Final report

Project title:

Integrated pest management of citrus gall wasp and Fuller's rose weevil

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Project code:

CT19009

Date:

30 May 2025

Disclaimer:

Spinosad and spinetoram are not registered for the control of the citrus gall wasp and therefore cannot be used on commercial properties for this purpose. The products were used at the DPIRD research station, which has permission to test off-label products for efficacy studies under experimental conditions.

Funding statement:

This project has been funded by Hort Innovation, using the citrus industry research and development levy, contributions from the Australian Government, and co-investment from NSW DPIRD, UQ, and USQ. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

Published and distributed by: Horticulture Innovation Australia Limited
ABN 71 602100149

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

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Public summary

Citrus gall wasp (CGW) and the Fuller's rose weevil (FRW) are two significant insect pests of citrus in Australia. CGW infestations lead to the formation of woody galls on trees, reducing fruit size and yield. FRW is a flightless root weevil that lays eggs under the calyces of citrus fruits. The presence of FRW eggs can result in the rejection of fruit in key export markets, particularly in Asia. Management tools are available for both pests, but their adoption is limited due to cost and application constraints. This project provides growers with essential tools and new knowledge to more effectively detect and manage these pests.

Monitoring is a key component in any integrated pest management (IPM) system. Currently, the standard monitoring method for FRW is branch shaking, which is highly variable and difficult to standardize. To provide a more reliable alternative for FRW monitoring, we investigated the performance of a free-standing trap called Tedders trap. We found that Tedders trap data were less erratic and provided a more consistent indication of local FRW population peaks than branch-shaking data. Tedders traps can be used alone without needing any attractant or lure.

For CGW, we developed an easy-to-use guide to rate local CGW infestation levels. The rating guide enables growers and pest consultants to quickly assess the local CGW infestation level (low, moderate, or high) and take appropriate management actions. The CGW online timing tool, developed in a previous Hort Innovation project (CT15006 - Development of national strategies to manage citrus gall wasp), has been updated to improve stability and expanded its application range to include Central Burnett, QLD, and Perth, Western Australia.

Machine-based monitoring is being increasingly used in the management of agricultural pests. However, it is not yet available for pests like CGW and FRW. In this project, we demonstrated the potential of machine vision technology to identify CGW galls, count FRW adults on drop sheets, and detect FRW damage on leaves. Further work is needed to refine these techniques and develop commercial automated monitoring tools.

Two chemicals currently registered for CGW control have long withholding periods and are costly, which discourages some growers from using them. We identified two biologically derived chemicals, spinosad and spinetoram, as promising alternatives with short withholding periods suitable for all citrus varieties. However, these chemicals are not yet registered for CGW control and require further regulatory approval before growers can use them.

A Ph.D. study provided new insights into the biology and ecology of CGW. Genetic analyses revealed distinct regional CGW populations but no strong evidence of multiple species within commercial orchards, except for a divergent population on Desert lime in Queensland which needs further investigation. Genetic data collected in this study do not seem to support the hypothesis that CGW populations in southern Australia originated from the movement of citrus seedlings in recent years. Parasitism rates by key biocontrol agents, *Megastigmus brevivalvus* and *M. trisulcus*, varied widely across regions and years, with generally low success in New South Wales. In many cases, larval mortality from unknown natural causes exceeded parasitoid-induced mortality, suggesting other environmental or biological factors play important roles in CGW population control. CGW reproductive rates also differed between regions and seasons, suggesting that local environmental or ecological drivers, such as parasitoid adaptation or environmental cues, play a role in population dynamics. Field studies in Queensland showed gall density is only weakly aggregated within orchards and parasitism rates vary with gall size, site, and year. Two additional parasitoids, *Amerostenus* sp. and *Eupelmus* sp., were recorded attacking CGW but caused relatively low mortality compared to other factors.

This project provides growers with better monitoring tools and valuable biological knowledge to improve pest management decisions for CGW and FRW. Growers are encouraged to use Tedders traps and the updated rating and timing tools to manage these pests more effectively. Continued research and efforts to register new chemical options, along with the development of automated monitoring technologies, will be important for sustainable pest management in the future.

Keywords

Citrus gall wasp, *Bruchophagus fellis*, Fuller's rose weevil, *Asynonychus cervinus*, monitoring, chemical control

Introduction

Citrus is a major horticultural crop in Australia, with commercial production in all five states and one territory and a total farmgate value of over AU$1 billion. As one of the country's largest fresh produce exports, citrus generated $543 million in export value in 2023/24 (Horticulture Statistics Handbook, 2023/24), primarily from oranges and mandarins. Oranges account for 63% of all citrus varieties in Australia (by volume) and are primarily grown in the Riverland, Murray Valley, and Riverina in southern Australia.

Many insects and mites have been reported attacking citrus in Australia. However, only a few require occasional human interventions. Among them are the citrus gall wasp (CGW, *Bruchophagus fellis*, Hymenopter: Eurytomidae) and the Fuller's rose weevil (FRW, *Asynonychus cervinus*, Coleoptera: Curculionidae).

Citrus gall wasp

CGW is an endemic pest of citrus in Australia. The adult wasps emerge from galls in the spring (Figure 1, right bottom). The female wasps lay eggs in spring shoots (Figure 1, right top). After hatching, the larvae burrow into the soft bark tissue and feed there in individually constructed cells until pupating. The area of a shoot housing multiple feeding larvae swells as the season progresses and eventually forms a characteristic gall. The lifecycle completes in one year. Citrus is the only recorded host group of CGW. Heavily infested trees can be covered with galls (Figure 1, left), causing yield loss and a reduction of fruit size.

There are few published studies on CGW. It was first reported as a citrus pest in the late 1890s (McKeown, 1898). Subsequent surveys showed it was present in the coastal districts of southern Queensland (Qld) and New South Wales (NSW) (Noble, 1936). The first detailed accounts of the morphology and life cycle of CGW and its parasitoids were provided by Noble (1936, 1938). It was first detected in the Murray Valley in southwest NSW in the late 1990s and has since spread rapidly in southern Australia, including the Riverina and Riverland. The infestation level is still rising in many places, threatening the viability of citrus production in Australia. Two recent Hort. Innovation-funded projects investigated the management of CGW. Project CT10021 (Managing citrus gall wasps in southern citrus regions) investigated the biology of the CGW, developed a preliminary phenology model for adult wasp emergence, and identified two potential chemical options (Mo, 2012; Mo and Stevens, 2014). Project CT15006 (Development of national strategies to manage citrus gall wasp) estimated the optimal time for pruning, identified population hot spots of CGW parasitoids, investigated rearing of parasitoids, identified three new chemical options for controlling the wasp larvae and a non-insecticidal product for repelling the adult wasps, and developed an online timing tool for the emergence of the adult gall wasp (Mo, 2018).

Figure 1. Galls of the citrus gall wasp (left), female gall wasp (right, top), and a dissected gall showing adult gall wasps ready to emerge (right, bottom).

Fuller's rose weevil

FRW (Figure 2, left) is a flightless weevil found in most citrus growing areas of Australia (Smith et al. 1997). The larvae feed on plant roots and pupate in the soil. Upon emergence, the adults climb up the tree to feed on foliage and lay eggs, mostly under fruit calyces (Figure 2, right) (Hely, 1948). Although FRW causes little direct damage to the trees and fruit, it is a declared quarantine pest in major export markets of Australian citrus in Asia. These countries require either zero detection of FRW in orchards or expensive fumigation treatment of the fruit. FRW is highly polyphagous. In addition to citrus, it also feeds on apples, peaches, and avocados.

Figure 2. An adult Fuller's rose weevil (left) and its eggs under the fruit calyx (right).

Previous Hort Innovation-funded research (CT13010 – In-line approaches to control surface pests of concern from export citrus; CT11002 – Citrus market access solutions for FRW and Island fly; CT07045 – Managing pests of quarantine concern for citrus market access; CT12016, CT06040) on FRW has focused primarily on surveillance and managing it as a post-harvest contaminant, but did not incorporate IPM. Recommended management strategies include skirting, weeding, and trunk sprays (Baker et al., 2011; Falivene and Creek, 2017; Mo and Stevens, 2013). Some growers also use foliar insecticide sprays and soil drenches with systemic insecticides. Chemical controls can be disruptive to the natural enemies (beneficials) of citrus, leading to outbreaks of secondary pests, and frequent chemical applications may result in chemical residue issues.

IPM provides the framework for sustainably managing pests. It is based on a good understanding of pest biology and ecology, effective monitoring of the pest population, and integration of different control options, including cultural, biological, and chemical. To sustainably manage CGW and FRW, the project focused on:

Monitoring and management of Citrus gall wasp and Fuller's rose weevil

For sustainable management of CGW and FRW, the project focused on:

- Investigating new monitoring methods for CGW and FRW,
- Exploring novel biological control options for CGW and FRW,
- Updating the online timing guide for CGW,
- Investigating new chemical options for controlling CGW and FRW, and
- Studying the biology and ecology of CGW and modelling the future population trend of CGW in Australia.

Being a gall-forming pest, CGW can be easily monitored by the presence and prevalence of galls. However, a quantitative monitoring method is needed to determine if the infestation level is low, moderate, or high. Currently, some pest consultants use the 'Branch method' (Mo, 2018) to determine the infestation level. However, this method is tedious, and very few growers are using it. To investigate the problem, the project investigated an alternative monitoring method to determine CGW infestation level. FRW is monitored by shaking a branch and counting the number of dislodged FRW adults (Mo and Stevens, 2013). This method is difficult to standardise as the number of weevils dislodged is affected by the frequency and intensity of shaking. The project investigated a trap-based method and a new technique based on machine vision for the monitoring of FRW populations.

CGW is attacked by several parasitic wasp species, with *Megastigmus brevivalvus* (MBV, Hymenoptera: Torymidae) being the predominant species (Noble, 1938). MBV has been established in the main orange production regions, however, its population level is currently insufficient to reduce the CGW populations (Mo, 2018). Lucerne is planted as an intercrop in some citrus orchards in Australia. Recently, it has been reported that lucerne interplanting improved CGW control. Lucerne seeds are attacked by the Lucerne seed wasp (LSW, *Bruchophagus roddi*, Hymenoptera: Eurytomidae, Strong, 1962). LSW has several parasitoids; at least two are present in Australia (De Barro, 2001). The project investigated the likelihood of LSW parasitoids attacking CGW. If they do, these parasitoids can potentially be used to boost the biological control of CGW significantly.

The parasitoid *Fidobia citri* (Hymenoptera: Scelionidae, Morse et al. 1988; Madge et al., 1992) parasitises FRW eggs, but the parasitoid alone is unlikely to reduce FRW populations to levels acceptable for export. Some entomopathogenic nematodes and fungi have demonstrated potential for FRW control (McCoy and Boucias, 1989; Edwards, 1996). This project conducted a proof-of-concept study to verify the potential of entomopathogenic nematodes and fungi for FRW control.

An online timing guide was developed in project CT15006 (Mo, 2018; Mo and Stevens, 2021) and is currently available for predicting the emergence of adult CGW and egg hatching in the Riverina, Murray Valley, and Riverland. CGW is a national problem, and the online timing guide needed to be expanded to include other citrus production regions in Australia.

