crops. Carrots are also commonly sprayed with insecticides during peak flowering for the control of sap sucking insects (Spurr, 2003; Gaffney *et al.*, 2011; Cook *et al.*, 2020). These conditions share similar characteristics to those associated with protected cropping systems; hence hybrid carrot seed is recognised as an effective model crop for understanding how environmental stress could impact future pollination (Broussard *et al.*, 2017). Increased understanding of honey bee health and behaviour within seed crops is also essential for facilitating the projected expansion of hybrid seed production under protective covers (Cook *et al.*, 2020; Kendall *et al.*, 2021).

Monitoring individual behaviour is one of the most effective techniques for investigating the impact of stressors on honey bees in all crops (Nunes-Silva *et al.*, 2018). In recent years radio frequency identification (RFID) has become the predominant technology utilised for monitoring individual honey bees (Decourtye *et al.*, 2011; Nunes-Silva *et al.*, 2018). Small RFID tags can easily be attached to the thorax of honey bees using super glue (Nunes-Silva *et al.*, 2018). An RFID system consisting of antenna, reader and computer can then be deployed to stationary positions such as hive entrances or feeders (Van Geystelen *et al.*, 2016; Odemer, 2022). When a tagged bee closely (approximately 10-20 mm) passes an antenna, it is detected, with the reader/computer logging the unique tag ID, time and antenna number (Van Geystelen *et al.*, 2016). Bees can be autonomously monitored for their entire life, with the data analysed to determine key behavioural parameters such as, age at foraging, number of trips, time spent foraging and life span (Colin *et al.*, 2019).

Although RFID technology has facilitated significant advancements in bee research, there are still several limitations. RFID systems have been utilised in a wide range of laboratory and semi-field studies; however successful deployment to full-strength commercial colonies has been rare. This is due to poor reliability, bulky componentry and a reliance on mains power (de Souza *et al.*, 2018). Most studies to date have been restricted to small nucleus hives, which only contain 4-6 frames and approximately 2000 -5000 bees (Schneider *et al.*, 2012; Tenczar *et al.*, 2014; Chang *et al.*, 2015; Lach *et al.*, 2015; Perry *et al.*, 2015; Bordier *et al.*, 2018; Evans *et al.*, 2019; Klein *et al.*, 2019; Hesselbach *et al.*, 2020). Although these nucleus hives are easier to use for RFID based studies, they provide a limited representation of a full-size colony's ability to buffer the impact of stress. Furthermore, the success of RFID technology relies on the accurate detection of tags; however, this is rarely acknowledged or quantified. There are few published studies that discuss the accuracy of their system or provide an analytical methodology to confirm this (Odemer, 2022). Of the studies that did report system performance, tag retention and detection accuracy was commonly low, with less than half of the bees tagged going on to record a trip (Lach *et al.*, 2015; Dosselli *et al.*, 2016; de Guzman *et al.*, 2017; Bordier *et al.*, 2018; Evans *et al.*, 2019; Klein *et al.*, 2019). Such issues have restricted the practical application of RFID findings to date.

Honey bees are critical for agricultural production and the ecosystem, yet bee health is in decline. The most advanced agricultural production systems have been developed, with limited consideration or understanding of bee health and behaviour. Poor understanding has been attributed to a lack of field deployable monitoring systems to facilitate widespread research. Therefore, addressing the methodological shortfalls of RFID technology, represents immense potential for understanding the mechanisms of honey bee colony collapse, foraging efficiency in key crops and the impact of weather. The development of a reliable RFID system that is field deployable on full-strength hives, will contribute to improving pollinator management and honey bee health globally.

Methodology

This project was undertaken over a 4-year period in Southern Tasmania, Australia. Initial focus was on developing a radio frequency identification system (RFID), which was capable of monitoring full-strength bee hives in remote field locations. This was achieved by first sourcing the componentry for the system, including RFID readers, ceramic patch antennas and tags. Extensive testing was then undertaken to ensure system functionality, including the development of automated software and a full solar power system. To facilitate accurate detection of tagged bees, a unique 'maze' hive entrance was designed and optimised for use on a full-strength commercial hive. This entrance design was incrementally modified to ensure RFID tag detection was maximised, whilst minimising disruption to natural bee behaviour. Furthermore, extensive code and methodology was developed to enable efficient analysis and interpretation of the data generated.

Following successful development, the RFID system was deployed in several field locations in key agricultural sectors, over multiple seasons. The system was utilised to investigate the effect of netting and polythene rain covers on the health and behaviour of honey bees during the pollination of sweet cherry crops. Over two consecutive seasons, six full-strength hives were equipped with tagged bees and radio frequency identification (RFID) systems. The hives were equally divided between open control, netted and polythene (semi-permanent VOEN in 2019 and retractable Cravo in 2020) groups. Over 1300 individual bees were monitored for the duration of the commercial pollination period to determine behavioural parameters such as foraging commencement age, number and duration of trips and overall survival.

A similar methodology was also utilised to investigate the impact of hybrid seed cropping environments on the behaviour of the honey bee. Six full-strength bee hives were equipped with solar powered RFID systems and over 900 tagged bees per season. These hives were deployed to commercial hybrid carrot seed crops over two consecutive seasons. Hives were allocated to either an on-crop or off-crop group. Individual bees were again monitored for the pollination period to assess key parameters. Data collected in this experiment was then further utilised to investigate the impact of weather on bee foraging behaviour. The impacts of temperature, humidity, solar radiation, barometric pressure and windspeed on bee foraging, were successfully modelled using a series of nested linear mixed models. A comprehensive methodology is presented in the thesis provided as an appendix of this report.