Two of the three chemical options identified as promising for CGW control in project CT15006, a systemic insecticide and a repellent, have since been registered for its control. While proven effective, the two chemicals have their limitations. The systemic insecticide is recommended for spring application and has a 20-week withholding period in citrus, making it unsuitable for use in Valencia orange trees, which bear mature fruit in spring and are typically harvested during the recommended application window. Additionally, the systemic insecticide is a neonicotinoid, a group of insecticides recently banned in Europe and are likely to be withdrawn in other places. The repellent is non-toxic, but it might impede the biological control of red scale (*Aonidiella aurantia*, Homoptera: Diaspididae), another important citrus pest. The repellent is also considered too expensive by many growers. New chemical options, preferably softer options, are needed to improve the management of CGW.

Multiple factors are likely responsible for the sudden build-up of CGW populations in southern Australia. To understand the causes and future population trends, and ultimately develop sustainable management strategies, the project studied the biology and ecology of CGW and developed a population model for CGW and its primary parasitoid, MBV

The NSW DPIRD project team was Dr. Jianhua Mo, Scott Munro, Steven Falivene, Andrew Creek, and Dr. Meena Thakur. NSW DPIRD was supported by the University of Queensland (UQ), University of Southern Queensland (UniSQ), Cesar Australia, and Riverina IPM.

Methodology

CGW monitoring

This study aimed to estimate the CGW infestation level in a citrus orchard by the abundance of medium to large galls. To do this, we analysed the distributions of gall length in galls collected from low, moderate, and high infestation orchards. First, we compared different statistical distributions to see which provided the best-fit for the gall length data. We fitted the data to the best-fit distribution separately for each infestation level. We then plotted the fitted cumulative proportions of galls by gall length for each infestation level to see which value of gall length provided the largest separation of different infestation levels. Finally, we developed a set of criteria for identifying low, moderate, and high CGW infestations based on the percentage of galls at or above a threshold gall length.

FRW monitoring

To provide an alternative to branch shaking for FRW monitoring, we investigated the performance of Tedders trap (Figure 3). Tedders trap is a free-standing trap used for weevil monitoring overseas. It exploits the negative geotaxis (climbing upwards) behaviour of weevils. No lures are required for the Tedders trap. We assessed the performance of the Tedders traps relative to the branch-shaking method in five citrus blocks in Leeton in southwest NSW that are known to have FRW populations. Ten traps were placed at each site. Monitoring data were collected twice weekly to fortnightly from January 2021 to March 2024. The number of adult FRW caught in each Tedders trap was counted and recorded on each monitoring occasion. For comparison, branch-shaking data and fruit-contamination data were also collected. Branch shaking data were collected from 5 random branches at each site on each monitoring occasion. Fruit contamination data were collected monthly by examining 100–500 random fruits from each site for the presence of FRW eggs.

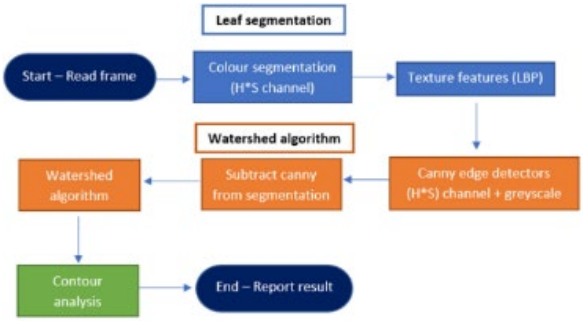
Figure 3. Tedders trap

To find out if an attractant can improve the efficiency of the Tedders traps, we investigated adult FRW movement in the field with respect to odours from the leaves of orange, rose, and clover, their essential oils, and in the laboratory using a Y-tube olfactometer and a 4-choice olfactometer. We also investigated trap enhancement directly by baiting the traps with two reported attractants of FRW, green leaf volatiles, and clover oil.

A detailed description of the method is provided in Appendix 1.

Monitoring with machine vision

This was a one-year, proof-of-concept study investigating the potential of automated monitoring of CGW and FRW using deep-learning machine vision models. The study focused on (1) CGW detection from the multi-spectral response, (2) FRW counting on a beat sheet, and (3) FRW detection from leaf damage. Consumer smartphones were used to collect in-field images to provide rapid, high-resolution image collection and emulate on-the-go image collection. A multi-spectral sensor and short-wave infrared camera were evaluated to sample the stems for CGW to identify wavelengths that could indicate gall presence and age of damage.



A detailed description of the method is provided in Appendix 2.

CGW timing guide

Data on adult gall wasp emergence were collected in Perth (WA) in 2019 and 2020 and in Mundubbera (QLD) in 2020. The observed emergence patterns were compared with the emergence pattern predicted by the online timing guide (https://citrusgallwasp.shinyapps.io/predict/). The timing guide was updated by adjusting the degree-day parameters and by adding two new sites: Gayndah in QLD and Perth City. Gayndah is the closest town to Mundubbera with an operational weather station. The timing guide automatically downloads temperature data for the site selected by the user. Previously, the timing guide downloaded the temperature data from the Australian Bureau of Meteorology (BOM: www.bom.gov.au). BOM recently introduced restrictions to automated data downloads. To overcome this, the temperature data source was moved to SILO (https://www.longpaddock.qld.gov.au/silo/).

Lucerne interplanting and CGW control

To determine if the parasitic wasps of the LSW also parasitises CGW, we collected CGW galls and galls of the lucerne seed wasps in a citrus orchard in central west NSW, where the benefit of lucerne interplanting for CGW control was reportedly observed**,** in October 2020. A total of 844 CGW galls were collected. The galls were kept in containers until all parasitic wasps had emerged. Identities and corresponding parasitism levels were determined for the gall samples. To assess whether lucerne planting reduced CGW infestation, we compared citrus rows with (Figure 4) and without lucerne interplanting within a large 14-hectare citrus block where lucerne had been established between rows.

Figure 4. Lucerne interplanted between citrus rows

Entomopathogenic fungi and nematodes

Two commercial products of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae* were investigated for controlling FRW larvae in the soil in two field trials in commercial orchards in the Riverina. Six experimental strains of the entomopathogenic fungi (Figure 5) *Beauveria bassiana* and *Metarhrizium anisopliae* were investigated for their potential in killing CGW and FRW adults or reducing their oviposition in a series of laboratory bioassays, and for controlling CGW larvae in a potted-tree trial.

A detailed description of the method is provided in Appendix 3.

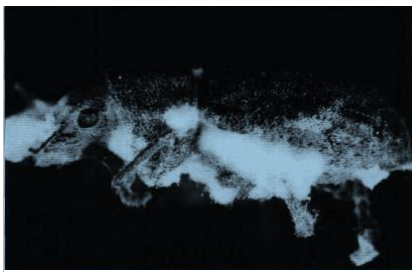


Figure 5. FRW adult infected with an entomopathogen

New chemical options for CGW control

Potential new chemical options for controlling CGW and FRW were first screened to identify promising options. Next, the promising chemicals were tested at a series of concentrations to estimate the minimal effective rates. These chemicals were then field-tested to determine their field efficacy. Finally, the promising chemicals were tested for their effects on key beneficial arthropods in citrus.

Both direct contact and residual activity of the chemicals were investigated. Direct contact activity was investigated by spraying the chemicals directly to test insects using a Potter spray tower. Indirect contact activity was investigated by spraying the chemical onto trees, collecting the leaves after a target period, and finally placing the leaves and test insects in a small container to ensure contact.

Screening trials identified spinosad and spinetoram as most promising for CGW control. An experimental product, DC-154, also showed efficacy against CGW when it was sprayed directly on the test wasps. The field efficacy of the three chemicals was investigated in a block of mature Valencia orange trees. To evaluate the effect of spray frequency and timing, spinetoram was tested in two additional treatment regimens: a double-spray treatment with the second application 7 days after the initial spray, and a single, late-spray treatment 11 days after the initial spray in other plots.

The beneficial impact study investigated the residual toxicity of spinosad, spinetoram, DC-154, and another experimental product, EXP-A, which has shown promise for FRW control in a separate study, on four important beneficial arthropods of citrus in Australia: the red scale parasitoid *Aphytis lingnanensis*, the ladybird beetle *Cryptolaemus montrouzieri*, the green lacewing *Mallada signatus*, and the predatory mite *Neoseiulus californicus*. Only residual toxicity was investigated.

A detailed description of the method is provided in Appendix 4.

Optimal timing for applying a foliar insecticide against CGW

MBV is the primary parasitoid species of CGW. It emerges from the galls 2-3 weeks after CGW. Taking advantage of this asynchrony, we modelled the effect of spray timing for a foliar-applied, non-systemic insecticide on the relative exposures of CGW and MBV based on their emergence distributions and estimated the optimal spray timing that maximises the control of CGW while minimising the effect on MBV. Three temporal emergence distribution scenarios for the two species and 28 residual activity durations of the insecticide were considered in the investigation.

A detailed description of the method is provided in Appendix 5.

New chemical options for FRW control

Similar methods were used to identify promising new chemical options for FRW control. In addition to mortality, candidate chemicals were also investigated for their effects on disabling the test weevils and their oviposition. The presence of FRW eggs in the fruit calyx is the only damage of concern for FRW. Follow-up investigations on rate-response, field efficacy, and beneficial impact were targeted at the experimental product Exp-A only. Exp-A was one of the two chemicals found promising in the initial screening. The other chemical found promising was Indoxacarb. It was not further investigated after the initial screening, considering the absence of MRLs for this chemical in any of Australia's citrus export markets.

A detailed description of the method is provided in Appendix 6.

Future CGW population trends

A discrete population model was developed to predict future CGW population patterns in the southern citrus production regions. This is a variation of the Nicholson-Bailey model, which is popular for describing pest-parasitoid interactions and includes density dependence in CGW population growth. It assumes that (1) CGW population growth follows the logistic function, (2) the total number of parasitising attempts by a parasitoid is governed by Holling's type II equation, and (3) the number of parasitising attempts per host follows the negative binomial distribution (NBD). A combination of unpublished and published data was used to estimate the fecundity, sex ratio, and stage-specific mortalities of the two species.

PhD study - CGW biology and ecology

As part of this project, a PhD study was undertaken to investigate the biology, ecology, and population genetics of the CGW. The study aimed to address key gaps in knowledge around CGW taxonomy, host range, and the variability in biological control outcomes across different regions. A particular focus was placed on investigating whether cryptic species exist within CGW populations, which could help explain inconsistent parasitism and management outcomes.

Mitochondrial COI barcoding and a high-resolution genomic approach known as genotyping-by-sequencing (GBS) were used to investigate this. CGW adults were collected from 21 locations across QLD and NSW over three consecutive years (seven sites in 2021/2022, five in 2022/2023, and nine in 2024) from commercial orchards and a native citrus species, Desert Lime (Citrus glauca), in Dululu, QLD.

Laboratory experiments were conducted to test the effects of different constant temperatures on CGW and different photoperiod regimes on CGW and *M. brevivalvus* development. Galls were maintained under controlled environmental conditions with varying temperatures and photoperiods to assess impacts on gall growth and adult emergence.

Field collections of CGW galls were carried out across different sites and seasons to evaluate the relative importance of different mortality factors (larval mortality and parasitism by known egg- pupal parasitoids) e affecting CGW. Galls were dissected to estimate stage-specific mortality, and life tables were constructed to calculate factor specific marginal mortality rates, sex ratios and net reproductive rates (R_o).

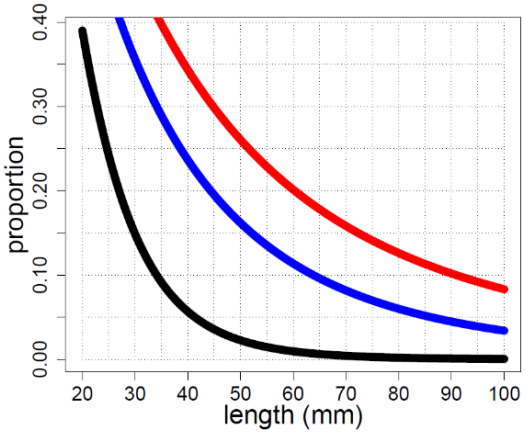
A two-season field experiment was conducted in lemon and tangelo orchards across selected Queensland sites to better understand the distribution of CGW and its parasitoids within orchards. The study examined the effect of gall size, gall density, and the spatial distribution of infested trees on parasitism rates. Satellite imagery was used to randomly select trees for sampling, and gall density was measured using a cubic frame inserted into the canopy. Galls were classified by size, and emergence data from CGW and parasitoids were recorded to analyse parasitism by the different species found attacking CGW, *M. brevivalvus, M. trisulcus*, *Amerostenus sp*. and *Eupelmus sp.*

A detailed description of the method is provided in Appendix 7.

Results and discussion

CGW monitoring

More large galls are present in a heavily infested orchard than in a lightly infested orchard. The proportion-gall size curves for different infestation categories are nicely separated (Figure 6). Based on the separation, CGW infestation level can be quickly determined by collecting 50 or more random, current-season galls and counting the number of 50 mm or longer galls. If the percentage of these galls is less than 3%, the infestation can be rated as low. If the percentage is over 25%, the infestation can be rated as high. The infestation level can be rated as moderate if the percentage is between 3% and 25%.



Citrus gall wasp infestation rating table

	Percentage of galls ≥50mm		
Gall size category	≤3%	≥25%	3-25%
Infestation rating	Low	High	Moderate

Figure 6. Left: Relationship between gall length and the cumulative proportion of galls above that length for low (black), moderate (blue), and high infestations (red); Right: the rating table based on the relationships.

FRW monitoring

Compared with data collected from branch shaking, data collected from Tedders traps are less erratic and provide a more consistent indication of the timing of local FRW population peaks (Figure 7). Tedders traps also appear to be more efficient than branch shaking in estimating local FRW density, approaching a target precision faster for sample size. Despite the differences, catches from the two monitoring methods followed similar patterns at most sites and in most years. Both produced seasonal patterns of FRW activity similar to those reported in a previous project, with relatively high FRW activity from February to July, relatively low activity from August to January, and the highest activity during March and April (Figure 8).

It has been reported that FRW adults are attracted to a synthetic blend of green leaf volatiles and clover leaves. We tested this in the field and found that baiting the Tedders traps with the two essential oils did not enhance FRW trapping.

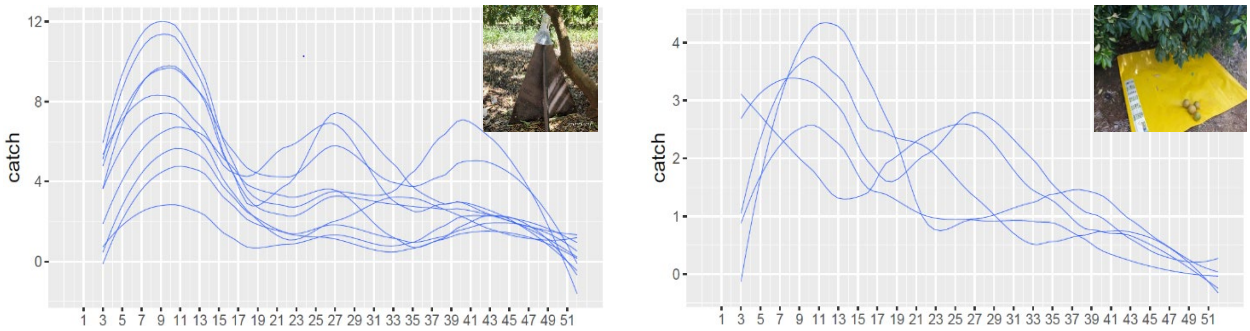


Figure 7. Number of Fullers rose weevil adults caught by Tedders traps (left) and branch shaking (right) at the monitoring site 'IM' in 2021.

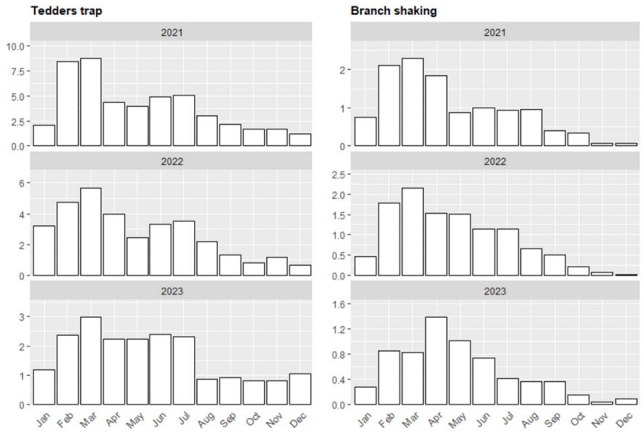


Figure 8. Average monthly catches of FRW at the five monitoring sites using Tedders trap (left) and branch shaking (right) in the three monitoring seasons.

In conclusion, we believe Tedders traps offer an attractive alternative to branch shaking for FRW monitoring. They can be used to identify local FRW hotspots of FRW infestation and indicate the timing of local FRW population peaks. Once they are set up, Tedders traps are easy to use and require minimal maintenance. With some modification, Tedders traps can potentially be used to gradually remove FRW adults from the local population.

Detailed results and discussion are provided in Appendix 1.

Monitoring with machine vision

For CGW, machine learning classification models achieved 75% validation accuracy in separating new galls, old galls, and healthy branches. Deep learning object detection was able to estimate total gall density with high correlation to manual inspection of images (Figure 9, left). The CGW status and age could be identified from point multi-spectral sensors with 75% precision and recall, and potential for detection demonstrated on camera at wavelengths 1050 and 1450 nm. In the future, maps of CGW pressure could be generated in orchards by mounting an automotive camera with GPS logging on the side of a small orchard vehicle and a processor to apply image analysis. This could be implemented in orchards with active multi-spectral sensors or short-wave infrared cameras in shaded lighting conditions using wavelengths identified as sensitive for galls.

Machine vision algorithms developed to count FRW from beat sheets had an accuracy of 78% on images captured in the field at Yanco, and 97% on images captured under controlled lighting conditions. Leaf edge analysis was implemented to automatically identify FRW chewing damage as an indication of FRW feeding (Figure 9, right). A segmentation-based approach to separating leaves was shown to be able to segment individual leaves and, with further analysis of leaf edges, has the potential to identify FRW feeding. In the future, an FRW beat sheet detector could be deployed with a robotic branch shaking system. FRW chewing damage could be automatically detected by adding leaf contour analysis to the developed leaf segmentation algorithm and run on an on-the-go camera and vehicle.

Figure 9. Detection of CGW galls with machine vision (left) and automated segmentation of citrus leaves to detect FRW damaged leaves (right).

Detailed results and discussion are provided in Appendix 2.

CGW timing guide

The updated CGW emergence timing guide can be found at https://citrusgallwasp.shinyapps.io/predict. To get the predicted date of peak CGW emergence, select from a drop-down list of towns nearest to your orchard and then click either the 'CURRENT STATUS' or 'PREDICTED DATES' tab. Towns currently included in the drop-down list are: Yanco, Griffith, Mildura, Loxton, Perth, Dubbo, and Gayndah. If you click the 'CURRENT STATUS' tab, the following chart will be shown (Figure 10).

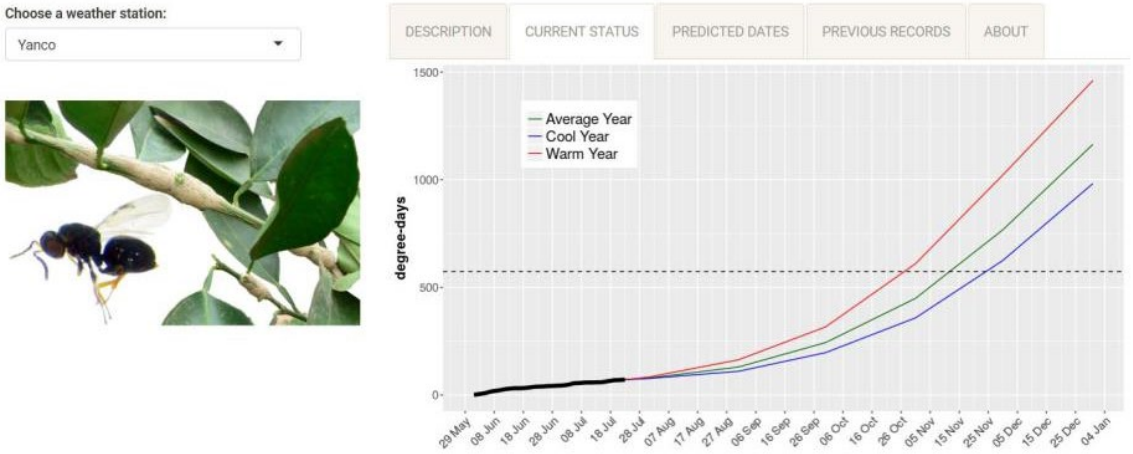


Figure 10. Homepage (https://citrusgallwasp.shinyapps.io/predict) of the online timing guide for CGW emergence

A dashed horizontal line indicates the required degree days (DD) for peak adult emergence. The thick black line shows the progression of observed degree days to date. Red, green, and blue lines show future DD projections in warm, average and cool years. If you click the 'predicted dates' tab, the peak emergence dates for an average, warm, and cool year will be shown.

Lucerne interplanting and CGW control

A total of 28,612 CGW adults and 3,754 parasitic wasps emerged from the collected galls. Random dissection of the galls revealed 11,755 un-emerged CGW adults and 466 un-emerged parasitic wasps, bringing the total number of CGW adults to 33,617 and that of the parasitic wasps to 4,220. The overall parasitism level was about 11%. The parasitic wasps were exclusively MBV, the primary parasitic wasp species of CGW in Australia (Figure 11). No known parasitic wasp species of lucerne seed wasp (LSW) were recovered (Figure 8). CGW infestation level was similar in citrus rows next to lucerne plantings and citrus rows not next to lucerne plantings ($P > 0.1$). We also collected sweep-netting data and lucerne seed pods. We did not find any known parasitic wasp species of either LSW or CGW.

Figure 11. Top - Citrus gall wasp (left) and its parasitic wasps (middle and right); Bottom – Lucerne seed wasp (left) and its parasitic wasps (middle and right).

In summary, we did not recover any parasitic wasps of LSW from CGW galls, nor did we observe reduced CGW infestation in citrus rows adjacent to lucerne plantings. While we do not rule out the possibility that parasitic wasps of LSW may parasitise CGW, the likelihood of such cross-species parasitism appears low. Most parasitic wasps are host-specific. In addition, parasitic wasps of LSW and CGW emerge at different times of the year, further reducing the chances of cross-species parasitism

Detailed results and discussion are provided in Appendix 8.