Photos/images/other audio-visual material

Please refer to supplementary thesis attached for images pertaining to this project.

Results and discussion

The thesis produced as part of this project presents a collection of tools, methodologies and findings, which can assist growers, beekeepers and researchers to further understand the impact of modern agricultural practices on honey bees.

Enhancing RFID system design and data analysis

We describe significant advancements in the methodology for collection and analysis of radio frequency identification (RFID) data from honey bees. In the past, field-based research has been limited by a reliance on small experimental nucleus hives, which may not truly represent the resilience of large hives in agricultural environments. To address this, the RFID system presented in this project was successfully developed for use on full-strength hives (3-8 ideal depth Langstroth boxes), that are approximately 2-40 times larger than a standard nucleus hive. To achieve deployment on these large hives, significant modification to the hive entrance was required to accommodate increased bee traffic. Through several design iterations, a novel split entry/exit maze that can be retrofitted to any Langstroth hive entrance, was found to be the most effective design for both traffic management and data quality.

For the first time all facets of RFID system functionality were considered in entrance design. This included addressing previously identified issues such as hive ventilation, antenna coupling, rapid succession detections, electromagnetic interference and the failure of bees to pass in close proximity to the antenna (Van Geystelen *et al.*, 2016; de Souza *et al.*, 2018; Nunes-Silva *et al.*, 2018; Susanto *et al.*, 2018; Alburaki *et al.*, 2021). The new system was proven to be capable of accommodating a large amount of bee traffic, whilst generating easily interpretable data. Further improvements were also gained through the selection of smaller RFID tags, that dislodged less frequently, were lighter and more readily available, as compared to those used in recent studies (de Souza *et al.*, 2018; Alburaki *et al.*, 2021). Overall, the consideration of these factors resulted in the highest RFID detection accuracy reported to date, with 90.5% of all passing bees detected.

Field deployment of the RFID system was further facilitated by the design and testing of a complete solar power system. This ensured data could be reliably collected in field locations over long periods of time, without the need for mains power. Indeed, the overall effectiveness of the remote functionality was demonstrated by its continuous operation over 280 days and the collection of over 300,000 successful detections. The complete RFID system with solar power remained relatively cost effective, being priced at approximately \$2000 AUD. Despite these advancements, future improvements in functionality may include the integration of telemetry to facilitate remote data download and system monitoring. It is also suggested that reliable, low-cost, high-resolution hive scales and a weather station with automated data logging be developed and integrated into the system.

The ability to generate large amounts of data creates the need for efficient methodologies for filtering, trip construction and analysis. This is critical for understanding biological/practical relevance and verifying system accuracy. A lack of standardised methodology has been acknowledged as a significant issue for RFID data analysis, with very few prior studies presenting critical understanding (Odemer, 2022). As part of system development, we present a comprehensive methodology and code for analysing the data collected. This enables the efficient quantification of key parameters linked to honey bee ontogeny and foraging performance such as: age at onset of foraging, survival and the number and duration of orientation and foraging flights. This represents a significant advantage for future studies, as the RFID data generated can be easily evaluated using the widely accessible R program.

Impact of protective cropping on bee health and behaviour (sweet cherry)

This project represents the first time an RFID system has been deployed on full-strength hives as part of a commercial pollination service, within an agricultural crop. The deployment of this system enabled the monitoring of individual bee behaviour to address the distinct lack of knowledge related to pollinator health under protective netting and polythene rain covers (Kendall *et al.*, 2021). Polythene covers have long been hypothesised to impact honey bee orientation; however, this has rarely been quantified (Lang, 2014; Evans *et al.*, 2019; Kendall *et al.*, 2021). This research provides the first evidence that such covers significantly increase the total time required for young bees to orientate.

Although covered cropping systems appear to represent difficult environments for honey bee orientation, they were not found to be detrimental to foraging behaviour in sweet cherry crops. Being placed within a commercial cherry orchard had little impact on age at onset of foraging or overall survival, with all groups demonstrating healthy parameters regardless of the covering type. Once bees were acclimatised under polythene covers, they generally conducted more foraging trips and spent a greater cumulative time outside of the hive. However, differences were observed between the two types of polythene rain covers investigated: semi-permanent VOEN (season 1) and retractable Cravo (season 2). Bees under VOEN collected the greatest proportion of cherry pollen due to being physically restricted to the crop. This was correlated with significantly shorter trip durations, as the bees flew shorter distances. In contrast, the Cravo system's walls, and roof are fully open when conditions are favorable. This resulted in longer trips and collection of a lower proportion of cherry pollen, as bees were able to easily forage from other floral resources in the surrounding environment.

Bird netting also offered improvements for increasing bee activity as compared to the open field site. In general, placing bees out in the neighboring open field was the least effective strategy for sweet cherry pollination. The bees undertook less trips, spent less cumulative time outside of the hive and collected a higher proportion of non-target pollen. While increased bee activity is generally associated with improved crop pollination, it is suggested that future studies incorporate an assessment of pollination success and fruit set, to support the RFID data collected. Experiments should also be expanded to encompass more hives over multiple seasons at the same orchard, along with increased pollen sampling to glean a greater understanding of temporal variations in the bee's forage selection.