Entomopathogenic fungi and nematodes

Two commercial products of entomopathogenic nematodes, one each from the species of *Heterorhabditis bacteriophora* and *Steinernema feltiae*, were investigated for controlling FRW larvae in the field and FRW adults in the laboratory. Two field trials were conducted, and neither provided conclusive evidence confirming the efficacy of the two nematode products against the FRW larvae in the soil. Results from one of the trials suggest that the nematodes might slightly reduce the survival of FRW larvae in the soil, but the reduction might not be sufficient for satisfactory control of FRW infestation. The two nematode products showed some potential for controlling the adult FRW in the laboratory in a high-humidity environment.

Five experimental strains of the entomopathogenic fungi *Beauveria bassiana* and *Metarhrizium anisopliae*, and a commercial mix of *B. bassiana*, *M. anisopliae*, and *Lecanicillium lecanii* were investigated in the laboratory for controlling FRW adults. One *B. bassiana* strain showed evidence of disabling/killing the adult weevils. The commercial product showed no efficacy against the adult weevils.

In summary, we investigated the potential of entomopathogenic nematodes and fungi for the control of FRW and CGW. We did not find conclusive evidence of their efficacy against the two pests. FRW larvae live in the soil and soil moisture is critical for microorganisms such as nematodes and fungi. Although citrus orchards are irrigated, maintaining adequate soil moisture for the microorganisms is challenging in the semi-arid regions where most of Australia's oranges are grown. The prospect of entomopathogenic nematodes or fungi being used for FRW control in the main orange production regions of Australia appears to be low.

Detailed results and discussion are provided in Appendix 3.

New chemical options for CGW control

Of the 10 new chemical options screened, spinosad and spinetoram showed the highest foliar toxicity to adult CGW, both via direct contact and indirect contact. The two chemicals also demonstrated excellent residual activity, with sprayed leaves remaining toxic to adult CGW up to 21 days after the spray (Figure 12). The actual residual activity in the field is likely to be much shorter due to rain or UV exposure and can also vary depending on the type of sprayer used.

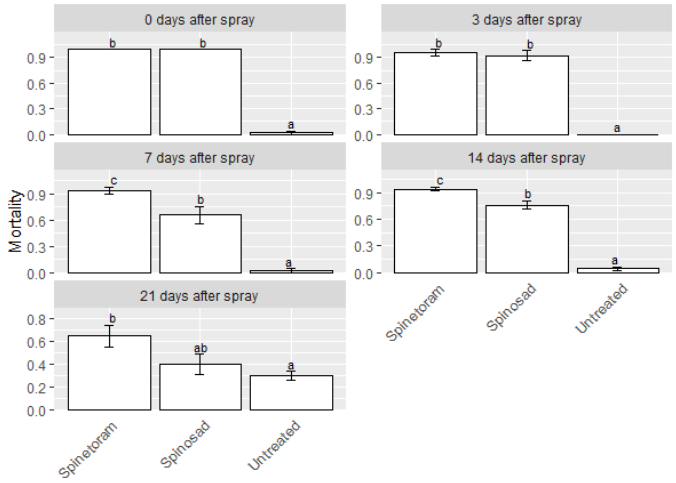


Figure 12. Mean mortality of CGW when exposed to leaves treated with spinosad at 40 mL/100L, spinetoram at 40 mL/100L, or water only (untreated) at 0, 3, 7, 14, or 21 days post spray. Wire bars show the standard errors. Bars labelled with different letters are significantly different at α = 0.05 by Tukey's multiple comparison test.

Spinetoram appeared to be more toxic to adult CGW than spinosad. The highest tested rate for both chemicals was 40 mL/100L, which is the recommended rate on the labels of registered insecticides containing these two chemicals. This rate was much lower than the required rate for spinosad to kill 90% or more of adult CGW at one day post-treatment but was appropriate for spinetoram to achieve this level of efficacy. The required rate for 90% mortality can be greatly reduced for both insecticides if the mortality data is expanded to include those that died after 2 or 3 days. Considering that adult CGW only lives for about 5 days and can mate and lay eggs on the same day, the decision to select an application rate should be based on 1-day mortality rather than 2 or 3-day mortality.

The field trial confirmed the efficacy of spinosad and spinetoram. A single application of either chemical just before the peak emergence of adult CGW reduced the total weight of the next season's galls by over 44%. Treated trees had a similar number of galls as the untreated trees, but the proportion of large galls (length ≥ 50 mm) was significantly lower. Of the 5 treatments, the double-spray of spinetoram achieved the best control of CGW, reducing the total gall weight by 66% and the proportion of large galls by 84%, compared with the untreated control.

Spinosad and spinetoram are all highly toxic to the red scale parasitoid *A. lingnanensis*, with a residual toxicity period of at least 7 days (Figure 13). Spinosad and spinetoram generally have low toxic effects on the ladybird, *C. montrouzieri* and the green lacewing *M. signatus*, but they are highly toxic to the predatory mite *N. californicus* at application rates that are potentially relevant to the citrus industry (Figure 10). However, it needs to be pointed out that chemicals found to be harmful in the laboratory might not always show toxic effects in the field.

Chemical	 Aphytis	 Ladybird	 Lacewing	 Predatory mite
Spinosad	Very high	Low	Low	High
Spinetoram	Very high	Low	Low	Very high

Figure 13. Toxicity ratings are based on the standard of the International Organisation for Biological Control (IOBC). Images sourced from Bugs for Bugs.

In conclusion, we believe that spinosad and spinetoram are effective new chemical options for controlling CGW. They complement currently registered chemical options for CGW control in that they can be used in all citrus varieties, including Valencia, because of their relatively short withholding period. More than one spray may be needed to achieve satisfactory control. Where a single spray is preferred, the spray should be put out just before the peak emergence of

the gall wasp to maximise its effect. Caution should be exercised when using the two insecticides in orchards with a history of red scale or mite problems, considering their negative impacts on *Aphytis* and predatory mites.

Detailed results and discussion are provided in Appendix 4.

Disclaimer: Spinosad and spinetoram are not registered for the control of the citrus gall wasp and therefore cannot be used on commercial properties for this purpose. The products were used at the DPIRD research station, which has permission to test off-label products for efficacy studies.

Optimal timing for applying a foliar insecticide against CGW

In this study, the optimal spray timing was consistently found to be before the peak emergence of CGW. Depending on the residual activity of the insecticide, the optimal spray date preceded the peak emergence of CGW by 1-24 days, with longer lead times for insecticides with longer residual activity. In contrast, the optimal spray date varied little across the different emergence scenarios, suggesting that the results are applicable over a wide range of locations and seasons. A single insecticide application at the optimal timing might not guarantee satisfactory CGW control. When the residual activity of the insecticide is short, multiple applications with a combined residual activity of 16 days are needed to ensure insecticide contact with ≥ 90% of all CGW that emerge in a season.

Detailed results and discussion are provided in Appendix 5.

New chemical options for FRW control

Of the nine new chemicals screened, EXP-A and indoxacarb were the only two showing potential for FRW control. EXP-A demonstrated high toxicity to the adult weevils, whereas Indoxacarb did not immediately kill the weevils but incapacitated them. Some of the incapacitated weevils later recovered, but their survival out in the field would be low as they would be more vulnerable to predators than healthy weevils. Despite the difference in their effects, both have shown the potential for reducing FRW egg contamination in citrus fruit, which is a key measure of a successful FRW control strategy. However, due to the lack of MRLs for indoxacarb in any of Australia's citrus export markets, only EXP-A was further investigated for rate response, field efficacy, and effect on beneficial arthropods in citrus.

Based on the rate-response data collected in the laboratory, EXP-A can achieve satisfactory control of FRW at the rate of 25 mL/100L or higher. This is half of the recommended rate for the chemical. Results from the field trial showed that EXP-A achieved a similar level of FRW control as the registered product cyantraniliprole (Exirel®). However, we were unable to confirm its field efficacy due to large random variations in the data. While investigating new chemicals for CGW control, we found that EXP-A might be highly toxic to predatory mites and ladybirds and might also be toxic to lacewings. However, laboratory bioassays of insecticide toxicity often reflect the worst-case laboratory conditions, and caution should be taken when extending findings of this study to field conditions.

In conclusion, EXP-A and indoxacarb are effective against FRW. If EXP-A is to be pursued for registration, we recommend it be further investigated for field efficacy and the effect on beneficial arthropods in citrus. Indoxacarb will also be a worthwhile candidate for consideration of registration for FRW control when its MRL data become available in Australia's export markets.

Detailed results and discussion are provided in Appendix 6.

Future CGW population trends

The model produces a variety of future CGW population trends in the absence of any human interventions (Figure 14). Without the parasitoid, the population is predicted to steadily increase until it reaches a maximal level determined by the capacity of citrus trees and stays at the maximal level afterwards. In the presence of the parasitoid, the CGW population is predicted to drop to a lower level before stabilising or undergoing cyclic fluctuations depending on several factors. A key factor in determining the outcome is the host-searching strategy of the parasitoid. If the parasitoid concentrates its search in places with high host density, the CGW population tend to stabilise after the initial increasing phase. On the other hand, if the search is more random, the CGW population can become unstable and undergo cyclic fluctuations. The initial population peak is expected to be reached in 10–15 years following the initial CGW incursion.

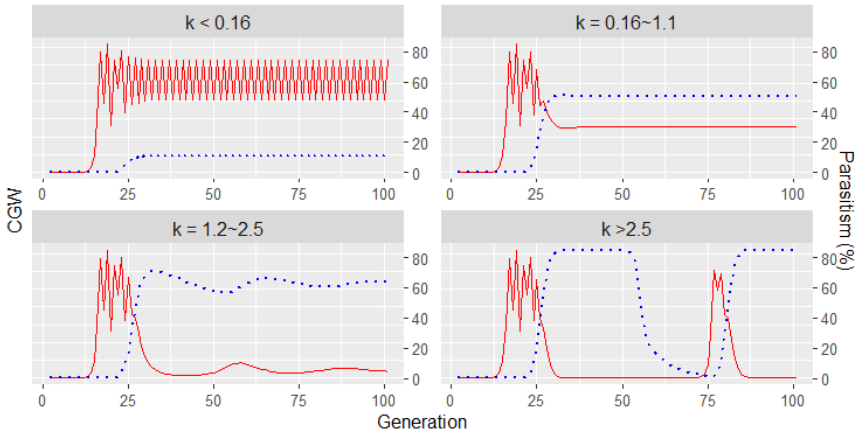


Figure 14. Predicted population patterns for the CGW (solid red line) and corresponding parasitism (dotted blue line) under different aggregation levels of the parasitoid, as indicated by the k value.

Detailed results and discussion are provided in Appendix 9.

PhD study - CGW biology and ecology

Screening for cryptic species in CGW – implications for biocontrol.

A total of 266 CGW individuals from Qld and NSW were genetically analysed. The mitochondrial DNA analysis revealed 20 haplotypes (Figure 15). The sample from Desert lime in Dululu (QLD) showed a 4% genetic divergence from the other samples, suggesting it may represent a distinct lineage. The remaining CGW samples showed a maximum divergence of only 1.2%.

Principal Component Analysis (PCA) and population structure studies found three clear genetic groups of CGW, which mostly matched their locations:

- Group 1: Northern Qld (Gayndah, Sunshine Coast, Bundaberg)
- Group 2: Southern NSW (Yanco, Leeton, Griffith)
- Group 3: A mixed group with samples from Bundaberg and Duingal (QLD) and Dareton (NSW)

These results indicate that CGW is likely one species with geographic population structure, rather than a complex of cryptic species. The variation in parasitism success across regions, alongside this genetic structure, suggests some level of population differentiation; however, it is insufficient to warrant taxonomic reclassification of CGW.

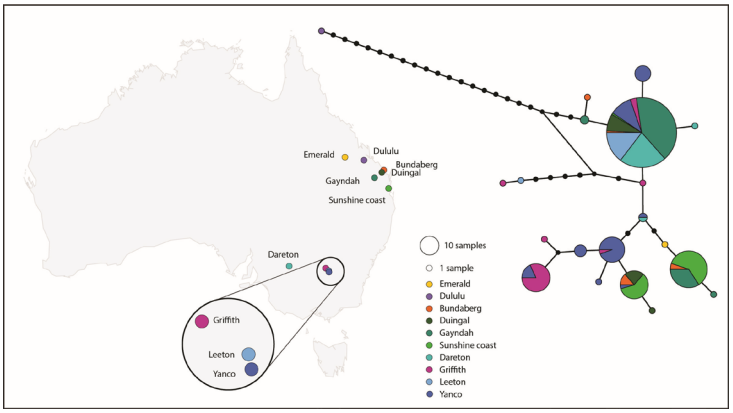


Figure 15. Map showing sampling locations (left) and haplotype network of the mitochondrial COI data (right). The black dots indicate mutations that were not present in the sample sequences.

One sample from Desert Lime in Dululu had a highly divergent COI gene sequence. This could mean it belongs to a separate population or species. In the population genetics analysis, this sample occurs outside the three clusters of CGW yet falls within the genetic variation that seems to encompass CGW. Establishing the species identity of the Dululu sample will require further sampling of wasps from Desert lime. This genetic insight supports the need for region-specific management strategies, particularly in optimizing the use of parasitoids, as variation in CGW population genetics and biology may be influencing biocontrol success across regions.

Effects of temperature and photoperiod on the growth and development of CGW

Two laboratory experiments were conducted to test the impact of different constant temperatures and photoperiods on CGW development. The constant temperature experiment revealed that gall growth rate was highest at 22°C but that CGW could not complete development at any of the tested temperatures (16°C, 19°C, 22°C, and 25°C) under a constant 14:10 light: dark photoperiod. The photoperiod experiment during 2023–24 tested both constant and variable photoperiods in conjunction with parasitoid exposure. However, by March 2025, no adult insects had emerged from any treatment, while control galls kept under glasshouse conditions began to produce adult CGW in October 2024. These results suggest that CGW development depends on environmental cues such as fluctuating light cycles and possibly exposure to low temperatures and that laboratory conditions must closely mimic natural environments to support normal CGW development.

CGW mortality and life table construction

Field collections and gall dissections were carried out at multiple sites in Queensland and NSW over two years and at further Queensland sites over an additional 2 years. In 2021, over 36,000 wasps emerged from collected galls, of which 94% were CGW and the remainder were the parasitoids *M. brevivalvus* and *M. trisulcus*. Overall, CGW mortality varied substantially between sites and between years. Parasitism rates were generally low in NSW, supporting the hypothesis that biocontrol success varies regionally and may be influenced by genetic differences in CGW populations. Dissections of single galls revealed further immature stages, including un-emerged CGW and parasitoids, allowing mortality estimates at various developmental stages. Natural larval mortality was higher than parasitism rates at all sites across both regions, except for Gayndah, where marginal death rates from parasitism exceeded the marginal natural death rates for larval mortality in both years.

The life table analysis showed considerable variation in CGW reproduction and survival across different locations and seasons. In Emerald, the CGW population remained relatively stable between years, while in Gayndah, a noticeable decline in net reproductive rate was observed. In NSW, sites like Leeton and Griffith experienced sharp declines in net reproductive rate in 2022 compared to the previous year. At most sites, natural larval mortality exceeded parasitoid-induced mortality, except in Gayndah, where parasitism was the primary cause of mortality over two seasons. Overall, Queensland sites showed more stable reproductive rates between years, while greater fluctuations were recorded in NSW. These findings suggest that regional environmental and biological factors, such as parasitoid activity, might significantly influence the dynamics of CGW populations.

Spatial distributions of CGW and its parasitoid

Gall sizes were categorized in 2023 as small, medium, or large, and galls were collected, incubated, and monitored for the emergence of both CGW and parasitoid wasps. Gall densities varied between sites and years, with gall distribution showing weak aggregation rather than randomness across orchards in 2022, however, in 2023, dispersion indices were closer to 1, suggesting a more random distribution of galls across sampled trees. These changes may reflect inter-annual variation in CGW population dynamics or environmental and management influences. There was a very strong linear relationship between gall volume and the number of CGW cells per gall, except at one site where many large galls contained no CGW cells.

Parasitism rates by the parasitoid *M. brevivalvus* varied widely, ranging from very low to moderate levels. Strong linear relationships were observed between the number of adult CGW emerging and the number of CGW cells per gall. Similarly, the number of adult *M. brevivalvus* emerging was strongly linked to CGW cell numbers at some sites but

not at others. This indicates that parasitism by *M. brevivalvus* might be highly site-specific, potentially influenced by microclimatic conditions, orchard management practices, or local parasitoid populations.

The analysis of 2023 samples are still underway. Gall density data has been assessed, and gall volume measurements and insect emergence data have been collected. Detailed emergence and parasitism rates have been determined and two additional species of parasitoid, *Amerostenus sp*. and *Eupelmus sp.* have been recorded attacking CGW. Final analyses of these data are underway. The categorisation of galls by size in 2023 (small, medium, large) will provide further resolution on how gall morphology influences parasitism success and CGW development.

This dataset, including 2023 parasitism and emergence data, provides a more robust understanding of inter-annual variability in CGW population structure and natural enemy effectiveness, and may inform targeted management strategies based on gall density, size distribution, and site characteristics.

Conclusions

The key finding from these studies is the clear regional variation among CGW populations across Queensland and NSW. However, there is no strong evidence for the presence of multiple CGW species within commercial citrus orchards. An exception is a highly divergent population associated with Desert lime in Queensland, indicating greater genetic diversity than previously recognized.

The commonly held hypothesis that CGW recently spread to southern citrus-growing regions along with imported citrus trees is not supported by the population structure revealed through genotyping-by-sequencing (GBS) analysis. Instead, the results suggest that distinct CGW populations were already present in NSW and Queensland prior to the widespread introduction of citrus, or that these populations have been geographically and genetically separated for some time.

This underlying genetic variation might partly explain the variation in parasitism rates of *Megastigmus* spp. parasitoids in southern citrus regions, despite repeated introductions. Host specificity or regional mismatches between parasitoid and CGW lineages might be limiting the biological control success in these regions, introductions, although climatic differences between the regions also likely affect parasitoid distributions and performance.

These findings highlight the need for a regionally tailored approach to CGW management, particularly in optimising biological control strategies. To build on these insights, we recommend expanded sampling across a wider range of native citrus hosts, especially Desert lime, and a formal taxonomic review that incorporates genetic, morphological, and ecological data.

For details, refer to Appendix 7. A scientific publication detailing the biology and ecology of CGW, from this Ph.D. research, is currently in preparation and will represent a key outcome of the project.

Outputs

Table 1. Output summary

Output	Description	Detail
A CGW monitoring guide	There was no standard method for CGW monitoring. We developed an easy-to-use guide to determine the CGW infestation levels. Target audience: citrus growers, and pest consultants.	CGW infestation level can be quickly determined by collecting 50 or more random, current-season galls and counting the number of galls ≥50 mm long. If the percentage of these galls is less than 3%, the infestation can be rated as low. The infestation can be rated as high if the percentage is over 25%. If the percentage is between 3% and 25%, the infestation level can be rated as moderate.
A trap-based monitoring tool for FRW	Branch shaking is the industry standard for FRW monitoring. We have demonstrated that Tedders traps are an attractive alternative to branch shaking for FRW monitoring. Target audience: citrus growers, and pest consultants.	Tedders traps are free-standing traps used for weevil monitoring overseas. Data collected by Tedders traps are less erratic and provide a more consistent indication of the timing of local FRW population peaks than data collected by branch shaking. See Appendix 1 for details.
An updated timing guide for CGW emergence	The timing guide allows users to easily predict the dates of peak emergence of CGW adults by simply selecting the closest weather station. Target audience: citrus growers, pest consultants, and R&D providers.	Compared with the previous version, the new timing guide has a wider application range and a more stable source of weather data. The timing guide can be accessed at: https://citrusgallwasp.shinyapps.io/predict.
Data on cross-parasitism of CGW galls by parasitoids of the Lucerne seed wasp	CGW galls were collected from the site where the benefit of lucerne interplanting was reported to check for evidence of cross-parasitism. Target audience: industry bodies and R&D providers.	A total of 3,754 parasitic wasps emerged from the collected galls. The parasitic wasps were exclusively *Megastigmus brevivalvus*, the primary parasitic wasp species of CGW in Australia. No known parasitic wasp species of lucerne seed wasp were recovered. See Appendix 8 for details.
A protocol system for automated detection of FRW activity	Machine vision algorithms have been detected to automatically count FRW on beat sheets and identify FRW-damaged leaves. Target audience: citrus industries, funding bodies, R&D providers.	The protocol system achieved an accuracy of 78% in counting FRW adults on drop sheets in field images and 97% on images captured under controlled lighting conditions. Leaf edge analysis was implemented to automatically identify FRW chewing damage as an indication of FRW feeding. See Appendix 2 for details.
Efficacy data of entomopathogenic nematodes and fungi for FRW	Efficacy data have been collected on the control of FRW by two commercial products of	Neither nematode product provided satisfactory control of FRW in the field, although they showed some potential for controlling the adult FRW in the laboratory in an artificially high-humidity environment. One experimental strain of

control	entomopathogenic nematodes and five experimental strains of entomopathogenic fungi. Target audience: citrus industries, R&D providers.	entomopathogenic fungi provided some control of FRW adults. See Appendix 3 for details.
A list of new chemical options for CGW and FRW control	Two new chemical options have been identified for CGW control and one for FRW control. Target audience: citrus industries, chemical companies	• Spinosad and spinetoram are promising new chemical options for CGW control and the experimental product EXP-A for FRW control. • Spinosad and spinetoram are relatively safe to the predatory ladybird *Cryptolaemus montrouzieri* and the green lacewing *Mallada signatus,* but are highly toxic to the red scale parasitoid *Aphytis lingnanensis* and the predatory mite *Neoseilus californicus.* EXP-A might be highly toxic to predatory mites, ladybird beetles and lacewings. See Appendix 4 and 6 for details.
Milestone reports	Annual reports were prepared to report on the project's progress.	MS102, MS103, MS104, MS105, MS106, MS107, MS108, MS109, and MS110 were submitted to Hort Innovation. These reports aligned with the project monitoring and evaluation plan.
A PhD thesis - CGW biology and ecology	To be submitted by 30th September 2025.	
Journal papers	Peer-reviewed articles	Mo J, Furlong MJ, Kirkland LS and Stevens MM (2023) Exploiting asynchronous host-parasitoid emergence distributions to optimise insecticide applications and protect beneficial insects– a case study using the citrus gall wasp *Bruchophagus fellis* (Hymenoptera: Eurytomidae). *Crop Protection*, 174: 106428. Kirkland LS, Thakur M and Mo J (2025) Effectiveness of spinosad and spinetoram against the citrus gall wasp, *Bruchophagus fellis* (Hymenoptera: Eurytomidae), and their impacts on beneficial arthropods in citrus. *Austral Entomology*, 64(2): e70005. https://doi.org/10.1111/aen.70005
Extension activities		
Industry articles	Articles accepted for publication in the *Australian Citrus News* magazine.	• Integrated pest management of citrus gall wasp and Fuller's rose weevil. *Australian Citrus News*, Summer 2021, pp 28–29. • Assessing potential enemies of the citrus gall wasp. *Australian Citrus News*, Autumn 2021, p 31–33. • Using smartphones to count Fuller's rose weevil in orchards. A*ustralian Citrus News*, 2022, 2: 28–29. • Potential chemical options for controlling CGW and FRW. *Australian Citrus News*, 2023, Issue 2: 29–30. • How to manage citrus gall wasp sustainably *Australian Citrus News*, 2025 (May edition, in publication process)
	Citrus Connect e-newsletter	• Citrus gall wasps: future trends and sustainable