A large proportion of variation in foraging activity was attributed to differences in the climatic conditions created by the protective crop coverings. This conformed to the understanding that honey bee activity is directly correlated with weather conditions (Vicens and Bosch, 2000; Clarke and Robert, 2018). We successfully demonstrated that barometric pressure, wind, and temperature have a significant impact on honey bee trip duration. This supported the observations made in sweet cherry, with bees placed in the warmer sheltered conditions (under covers), spending significantly more total time foraging as compared to those placed in the open field locations. Furthermore, despite being placed under rain covers, no bees were recorded undertaking trips when it rained. This observation conforms to the notion that bees utilise changes in barometric pressure to perceive incoming rainfall. It also indicates that placing hives under rain covers does not promote improved crop pollination during rainy periods. Although clear trends were identified, the relationship between bee activity and climatic conditions under protective covers, requires further investigation.

We revealed that polythene covers had the greatest impact on bee behaviour within the cool-temperate environments assessed. Thus, it is suggested that crops using these types of covers are a critical priority for future pollination research. A specific area identified is the rapidly expanding berry industry, which is reliant on both broad acre polythene tunnels and honey bees during long pollination periods (Hall *et al.*, 2020). Berry pollination can last for over 5 months, representing an opportunity to understand the long-term impacts of covers, especially in warm summer conditions. The new RFID system represents immense potential for quantifying behavioural and developmental characteristics within these environments.

Impact of isolated and unattractive crops on bee health and behaviour (carrot)

Hybrid crops are essential for agricultural production; however, they represent significant challenges for pollination. This project describes the first ever investigation into the direct impact of hybrid carrot seed crops on honey bee health and behaviour. Although never quantified, anecdotal evidence suggested that hybrid carrot crops have a negative impact on bees, due to poor floral attractiveness, low nutrition, isolated cropping environments and regular insecticide exposure (Spurr, 2003; Broussard *et al.*, 2017; Mas *et al.*, 2018; Gaffney *et al.*, 2019; Cook *et al.*, 2020; Gaffney *et al.*, 2020). We were able to successfully utilise the RFID system to investigate the hypothesis that foraging in hybrid cropping environments has a negative impact on honey bees.

Through comparison with off-crop (control) groups, we established that hybrid carrot crop pollination can significantly impact foraging behaviour. The bees positioned near the carrot crop consistently undertook fewer trips, that were longer in duration. A substantial amount of this variation was attributed to differences in the collection of pollen and nectar. Carrot flowers were found to be highly unattractive for pollen foragers, whilst relatively attractive for nectar foragers. Overall, longer trip times were also associated with carrot bees visiting significantly more types of flowers during a trip, including several weedy species. These results highlight the importance of supplementing RFID data with additional biological information in order to garner a deeper understanding of pollinator behaviour within agricultural systems.

Although carrot crops were found to influence foraging behaviour, being placed on the crop did not significantly impact bee survival or development. In both seasons, the bee's median survival age was higher on the carrot crops and exceeded the median life expectancy (10-22 days) reported in other RFID studies (Bordier *et al.*, 2018; Colin *et al.*, 2019; Coulon *et al.*, 2020; Prado *et al.*, 2020; Colin *et al.*, 2021). The bees located on the carrot crop also orientated effectively and began foraging at a significantly older age (4.3-5.8 days) in both seasons. This provides the first quantifiable evidence that contradicts the anecdotal understanding that hybrid carrot crops are fundamentally detrimental to bees.

One of the more controversial aspects of hybrid carrot production in Australia is multiple night-time applications of the insecticide *tau*-fluvalinate, during the pollination period for the control of rutherglen bug (*Nysius vinitor*). Our data did not provide any evidence of either acute or sublethal impacts on the tagged bees within this study. Although exposure was unlikely, future studies should include residue testing of honey, bees and wax. Exposure to some pesticides is also known to have intergenerational impacts, through the feeding of contaminated resources (Tomé *et al.*, 2020; Milone and Tarpay, 2021). The methodology used did not capture any of the potential intergenerational impacts of pesticides. However, the future use of RFID systems in field-based experiments, represents immense potential for furthering the understanding of this phenomena.

Despite the advancements this study provides, further experimentation is required to develop a broader understanding of pollination in carrots and other hybrid crops. This should include monitoring a greater number of hives, across multiple carrot growing regions and seasons. The characteristics of hybrid carrot crops are known to vary significantly according to the type of parent line used (Broussard *et al.*, 2017). Therefore, future studies should also incorporate a wide variety of differing hybrid parents and draw comparisons to open pollinated varieties, which are less challenging for pollination.

Influence of weather on honey bee foraging duration

This project successfully demonstrated that honey bee trip duration can be modelled by combining RFID data and high-resolution weather recordings. The temperature, humidity, pressure, solar radiation and windspeed observed during a foraging trip were all found to have a significant impact on the overall trip duration. A complex relationship was revealed, with longer trips linked to both taking advantage of optimal conditions or struggling to return to the hive during poor conditions. When only considering individual weather variables, change in barometric pressure was the most effective parameter for modelling trip duration. Generally, the greater the pressure change, the longer the trip regardless of improving or worsening conditions. We also successfully incorporated other biotic factors such as bee age, 'individuality' and hive in the analysis of trip duration, which explained an additional 32.9% of variation.

We present an exploratory approach, which substantially advances the understanding of bee behaviour in relation to weather and paves the way for several further studies. Successful pollination of horticultural crops is known to be directly linked to weather conditions during the flowering period. It is therefore suggested that future studies focus on understanding the correlation between longer and shorter bee trips and their practical pollination effectiveness. The understanding of weather must also be directly linked to practical outcomes regarding horticultural production, such as total fruit set and yield.

Photos/images/other audio-visual material

Please refer to supplementary thesis attached for images pertaining to this project.

Outputs

Table 1. Output summary

| Output | Description | Detail |
|--|--|---|
| Tracking technology and its potential for improving bee health and crop pollination. AG Institute of Australia division meeting. | Oral presentation regarding the planned project. Presented to ~20 beekeepers, growers and state government advisors. | This presentation was delivered on the 5 th June 2018 in North Hobart, Tasmania, Australia. A recording was uploaded to the AG institute of Australia's intranet for members to view. Engagement was facilitated by audience questions and open discussion regarding the project. |
| New RFID techniques to track the life history of individual bees. Macquarie University Neuroethology group presentation. | Oral presentation discussing RFID technology and the project. Presented to ~15 academics who specialise in bee research. | This presentation was delivered in person on the 26 th of June 2018, at Macquarie University, Sydney, Australia. Engagement was achieved through discussion of questions and suggestions from the expert audience. |
| "Research creates a real buzz". News article. | Article detailing the research project and background. Published in the Tasmanian Country newspaper and available to entire readership. | Article printed on the 25 th of May 2018 in the Tasmanian Country newspaper. Online abstract available at: https://www.utas.edu.au/tia/news-events/news-items/bees-with-backpacks-move-from-lab-to-real-world |
| Bee monitoring system creates a buzz. Cherry Magazine, Summer 2019. | Article describing the research project, published in a cherry industry magazine, produced by Horticulture Innovation Australia | The article was published in the summer 2019 edition of the Australian Cherry Magazine. |
| Monitoring Bee Health within Cropping Environments, Poster. Presentation. | Scientific poster presentation covering project design and preliminary outcomes. Presented to a scientific/academic audience of ~200 people. | Poster presented at the STEM State Future Forum, Hobart, Tasmania, Australia, 3 rd September 2019. Effective engagement was confirmed by receiving the second-place award for best academic poster. |
| Carrot pollination industry workshop. | Oral presentation and workshop with key members of the hybrid seed industry. | Presented via Zoom during Covid lockdowns (2020). Engagement was ensured via a workshop format, involving key stakeholders. |
| Bees with backpacks and other projects. Southern Beekeepers Association presentation. | Oral presentation about project and key findings. Delivered to ~40 members of the Southern Tasmanian Beekeepers Association. | Presented on the 31 st May 2021 in Claremont, Tasmania, Australia. Engagement was enabled through audience questions and open discussion regarding the project. |
| Tracking device could | Magazine article detailing | Published on the 21 st of December 2020 (Issue 51). |

| | | |
|--|---|---|
| bring sweet success. Alumni Magazine. | the research and candidate. Published in the University of Tasmania's Alumni Magazine with a readership of over 50,000 people. | https://www.utas.edu.au/alumni/news-and-publications/news-items/tracking-device-could-bring-sweet-success |
| Monitoring bee behaviour and health using RFID. Oral presentation, TIA pollination workshop. | Oral presentation focusing on pollination in cherry and hybrid seed crops. Presented to ~30 members of the horticultural industry. | Presentation delivered in person at the TIA pollination industry workshop, Sandy Bay, Tasmania, Australia, 2 nd July 2021. Evidence of engagement was garnered from personal communication with attendees. |
| Pollination under covers. Industry field day/Orchard walk. | A presentation detailing pollination was given at an 'orchard-walk' field day organised by Serve-Ag. There were approximately 50 attendees, representing all major stakeholders of the Tasmanian cherry industry. | A presentation was given at Woodmere Cherries in the Tamar Valley, Tasmania on the 26 th of February. |
| Honey bee health within cropping environments (Carrots). Conference presentation. | Industry presentation regarding project findings for bees in Hybrid carrot crops. Delivered to the South Pacific Seeds Production Conference. | Online international conference presented via Zoom on the 1 st September 2021. Evidence of engagement was obtained through audience participation and follow-up communication after the event. |
| Evaluating the foraging performance of individual honey bees in different environments with automated field RFID systems. Journal Article. | Academic journal article describing the RFID system. Published in Ecosphere, with open access for a wide academic audience. | Published in Ecosphere, Volume 13, Issue 5 on the 19 th May 2022. Available at https://doi.org/10.1002/ecs2.4088 |
| Optimising and applying RFID technology to monitor individual honey bee behaviour in agricultural field settings. Thesis. | Formal academic thesis describing the entire project. Written for an academic audience. | Submitted to the University of Tasmania for assessment. Available as an appendix to this report. |

Photos/images/other audio-visual material

N/A

Outcomes

This project was initially funded by the now defunct National Leaders in Horticulture Program. As a result, there are no fund outcomes, strategies or KPI available. Instead, those found in the Hort Frontiers Pollination Fund were utilised for this table.