	article	management
Factsheets	NSW DPIRD released two fact sheets with detailed coverage of CGW and FRW	CGW fact sheet https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0010/1457848/Citrus-gall-wasp.pdf FRW fact sheet https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0004/1472377/Fullers-rose-weevil.pdf
Videos	Video outputs with the latest information gained from the project	One YouTube video on CGW monitoring: https://youtu.be/H9D3sHy2Jnk?si=qxxZkH8WL8zgWsAX Two Webinar videos on citrus gall wasp management: https://www.youtube.com/watch?v=o6cNJpq5TS8andt=11s https://www.youtube.com/watch?v=KFAMHeXTXr8
Poster presentations	Posters accepted for presentations to industry and scientific community	• Poster presentation at Citrus Congress 2025 titled '**New chemical options for managing citrus gall wasps and their effect on beneficial arthropods**' (Appendix 4) • Poster presentation at the Citrus Congress 2024 titled '**Optimal spray timing for the citrus gall wasp using a foliar insecticide**' (Appendix 5) • Poster presentation at the 25th International Congress 2023 on Modelling and Simulation titled '**Modelling the future trend of the citrus gall wasp population in southern Australia** (Appendix 9)
Field days/workshops	Events specifically organized by NSW DPIRD or another industry group	• Seasonal walks on CGW in Griffith and Yanco were organized on 19 October 2022 in the IPDM project CT19011. Participants represented different sectors of the industry, including growers, horticulturists, and pest consultants. • Dareton Citrus Field Day, Dareton, held on 13/09/2023. 80 people, 60 of whom were growers, attended the field day. • Conducted a demonstration trial of registered chemicals for CGW control. Scott Munro presented results to growers on 13 October 2023 in Leeton. • Presented to Citrus Regional Forum, Griffith, 2024 • Presented latest research findings on gall wasp management at WA Field Day on 25/02/2025. Participants at the events represented different sectors of the industry, including growers (representing 80% of WA citrus production), horticulturists, pest consultants, and chemical company reps. • Citrus Congress 2025 field day. Participants at the events represented different sectors of the industry, including growers, horticulturists, pest consultants, students, and extension workers.

Outcomes

Table 2. Outcome summary

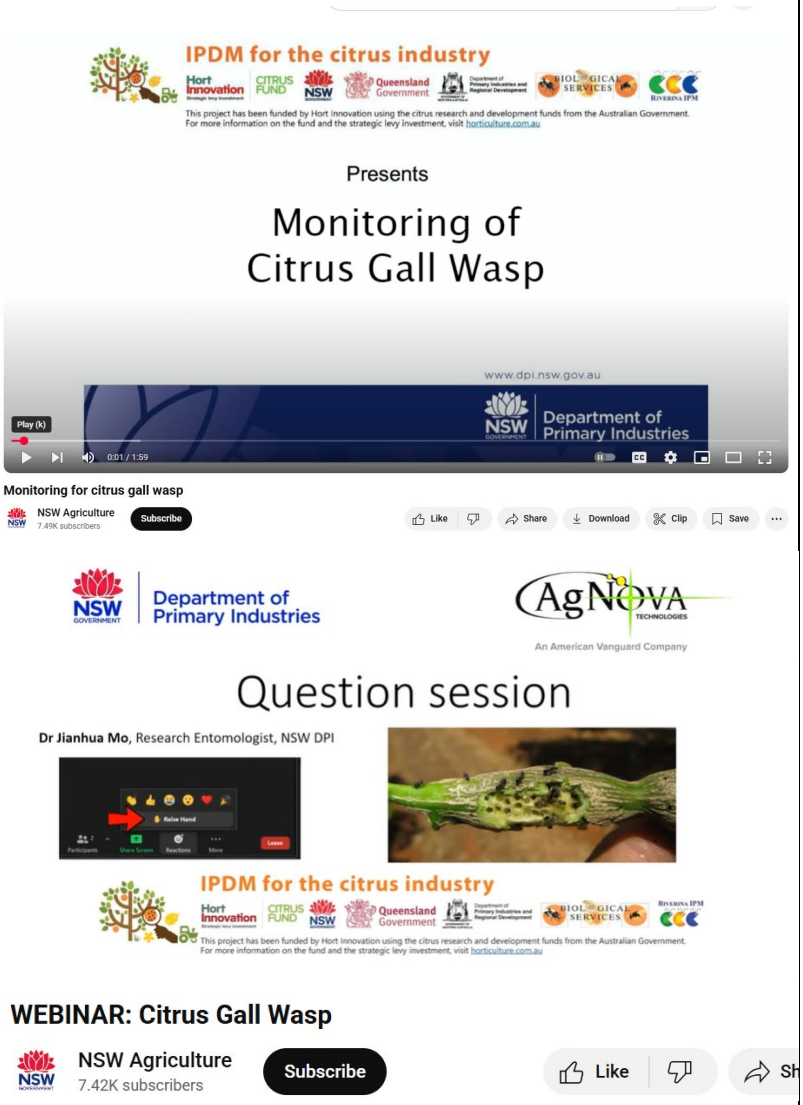
Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
The feasibility of monitoring FRW with traps and machine vision technology has been determined.	Citrus SIP 2022–2026 Outcome 1, strategy 9: Develop and optimise a whole-systems approach to integrated pest and disease management (IPDM)	Three-year monitoring data showed that Tedders traps tracked the local FRW population more effectively than branch-shaking. A 1-year scoping study demonstrated the potential of automated monitoring of FRW with machine vision technology.	Appendix 1. Appendix 2
The applicability of the CGW timing tool in regions has been determined	Citrus SIP 2022–2026 Outcome 1, strategy 9	We have updated the timing guide and expanded its application range from originally the Riverina, Murray Valley, and Riverland to also cover the Central Burnett (QLD) and suburbs of Perth (WA).	The online timing guide can be found at https://citrusgallwasp.shinyapps.io/predict.
New biological control options have been evaluated for CGW and FRW control	Citrus SIP 2022–2026 Outcome 1, strategy 9 Citrus SIP 2022–2026 Outcome 3, strategy 1: Deliver communication and extension programs to create positive change in the areas of biosecurity preparedness, varieties that meet consumer demand, sustainable production, pest and disease management, and export protocols and markets	For new biological options against CGW, we investigated the cross-parasitism of CGW by the parasitoids of the lucerne seed wasp. We did not find any evidence of cross-parasitism. For new biological control options against FRW, we investigated entomopathogenic nematodes (EPN) and fungi (EPF). We found neither EPN nor EPF promising for FRW control.	Appendix 8 Appendix 3
New chemical options have been evaluated for CGW and FRW control	Citrus SIP 2022–2026 Outcome 1, strategy 9 Citrus SIP 2022–2026 Outcome 3,	Spinosad and spinetoram demonstrated good control of CGW adults. An experimental product, Exp-A, provided significant control of FRW	Appendix 4 Appendix 6

	strategy 1	adults.	
Improved understanding of CGW biology and ecology	Citrus SIP 2022–2026 Outcome 1, strategy 9	The findings of PhD study showed that (1) there is higher natural mortality of immature CGW due to causes other than parasitism inside the galls; (2) temperature alone does not explain CGW development, which appears to be influenced by natural environmental cues such as fluctuating photoperiod; (3) CGW parasitism levels vary significantly by region, with particularly low rates in southern citrus-growing areas, (4) the spatial distribution of CGW galls revealed genetic variation among regional CGW populations, including a highly divergent lineage associated with Desert lime in Qld, suggesting greater genetic diversity than previously recognized; (5) The parasitism by *M.brevivalvus* is influenced by gall size, gall density, and orchard-level gall distribution, with parasitism effectiveness varying between locations and seasons.(6) In Queensland CGW can also be attacked by the parasitoids *Amerostenus* sp. and *Eupelmus* sp. but attack rates are typically low and vary considerably through space and time	Appendix 7
Australia has increased capacity in researching IPM solutions for citrus pests.	Citrus SIP 2022–2026 Outcome 1 Citrus SIP 2022–2026 Outcome 3	The PhD student will have the knowledge and skills, and the University of Queensland will have an increased capacity to research solutions for CGW and other citrus pests.	A scientific paper on the biology and ecology of CGW is underway and will be completed after the PhD thesis is submitted.

Monitoring and evaluation

Table 2. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project achieved its expected outcomes?	Yes, the project has identified new tools and information that will help with better CGW management. • The project identified two potential new chemical options for CGW control and one for FRW control (pending registrations or off label permits). • Expanded the application range of the online CGW emergence timing tool to include WA and QLD. • Identified Tedders trap as a viable alternative for FRW monitoring. • Developed a simple monitoring tool to rate CGW infestation levels. • Demonstrated feasibility of using machine vision for automated monitoring of FRW and CGW. • Disproved the hypothesis that lucerne inter-rows improve CGW biological control. • Determined that entomopathogenic nematodes/fungi are unlikely to be viable for FRW control in southern regions due to semi-arid conditions.	• With respect to new chemical options for CGW control, spinetoram appears to be more efficacious than spinosad. As such, future work should focus on spinetoram. Future field trials are needed to optimise spray timing and rates for spinetoram. • Although the Tedders trap can be made by DIY, commercialising it would support greater industry adoption. • Automated monitoring is the future of pest monitoring. This project has demonstrated the potential of technology for FRW and CGW monitoring. Further work is needed to develop prototypes and test them in the field.
How relevant was the project to the needs of intended beneficiaries ?	This project has addressed key pest challenges: FRW, a market-access pest, and CGW, a domestic biosecurity pest. • The project has improved the monitoring of FRW and identified a new chemical option for its control, and thus contributing to the improved market access of Australian export oranges (aligned with Citrus SIP 2017–2021 Outcome 1). • This project identified new chemical options to manage CGW enabling CGW control for juice citrus growers with minimal residue concerns, expanding chemical options (aligned with Citrus SIP 2017–2021 Outcome 2).	The project has generated efficacy and beneficial impact data that can support further data generation for label extension of products containing spinosad/spinetoram for CGW control. Regulatory approval may require additional trials or datasets.
How well have intended beneficiaries	We aimed to engage citrus growers and industry stakeholders throughout the project through the peak industry body, Citrus Australia, and through established NSW DPIRD channels.	We have successfully engaged regional growers and citrus industry stakeholders

been engaged in the project?	• We published five technical and extension-focused articles in Australian Citrus News, which reaches an estimated 5,000 readers quarterly across the national citrus industry. These articles provided updates on CGW and FRW research findings, monitoring techniques, and integrated pest management strategies, emphasising practical application for growers. We have also published about CGW management based on the current project findings through our CitrusConnect e-newsletter. • To support grower decision-making and enhance field diagnostics, we produced two fact sheets–one each for CGW and FRW designed for quick reference and distributed both online and in print at field days and extension events. • We developed three videos to improve the accessibility of technical content: one focused on CGW monitoring methods, and two webinar-style videos highlighting CGW management strategies. These resources were shared through Citrus Australia's online platforms and NSW DPIRD's media channels, broadening our outreach to growers unable to attend in-person events. 	through DPIRD, Citrus Australia, and various online platforms. However, a more targeted approach might enhance our reach to growers outside the tri-state region, particularly in Western Australia, where CGW is not yet a concern in commercial orchards but could become one. While CGW pressure is highest in the southern citrus regions, occasional state-specific webinars (e.g. CGW: management considerations for WA Growers) could help inform and prepare out-of-region growers and agronomists. These could highlight biosecurity risks, early detection, and management learnings applicable should incursions occur or expand.