Table 2. Outcome summary

| Outcome | Alignment to fund outcome, strategy and KPI | Description | Evidence |
|---|--|--|---|
| Improved management of bees operating in protected cropping environments. | <p>Outcome: Improved management of European Honey Bee for pollination.</p> <p>KPI: New information available on overall health and capacity of managed European Honey Bee populations.</p> | This project represents the most comprehensive study quantifying the impacts of protected cropping environments on honey bee behaviour. Improved understanding of how bees develop, forage and survive provides key information, which can be utilised by beekeepers and growers to improve the management of bees. | Feedback was received from direct consultation with pollination providers within the sweet cherry and hybrid seed industries. An increase in willingness to place hives under protective covers was observed, with an acknowledgement of nutrition as a future focus. |
| Ability to utilise real-time feedback to manage and maintain honey bee hives and pollination. | <p>Outcome: Improved management of European Honey Bee for pollination.</p> <p>KPI: Guidelines and tools developed for optimal hive set up and deployment.</p> | This project resulted in the development of the first precision RFID system, capable of field deployment on full-strength bee hives. This is an important tool, which generates information that can be readily used for pollination management. | Evidence is provided by a physical system, which has been tested in many field deployments. The effectiveness and relevance of this design is confirmed by publication in a peer-reviewed scientific journal. |
| Increased willingness by beekeepers to service the growing protected netting pollination industries. | <p>Outcome: Improved management of European Honey Bee for pollination.</p> <p>KPI: Recommendations based on R&D available for best practice management of European Honey Bees.</p> | The deployment of the RFID system facilitated the collection of quantifiable data regarding bee behaviour and survival under protected cropping systems. In general, these systems were found to have limited impact on bees. | Feedback was received via consultation with beekeepers that service the sweet cherry and hybrid seed industries. There was an increased willingness for these stakeholders to place hives in protected/modified cropping environments, as demonstrated by bees being placed under a 'Cravo' covering system for the first time. |
| Maintained profits for Australia's managed pollination and horticulture industry and reduced risk of partial or total pollination failure | <p>Outcome: Improved management of European Honey Bee for pollination.</p> <p>KPI: New information available on overall health and capacity of managed European Honey Bee populations.</p> | This project provides a critical baseline for establishing the impact of protected cropping on honey bees. The availability of this information provides reassurance to beekeepers, thus ensuring pollination continues. This stability can facilitate maintained or increased profits for both beekeepers and the horticultural industry. | Feedback was obtained directly from growers and beekeepers at industry events. Based on the evidence provided, all stakeholders were happy to maintain existing profitable relationships. |
| Building national capacity in honeybee and crop pollination biology by supporting postgraduate | <p>Outcome: Improved management of European Honey Bee for pollination.</p> | This project enabled the training of a postgraduate student in honey bee and crop pollination. | A completed thesis was submitted by a fully trained PhD candidate. |

| | | | |
|---------------------------|--|--|--|
| research degree training. | | | |
|---------------------------|--|--|--|

Monitoring and evaluation

Table 3. Key Evaluation Questions

| Key Evaluation Question | Project performance | Continuous improvement opportunities |
|---|---|---|
| Development of tools to reliably monitor foraging activity and bee health. | Resulted in the successful development of a highly accurate RFID system capable of monitoring honey bee behaviour in the field. This represents an important tool for future use. | In the future this system may be improved by incorporation of remote connectivity, continued development of analytical methodologies and the investigation of machine learning. |
| Enhanced pollination and honey bee health in protected cropping systems. | Provided one of the most comprehensive quantitative studies regarding honey bee behaviour in modified cropping systems to date. Facilitated the transfer of new knowledge to both growers and beekeepers. Directly facilitated management changes to ensure both bee health and pollination can be maintained and enhanced in the future. | Future work should utilise the methodology presented to investigate pollination of a range of other protected and modified crops e.g. a focus on the expanding berry industry. Issues such as optimising hive nutrition and minimising the impact of hive transport should also be a focus of further work. Overall, there is a need to more effectively understand the link between bee health and pollination outcomes. |
| Satisfy all PhD requirements e.g. confirmation, drafts, annual reviews and obtain a graduate certificate in research. | All requirements for PhD completion were met by the candidate. | Not applicable. |

Recommendations

Cherry growers

- Current pollination practices can continue. Protective covers were not found to be detrimental to bee health during the sweet cherry pollination window.

-Placing honey bees under both netted and polythene crop coverings generally resulted in more active foragers as opposed to those in the open. However, placing bees under covers will not ensure foraging during rain, yet it may create microclimatic conditions that are advantageous for foraging.

-Bees placed under increasingly restrictive crop coverings will generally collect more cherry pollen, however bees will still forage elsewhere. Flowering weeds, especially *Brassica* sp. should be managed within the orchard to maximise crop visitation. However, it is not suggested that all alternative resources are removed, to ensure a varied diet is available.

- Further investigation is required into optimal hive placement within an orchard, with relatively small separation distances from the crop (<200 m) causing significant variation in foraging behaviour.

- Further research should focus on the impact of weather on pollination outcomes, especially during a poor season. This could be used to predict pollination deficits and identify the need for supplementary pollination.

Carrot growers

- Being placed on a hybrid carrot crop can significantly impact individual foraging behaviour, resulting in bees consistently conducting fewer trips with a longer duration. Although causing variation in individual foraging behaviour, little evidence

was found to suggest that being placed on a hybrid carrot seed crop was determinantal to overall bee health and survival.

- Honey bees will regularly leave the carrots to visit a wide range of native and weed species, collecting pollen from multiple species in one trip. The strategy of selecting isolated cropping sites, appears to have limited effectiveness for restricting bees to the crop; hence should be reconsidered. Floral attractiveness should be considered in future selection of hybrid parent lines.
- No sublethal effects were identified from current pesticide management, however future studies should include residue analysis.
- Further studies should be undertaken to incorporate a greater number of hives, in multiple carrot growing areas, utilising different hybrid varieties. This includes investigation within pollination tents and other protective cropping systems.
- Future studies should collect high resolution weather and bee data from a variety of locations and differing seasons to generate a wider range of weather observations.

Beekeepers

- Placing hives underneath protective cropping systems, such as bird netting, VOEN and Cravo, will not cause rapid foraging bee losses. Such positioning may be beneficial rather than detrimental.
- Although conditions are often extreme, hybrid carrot seed crops will not necessarily have a negative impact on hive health.
- Further investigation is required into hive management strategies pre and post crop. This includes supplementary feeding, which may be effective for building up hive strength prior to deployment or artificially influencing the visitation of floral resources e.g. encouraging nectar foraging on carrots.
- There should be a focus on generating quantifiable evidence to investigate the impact of post crop recovery on honey yields and hive strength. This is especially relevant to long term monitoring using accurate hive scales.
- It must be acknowledged that compromises are required when providing commercial pollination. Although the crop itself may have limited impact on bees, factors such as hive movement may be highly disruptive. Providing commercial pollination is likely to impose some stress on bees, however minor bee losses must be considered in terms of financial return.
- Continue to request that the spraying of pesticides is minimised during the pollination window and insist on spraying when bees are not active.

Researchers

- For the first time a fully optimised RFID system has been developed for monitoring full-strength honey bee hives in the field. This now represents immense potential for a wide range of future studies in a wide range of protective cropping environments. It is suggested that a focus in cool temperate climates should be berry pollination.
- Further work is required to more explicitly link RFID foraging data with practical pollination outcomes. This can in part be achieved through comparing RFID data with variation in the collection of pollen and nectar.
- RFID systems should be utilised in the long-term monitoring of hives to further encompass the impacts of commercial pollination on hive recovery. As well as designing specific experiments to investigate the influence of hive transport on bee health. Underlying pesticide levels within hives should also be investigated utilising multigeneration monitoring to identify any legacy effects.
- Further development of the RFID system is suggested through integration of wireless data downloads, the direct

integration of an accurate weather station and measuring colony level foraging force dynamics.

Refereed scientific publications

Journal article

Colin, T., Warren, R.J., Quarrell, S.R., Allen, G.R., and Barron, A.B., 2022. Evaluating the Foraging Performance of Individual Honey Bees in Different Environments with Automated Field RFID Systems. *Ecosphere* 13(5): e4088. <https://doi.org/10.1002/ecs2.4088>

Intellectual property

No project IP or commercialisation to report

Acknowledgements

We acknowledge the dedication, guidance and expert opinions of Dr Théotime Colin and Professor Andrew Barron from Macquarie University. We thank Duncan MacIntyre of Tasman Apiaries for his beekeeping expertise and supplying the hives used in the experiments. Andrew Hall, Lochie and Dale, from Reid fruits Jericho and Howard Hansen and Ryan Hankin from Hansen Orchards for allowing access to the orchards used. Craig Garland from South Pacific Seeds for access to the carrot crops, Dr Cameron Spurr from Seed Purity for practical advice and Piñata Farms for providing access to the raspberry farm. We also thank Cameron Stone, Royce Warren, Madeleine Way and Mengyong Lim for assistance in conducting experiments and Powercom Solar, Rohan Windsor and Taylor Gatehouse for assistance with solar systems.

Appendices

Complete thesis provided as separate attachment.