- Project outcomes were showcased in poster format at both the 2024 and 2025 Citrus Congress events, providing high-visibility communication to a broad cross-section of the industry. In addition, we delivered oral presentations at key regional events, including the Dareton Citrus Field Day (2023) and the Western Australia Field Day (2025), where we engaged directly with growers and industry advisors on CGW and FRW management innovations. We also established a CGW demonstration site in Leeton, did a field tour at the demonstration site with growers, and presented the findings to the growers.

Scott Munro (in blue half jacket) with the growers at the CGW demonstration site in Leeton.

- The project team also participated in multiple field days and farm walks delivered under the IPDM project (CT19011), using these events to demonstrate monitoring tools, discuss trial results, and gather grower feedback. These engagements helped embed our findings within broader IPDM frameworks being adopted across citrus-growing regions.

To what extent were engagement processes appropriate	• Field days and demonstration trials are two of the preferred learning styles by growers. The project participated in 4 field days and set up a demonstration trial showing the control of CGW with registered products. • The project regularly updated the citrus industry though articles in the	

to the target audience/s of the project?	*Australian Citrus News*, which is an industry newsletter widely accessed by citrus growers.	
What efforts did the project make to improve efficiency?	• Applied existing knowledge, resources, and expertise to maximise project value. • The project regularly used online meetings to discuss and plan project activities.	We used a mix of communication tools including articles, fact sheets, videos, webinars, and field days to suit different audiences. We also timed our outreach to align with peak pest activity, ensuring information was timely and actionable. Collaboration across regions and projects allowed us to share resources and extend reach without extra cost. We also sought fresh ideas from growers and other networks to be more efficient and reachable.

Recommendations

- Spinetoram has been shown to be effective against adult CGW adults. Several insecticides with spinetoram as the main active ingredient are currently registered in citrus for controlling other citrus pests. It is recommended that chemical companies/citrus industries apply for label extension of one of these products for CGW control. The availability of spinetoram will provide Valencia citrus growers with a chemical option to control CGW without the concern of a long withholding period.
- Automation is the future of pest monitoring. This project demonstrated the potential of technology for FRW and CGW monitoring. Further work is needed to develop prototypes and test them in the field. Of interest are mobile phone-based apps that can automatically detect FRW damage and/or automatically separate current-season galls from previous-season galls; the latter can be used to inform growers/pest consultants if the local CGW population has increased or decreased over the previous season.
- Despite the recent surge of CGW populations in the southern citrus production regions, some coastal regions have reported little to no CGW issues despite favourable environmental conditions. It is worth doing surveys in those areas to find out why. Learning what is naturally keeping CGW numbers low could unlock new control ideas for the rest of the industry.
- During our work, we saw little to no CGW adults emerge from galls in potted trees, which were kept under constant temperatures, even though the temperatures were well within the reported range of CGW development. This anomaly suggests that temperature might not be the only factor affecting CGW development. Plant biochemicals such as the phytohormone gibberellin and others produced during flowering might also play a key role in triggering CGW emergence, especially since adult CGW typically emerge a few weeks after citrus bloom. It is recommended that controlled experiments be conducted to determine the level of different biochemicals in the citrus plants during flowering and investigate how they influence CGW development.
- Certain citrus varieties appear to be more heavily attacked by CGW than others. Several factors could be responsible for the varietal differences, such as the abundance and size of the oviposition sites (spring flush), texture of the oviposition sites, and plant volatiles. The difference might also be because immature stages of CGW survive better in some varieties than in others. However, plant biochemicals such as phenols, lipids, and other defence compounds

could also play a role. Finding the reasons could potentially lead to the development of CGW attractants/repellents and/or new chemicals (e.g. finding a hormone that can alter the production of these biochemicals) that can be topically applied to reduce CGW survival inside the galls. It is recommended that physical traits and biochemical profiles that affect CGW attack in different varieties be identified to understand how these traits influence egg-laying and larval survival to develop more targeted control or resistant varieties.

- Calcined kaolin is currently registered as a repellent of CGW but not many growers are using it due to its high costs. It is recommended that studies be conducted to see if kaolin can be used at lower rates alone or in combination with other chemicals and if the cost can be reduced by using kaolin in a push-pull strategy. Using a push-pull strategy can lead to reduced use of insecticides. It also complements kaolin sprays because adult CGW pushed away by kaolin are eventually killed by insecticides, not left alive to infest surrounding orchards. With the reduced use of kaolin, secondary pest issues (e.g. red scale) will also be managed well.
- mRNA-based pest control is a new and exciting technology that could be adapted for CGW. We recommend early-stage screening of mRNA tools against CGW to see if they can offer a targeted, environmentally friendly management option in the future.

Refereed scientific publications

Journal article

Mo J, Furlong MJ, Kirkland LS and Stevens MM (2023) Exploiting asynchronous host-parasitoid emergence distributions to optimise insecticide applications and protect beneficial insects – a case study using the citrus gall wasp *Bruchophagus fellis* (Hymenoptera: Eurytomidae). *Crop Protection*, 174: 106428.

Kirkland LS, Thakur M Mo J (2025) Effectiveness of spinosad and spinetoram against the citrus gall wasp, *Bruchophagus fellis* (Hymenoptera: Eurytomidae), and their impacts on beneficial arthropods in citrus. *Austral Entomology*, 64(2): 70005. https://doi.org/10.1111/aen.70005

Chapter in a book or paper in conference proceedings

Mo J (2023) Modelling the future trend of the citrus gall wasp population in southern Australia. In Vaze J, Chilcott C, Hutley L and Cuddy SM (eds) MODSIM2023, 25th International Congress on Modelling and Simulation. Modelling and Simulation Society of Australia and New Zealand, July 2023, pp. 597. ISBN: 978-0-9872143-0-0. https://doi.org/10.36334/modsim.2023.mo

References

Aghaee MA, Dorman SJ, Taylor SV, Reisig DD (2019) Evaluating optimal spray timing, planting date, and current thresholds for Lygus lineolaris (Hemiptera: Miridae) in Virginia and North Carolina Cotton. *Journal of Applied Entomol*ogy, 112:1207–1216. https://doi.org/10.1093/jee/toy407

Ahmad TR, Ali MA, Hamad BS (1995) Using degree-days model to determine the optimum spray timing for the codling moth Cydia pomonella (L.) (Lepidoptera: Olethreutidae). *Journal of Applied Entomol*ogy, 119, 143-144. https://doi.org/10.1111/j.1439-0418.1995.tb01260.x

Bargoti S, Underwood J (2017) Deep fruit detection in orchards. In: 2017 IEEE International Conference on Robotics and Automation (ICRA), Singapore, pp 3626–3633.

Brévault T, Oumaro Y, Achaleke J, Vaissayre M, Nibouche S (2009) Initial activity and persistence of insecticides for the control of bollworms (Lepidoptera: Noctuidae) in cotton crops. *Crop Protection* 28:401–406. https://doi.org/10.1016/j.cropro.2008.12.006

Candolfi MP, Bakker F, Canez V, Miles M, Neumann C, Pilling E, Primiani M, Romijn K, Schmuck R, Storck-Weyhermuller S, Ufer A, Waltersdorfer A (1999) Sensitivity of nontarget arthropods to plant protection products: could *Typhlodromus pyri* and *Aphidius* spp. be used as indicator species? *Chemosphere* 39:1357–1370.

Cannard M (2007) Native parasitic wasps released to control citrus gall wasp. *Citrep* 51:14–15.

Chaki J, Parekh R, Bhattacharya S (2018) Plant leaf classification using multiple descriptors: A hierarchical approach. *Journal of King Saud University – Computer and Information Sciences* (in press). https://doi.org/10.1016/j.jksuci.2018.09.014

Chauhan R, Monga S, Kumari B (2012) Dissipation and decontamination of bifenthrin residues in tomato (*Lycopersicon esculentum* Mill.). *Bulletin of Environmental Contamination and Toxicology* 89:181–186. https://doi.org/10.1007/s00128-012-0629-4

Citrus Australia (2023) About Australian Citrus. https://citrusaustralia.com.au/growers-industry

Colmenarez YC, Corniani N, Jahnke SM, Sampaio MV, Vásquez C (2018) Use of parasitoids as a biocontrol agent in the neotropical region: Challenges and potential. In: Baimey HK, Hamamouch N, Kolombia YA (eds) *Horticultural Crops*, IntechOpen, London, pp 171–185.

Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology* 52:81–106.

Ding W, Taylor G (2016) Automatic moth detection from trap images for pest management. *Computers and Electronics in Agriculture* 123:17–28.

Edwards M (1996) Biological control of Fuller's rose weevil in citrus with entomophagous nematodes and fungi. *Final Report*, Horticulture Innovation Ltd.

Eller FJ, Bartelt RJ (1996) Grandisoic acid, a male-produced aggregation pheromone for the plum curculio, *Conotrachelus nenuphar*. *Journal of Natural Products* 59:451–453.

Ehler LE (2006) Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. *Pest Management Science* 62:787–789. https://doi.org/10.1002/ps.1247

Duncan LW, McCoy CW, Stansly PA, Graham JH, Mizell RF (2001) Estimating the relative abundance of adult citrus root weevils (Coleoptera: Curculionidae) with modified Tedders traps. *Environmental Entomology* 30:939–946.

Fantke P, Gillespie BW, Juraske R, Jolliet O (2014) Estimating half-lives for pesticide dissipation from plants. *Environmental Science and Technology* 48:8588–8602. https://doi.org/10.1021/es500434p

Faria MR, Wraight SP (2007) Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control* 43:237–256.

Fettig CJ, Dalusky MJ, Berisford CW (2000) Nantucket pine tip moth phenology and timing of insecticide spray applications in seven southeastern states. *Research Paper SRS-18*, USDA Forest Service, Southern Research Station. https://doi.org/10.2737/srs-rp-18

Flett H (2011) Establishment of citrus gall wasp parasites in the Murray Valley region. *Final Report*, Project CT08000, Horticulture Australia Ltd, Sydney.

Ghidiu GM, Zehnder GW (1993) Timing of the initial spray application of *Bacillus thuringiensis* for control of the Colorado potato beetle (Coleoptera: Chrysomelidae) in potatoes. *Biological Control* 3:348–352. https://doi.org/10.1006/bcon.1993.1046

Grewal P, Georgis R (1999) Entomopathogenic nematodes. In: Hall FR, Menn JJ (eds) *Biopesticides: Use and Delivery*, Methods in Biotechnology, vol 5. Humana Press.