References

- Alburaki, M., Madella, S., Corona, M., 2021. Rfid technology serving honey bee research: A comprehensive description of a 32-antenna system to study honey bee and queen behavior. *Applied System Innovation* 4, 88.
- Bordier, C., Klein, S., Le Conte, Y., Barron, A.B., Alaux, C., 2018. Stress decreases pollen foraging performance in honeybees. *The Journal of Experimental Biology* 221, jeb171470.
- Broussard, M.A., Mas, F., Howlett, B., Pattemore, D., Tylianakis, J.M., 2017. Possible mechanisms of pollination failure in hybrid carrot seed and implications for industry in a changing climate. *Plos One* 12, e0180215.
- Chang, L.-H., Barron, A.B., Cheng, K., 2015. Effects of the juvenile hormone analogue methoprene on rate of behavioural development, foraging performance and navigation in honey bees (*apis mellifera*). *The Journal of Experimental Biology* 218, 1715-1724.
- Clarke, D., Robert, D., 2018. Predictive modelling of honey bee foraging activity using local weather conditions. *Apidologie* 49, 386-396.
- Colin, T., Forster, C.C., Westacott, J., Wu, X., Meikle, W.G., Barron, A.B., 2021. Effects of late miticide treatments on foraging and colony productivity of European honey bees (*apis mellifera*). *Apidologie* 52, 474-492.
- Colin, T., Meikle, W.G., Wu, X., Barron, A.B., 2019. Traces of a neonicotinoid induce precocious foraging and reduce foraging performance in honey bees. *Environmental Science & Technology* 53, 8252-8261.
- Cook, D.F., Voss, S.C., Finch, J.T.D., Rader, R.C., Cook, J.M., Spurr, C.J., 2020. The role of flies as pollinators of horticultural crops: An Australian case study with worldwide relevance. *Insects* 11, 341.
- Coulon, M., Dalmon, A., Di Prisco, G., Prado, A., Arban, F., Dubois, E., Ribière-Chabert, M., Alaux, C., Thiéry, R., Le Conte, Y., 2020. Interactions between thiamethoxam and deformed wing virus can drastically impair flight behavior of honey bees. *Frontiers in Microbiology* 11, 766-766.
- Dag, A., 2008. Bee pollination of crop plants under environmental conditions unique to enclosures. *Journal of Apicultural Research* 47, 162-165.
- de Guzman, L.I., Frake, A.M., Simone-Finstrom, M., 2017. Comparative flight activities and pathogen load of two stocks of honey bees reared in gamma-irradiated combs. *Insects* 8, 127.
- de Souza, P., Marendy, P., Davie, A., Budi, S., Hirsch, P., Barbosa, K., Nikolic, N., Gunthorpe, T., Pessin, G., 2018. Low-cost electronic tagging system for bee monitoring. *Sensors (Switzerland)* 18 (7), 2124.
- Decourtye, A., Devillers, J., Aupinel, P., Brun, F., Bagnis, C., Fourrier, J., Gauthier, M., 2011. Honeybee tracking with microchips: A new methodology to measure the effects of pesticides. *Ecotoxicology* 20, 429-437.
- Dosselli, R., Grassl, J., Carson, A., Simmons, L.W., Baer, B., 2016. Flight behaviour of honey bee (*apis mellifera*) workers is altered by initial infections of the fungal parasite *nosema apis*. *Scientific Reports* 6, 36649.
- Evans, L.J., Cutting, B.T., Jochym, M., Janke, M.A., Felman, C., Cross, S., Jacob, M., Goodwin, M., 2019. Netted crop covers reduce honeybee foraging activity and colony strength in a mass flowering crop. *Ecology and Evolution* 9, 5708-5719.
- Gaffney, A., Allen, G.R., Brown, P.H., 2011. Insect visitation to flowering hybrid carrot seed crops. *New Zealand Journal of Crop and Horticultural Science* 39, 79-93.
- Gaffney, A., Bohman, B., Quarrell, S.R., Brown, P.H., Allen, G.R., 2019. Limited cross plant movement and non-crop preferences reduce the efficiency of honey bees as pollinators of hybrid carrot seed crops. *Insects* 10(2):34.
- Gaffney, A., Bohman, B., Quarrell, S.R., Brown, P.H., Allen, G.R., 2020. It is not all about being sweet: Differences in floral traits and insect visitation among hybrid carrot cultivars. *Insects* 11, 402.
- Goulson, D., Nicholls, E., Botias, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*

- Hall, M.A., Jones, J., Rocchetti, M., Wright, D., Rader, R., 2020. Bee visitation and fruit quality in berries under protected cropping vary along the length of polytunnels. *Journal of Economic Entomology* 113, 1337-1346.
- Hesselbach, H., Seeger, J., Schilcher, F., Ankenbrand, M., Scheiner, R., 2020. Chronic exposure to the pesticide flupyradifurone can lead to premature onset of foraging in honeybees *apis mellifera*. *Journal of Applied Ecology* 57, 609-618.
- Hung, K.-L.J., Kingston, J.M., Albrecht, M., Holway, D.A., Kohn, J.R., 2018. The worldwide importance of honey bees as pollinators in natural habitats. *Proceedings of the Royal Society B: Biological Sciences* 285, 20172140.
- Kendall, L.K., Evans, L.J., Gee, M., Smith, T.J., Gagic, V., Lobaton, J.D., Hall, M.A., Jones, J., Kirkland, L., Saunders, M.E., Sonter, C., Cutting, B.T., Parks, S., Hogendoorn, K., Spurr, C., Gracie, A., Simpson, M., Rader, R., 2021. The effect of protective covers on pollinator health and pollination service delivery. *Agriculture, Ecosystems & Environment* 319, 107556.
- Khalifa, S.A.M., Elshafiey, E.H., Shetaia, A.A., El-Wahed, A.A.A., Algethami, A.F., Musharraf, S.G., Al Ajmi, M.F., Zhao, C., Masry, S.H.D., Abdel-Daim, M.M., Halabi, M.F., Kai, G., Al Naggar, Y., Bishr, M., Diab, M.A.M., El-Seedi, H.R., 2021. Overview of bee pollination and its economic value for crop production. *Insects* 12, 688.
- Klein, A.-M., Vaissière, 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, 303-313.
- Klein, S., Pasquaretta, C., He, X.J., Perry, C., Søvik, E., Devaud, J.-M., Barron, A.B., Lihoreau, M., 2019. Honey bees increase their foraging performance and frequency of pollen trips through experience. *Scientific Reports* 9, 6778.
- Kulhanek, K., Steinhauer, N., Rennich, K., Caron, D.M., Sagili, R.R., Pettis, J.S., Ellis, J.D., Wilson, M.E., Wilkes, J.T., Tarpy, D.R., Rose, R., Lee, K., Rangel, J., vanEngelsdorp, D., 2017. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. *Journal of Apicultural Research* 56, 328-340.
- Kurlus, R., Rutkowski, K., Łysiak, G.P., 2020. Improving of cherry fruit quality and bearing regularity by chemical thinning with fertilizer. *Agronomy* 10, 1281.
- Lach, L., Kratz, M., Baer, B., 2015. Parasitized honey bees are less likely to forage and carry less pollen. *Journal of Invertebrate Pathology* 130, 64-71.
- Lang, G.A., 2014. Growing sweet cherries under plastic covers and tunnels: Physiological aspects and practical considerations. VI International Cherry Symposium. *Acta Horti*, pp. 303-312.
- Lang, G.A., Sage, L., Wilkinson, T., 2016. Ten years of studies on systems to modify sweet cherry production environments: Retractable roofs, high tunnels, and rain-shelters. *Acta Horti* 1130, 83-90.
- Mas, F., Harper, A., Horner, R., Welsh, T., Jaksons, P., Suckling, D.M., 2018. The importance of key floral bioactive compounds to honey bees for the detection and attraction of hybrid vegetable crops and increased seed yield. *Science of Food and Agriculture* 98, 4445-4453.
- Mika, A., Buler, Z., Wójcik, K., Konopacka, D., 2019. Influence of the plastic cover on the protection of sweet cherry fruit against cracking, on the microclimate under cover and fruit quality. *Journal of Horticultural Research* 27, 31-38.
- Milone, J.P., Tarpy, D.R., 2021. Effects of developmental exposure to pesticides in wax and pollen on honey bee (*apis mellifera*) queen reproductive phenotypes. *Scientific Reports* 11, 1020-1020.
- Nunes-Silva, P., Hrnčir, M., Guimarães, J., Arruda, H., Costa, L., Pessim, G., Siqueira, J., De Souza, P., Imperatriz-Fonseca, V.L., 2018. Applications of rfid technology on the study of bees. *Insectes Sociaux* 66, 15-24.
- Odemer, R., 2022. Approaches, challenges and recent advances in automated bee counting devices: A review. *Annals of Applied Biology* 180, 73-89.
- Perry, C.J., Søvik, E., Barron, A.B., Myerscough, M.R., 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *Proceedings of the National Academy of Sciences of the United States of America* 112, 3427-3432.
- Prado, A., Requier, F., Crauser, D., Le Conte, Y., Bretagnolle, V., Alaux, C., 2020. Honeybee lifespan: The critical role of pre-foraging stage. *Royal Society Open Science* 7, 200998.
- Rubauskis, E., Skrīvele, M., Ruisa, S., Feldmane, D., 2013. Effects of voen cover on the growth and yield of two sweet cherry cultivars. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences* 67, 157-161.
- Ruisa, S., Feldmane, D., Skrīvele, M., Rubauskis, E., Kaufmane, E., 2017. The effect of rain protective covering on sweet cherry fruit quality. VII International Cherry Symposium. *Acta Horti*, pp. 143-148.
- Schneider, C., Tautz, J., Grünewald, B., Fuchs, S., 2012. Rfid tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *apis mellifera*. *Plos One* 7, e30023-e30023.
- Simone-Finstrom, M., Li-Byarlay, H., Huang, M.H., Strand, M.K., Rueppell, O., Tarpy, D.R., 2016. Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. *Scientific Reports* 6, 32023.
- Spurr, C., 2003. Identification and management of factors limiting hybrid carrot seed production in australia. University of Tasmania Australia, pp. 1-281.
- St. Clair, A.L., Zhang, G., Dolezal, A.G., O'Neal, M.E., Toth, A.L., 2020. Diversified farming in a monoculture landscape: Effects on honey bee health and wild bee communities. *Environmental Entomology* 49, 753-764.
- Susanto, F., Gillard, T., De Souza, P., Vincent, B., Budi, S., Williams, R.N., Engelke, U., Marendy, P., Hirsch, P., He, J., Almeida, A., Pessim, G., Arruda, H., 2018. Addressing rfid misreadings to better infer bee hive activity. *IEEE Access* 6, 31935-31949.
- Tenczar, P., Lutz, C.C., Rao, V.D., Goldenfeld, N., Robinson, G.E., 2014. Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. *Animal Behaviour* 95, 41-48.
- Tomé, H.V.V., Schmehl, D.R., Wedde, A.E., Godoy, R.S.M., Ravaiano, S.V., Guedes, R.N.C., Martins, G.F., Ellis, J.D., 2020. Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. *Environmental Pollution* 256, 113420.
- Van Geystelen, A., Benaets, K., de Graaf, D.C., Larmuseau, M.H.D., Wenseleers, T., 2016. Track-a-forager: A program for the automated analysis of rfid tracking data to reconstruct foraging behaviour. *Insectes Sociaux* 63, 175-183.
- Vávra, R., 2020. Sweet cherry fruit characteristic in covered orchards. *Acta Horticulturae et Regiotecturae* 23, 5-7.
- Vicens, N., Bosch, J., 2000. Weather-dependent pollinator activity in an apple orchard, with special reference to *osmia cornuta* and *apis mellifera* (hymenoptera: Megachilidae and apidae). *Environmental Entomology* 29, 413-420.