Hanula J, DeBarr G, Weatherby J, Barber L, Berisford C (2002) Degree-day model for timing insecticide applications to control *Dioryctria amatella* (Lepidoptera: Pyralidae) in loblolly pine seed orchards. *The Canadian Entomologist* 134:255–268. https://doi.org/10.4039/Ent134255-2

Hassan SA (1985) Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. *IOBC/WPRS Bulletin* 15:214–255.

Javaid I (1990) Application of insecticides on cotton in Zambia: timing of spray applications. *International Journal of Pest Management* 36:1–9. https://doi.org/10.1080/09670879009371425

Koirala A, Walsh KB, Wang Z, Anderson N (2020) Deep learning for mango (*Mangifera indica*) panicle stage classification. *Agronomy* 10:143. https://doi.org/10.3390/agronomy10010143

Kudon LH, Berisford CW, Dalusky MJ (1988) Refinement of a spray timing technique for the Nantucket pine tip moth (Lepidoptera: Tortricidae). *Journal of Entomological Science* 23: 180–186.

Li W, Wang D, Li M, Gao Y, Wu J, Yang X (2021) Field detection of tiny pests from sticky trap images using deep learning in agricultural greenhouses. *Computers and Electronics in Agriculture* 182:105994. https://doi.org/10.1016/j.compag.2021.105994

Li Z, Alves SB, Roberts DW, Fan M, Delalibera I, Tang J, Lopes RB, Faria M, Rangel DEN (2010) Biological control of insects in Brazil and China: history, current programs and reasons for their successes using entomopathogenic fungi. *Biocontrol Science and Technology* 20:117–136.

Loreau M, de Mazancourt C (2008) Species synchrony and its drivers: Neutral and nonneutral community dynamics in fluctuating environments. *The American Naturalist* 172:E48–E66.

Maiteki GA, Lamb RJ (1985) Spray timing and economic threshold for the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae), on field peas in Manitoba. *Journal of Economic Entomology* 78:1449–1454. https://doi.org/10.1093/jee/78.6.1449

McCoy CW, Boucias DG (1989) Selection of *Beauveria bassiana* pathotypes as potential microbial control agents of soil-inhabiting citrus weevils. *Memórias do Instituto Oswaldo Cruz* 84(Suppl III):75–80.

Mo J (2012) Managing citrus gall wasp in southern citrus regions. *Final Report*, HAL CT10021, Horticulture Australia Ltd, Sydney.

Mo J (2018) Development of national strategies to manage citrus gall wasp. *Final Report*, HAL CT15006, Horticulture Australia Ltd, Sydney.

Mo J, Stevens MM (2013) Vertical and horizontal movements of Fuller's rose weevil (Coleoptera: Curculionidae) in Australian citrus groves. *Entomological Science* 16:26–33.

Mo J, Stevens MM (2014) Emergence patterns of adult citrus gall wasp, *Bruchophagus fellis* (Hymenoptera: Eurytomidae), and its key parasitoids in southern Australia. *Journal of Asia-Pacific Entomology* 17:311–317. https://doi.org/10.1016/j.aspen.2014.02.006

Mo J, Stevens MM (2021) A degree-day model for predicting adult emergence of the citrus gall wasp, *Bruchophagus fellis* (Hymenoptera: Eurytomidae), in southern Australia. *Crop Protection* 143:105553. https://doi.org/10.1016/j.cropro.2021.105553

Muppala C, Guruviah V (2020) Machine vision detection of pests, diseases, and weeds: a review. *Journal of Phytology* 12:9–19.

Myers SW, Hogg DB, Wedberg JL (2005) Determining the optimal timing of foliar insecticide applications for control of soybean aphid (Hemiptera: Aphididae) on soybean. *Journal of Economic Entomology* 98:2006–2012. https://doi.org/10.1603/0022-0493-98.6.2006

Nauen R, Jeschke P, Velten R, Beck ME, Ebbinghaus-Kintscher U, Thielert W, Wölfel K, Haas M, Kunz K, Raupach G (2015) Flupyradifurone: a brief profile of a new butenolide insecticide. *Pest Management Science* 71:850–862. https://doi.org/10.1002/ps.3932

Ngugi LC, Abelwahab M, Abo-Zahhad M (2020) Recent advances in image processing techniques for automated leaf pest and disease recognition – a review. *Information Processing in Agriculture* (in press)

Noble NS (1936) The citrus gall wasp (*Eurytoma fellis* Girault). *Science Bulletin No. 53*, Department of Agriculture, NSW

Noble NS (1938) *Epimegastigmus (Megastigmus) brevivalvus* Girault. *Science Bulletin No. 65*, Department of Agriculture, NSW

Partel V, Nunes L, Stansly P, Ampatzidis Y (2019) Automated vision-based system for monitoring Asian citrus psyllid in orchards utilizing artificial intelligence. *Computers and Electronics in Agriculture* 162:328–336

PIRSA (2016) Fuller's Rose Weevil fact sheet. Viewed 15 July 2020. https://www.pir.sa.gov.au/__data/assets/pdf_file/0009/285534/Fullers_Rose_Weevil_in_Citrus_Fact_Sheet.pdf

Purves DW, Law R (2002) Fine-scale spatial structure in a grassland community: quantifying the plant's eye view. *Journal of Ecology* 90:121–129

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

Reddy GVP, Guerrero A (2001) Optimum timing of insecticide applications against diamondback moth *Plutella xylostella* in cole crops using threshold catches in sex pheromone traps. *Pest Management Science* 57:90–94. https://doi.org/10.1002/1526-4998(200101)57:1<90::AID-PS258>3.0.CO;2-N

Reissig WH, Weires RW, Onstad DW, Stanley BH, Stanley DM (1985) Timing and effectiveness of insecticide treatments against the San Jose scale (Homoptera: Diaspididae). *Journal of Economic Entomology* 78:238–248. https://doi.org/10.1093/jee/78.1.238

Rieske LK, Raffa KF (1993) Potential use of baited pitfall traps in monitoring pine root weevil, *Hylobius pales*, *Pachylobius picivorus*, and *Hylobius radicis* (Coleoptera: Curculionidae) populations and infestation levels. *Journal of Economic Entomology* 86:475–485

Reissig WH, Nyrop JP, Straub R (1998) Oviposition model for timing insecticide sprays against plum curculio (Coleoptera: Curculionidae) in New York State. *Environmental Entomology* 27:1053–1061. https://doi.org/10.1093/ee/27.5.1053

Rice RE, Weakley CV, Jones RA (1984) Using degree-days to determine optimum spray timing for the oriental fruit moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology* 77:698–700. https://doi.org/10.1093/jee/77.3.698

Ruppel RF (1984) Model for effective timing of an insecticide. *Journal of Economic Entomology* 77:1083–1085. https://doi.org/10.1093/jee/77.5.1083

Schumuff NR, Phillips JK, Burkholder WE, Fales HM, Chen CW, Roller PP, Ma M (1984) The chemical identification of the rice weevil and maize weevil aggregation pheromone. *Tetrahedron Letters* 25:1533–1534

Sharma A, Srivastava A, Ram B, Srivastava PC (2007) Dissipation behaviour of spinosad insecticide in soil, cabbage and cauliflower under subtropical conditions. *Pest Management Science* 63:1141–1145. https://doi.org/10.1002/ps.1437

Sparks TC, Crouse GD, Benko Z, Demeter D, Giampietro NC, Lambert W, Brown AV (2021) The spinosyns, spinosad, spinetoram, and synthetic spinosyn mimics – discovery, exploration, and evolution of a natural product chemistry and the impact of computational tools. *Pest Management Science* 77:3637–3649. https://doi.org/10.1002/ps.6073

Spearman C (1904) The proof and measurement of association between two things. *American Journal of Psychology* 15:72–101. https://doi.org/10.2307/1412159

Stansly PA (1997) Monitoring *Diaprepes abbreviatus* with Tedders traps in southwest Florida citrus. *Proceedings of the Florida State Horticultural Society* 110:30–35

Steel RGD, Torrie JH (1997) *Principles and Procedures of Statistics: A Biometrical Approach.* McGraw-Hill

Sterk G, Hassan SA, Baillod M, Bakker F, Bigler F, Blumel S, Bogenschutz H, Boller E, Bromand B, Brun J, et al. (1999) Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. *BioControl* 44:99–117

Sun Y, Liu X, Yuan M, Ren L, Wang J, Chen Z (2018) Automatic in-trap pest detection using deep learning for pheromone-based *Dendroctonus valens* monitoring. *Biosystems Engineering* 176:140–150

Tedders WL, Wood BW (1994) A new technique for monitoring pecan weevil emergence (Coleoptera: Curculionidae). *Journal of Entomological Science* 29:18–30

Tsukamoto M, et al. (2021) Chapter 10 – Cyclaniliprole: a novel diamide insecticide. In: Maienfisch P, Mangelinckx S (eds) *Recent Highlights in the Discovery and Optimization of Crop Protection Products.* Academic Press, pp. 213–219

Turney S (2024) Pearson Correlation Coefficient (r)/ Guide and Examples. Scribbr. Viewed 20 March 2024. https://www.scribbr.com/statistics/pearson-correlation-coefficient/

Wee SL, El-Sayed AM, Gibb AR, Mitchell V, Suckling DM (2008) Behavioural and electrophysiological responses of *Pantomorus cervinus* (Boheman) (Coleoptera: Curculionidae) to host plant volatiles. *Australian Journal of Entomology* 47:24–31

Western Australian Department of Agriculture (2020) Citrus gall wasp control fact sheet. Viewed 15 July 2020. https://www.agric.wa.gov.au/plant-biosecurity/citrus-gall-wasp-control

Veyron D, Fenigstein A, Melamed-Madjar V, Klein M (1988) Effects of concentration and application method on decay and residual activity of foliar chlorpyrifos. *Journal of Economic Entomology* 81:621–627. https://doi.org/10.1093/jee/81.2.621

Venables WN, Ripley BD (2002) *Modern Applied Statistics with S.* 4th edn. Springer, New York. ISBN 0-387-95457-0

Visnupriya M, Muthukrishnan N (2019) Persistent toxicity of newer molecule spinetoram 12 SC W/V (11.7 W/W) against *Helicoverpa armigera* and *Spodoptera litura* on tomato. *Journal of Entomology and Zoology Studies* 7:1025–1028

Zalucki MP, Adamson D, Furlong MJ (2009) The future of IPM: whither or wither? *Australian Journal of Entomology* 48:85–96. https://doi.org/10.1111/j.1440-6055.2009.00690.x

Zhong Y, Gao J, Lei Q, Zhou Y (2018) A vision-based counting and recognition system for flying insects in intelligent agriculture. *Sensors* 18:1489. https://doi.org/10.3390/s18051489

Intellectual property

Project IP: Machine vision algorithms for FRW and CGW detection

This project developed four machine vision algorithms for automated sensing of Fuller's rose weevil and citrus gall wasp in citrus trees. Commercialisation would require refinements in field testing, selection of a commercial partner and agreements. The IP will be managed by keeping it under embargo until reviewed by the IP owners (Hort Innovation) in consultation with project leader NSW DPIRD and IP developer University of Southern Queensland (UniSQ).

Acknowledgements

This project has been funded by Hort Innovation, using the citrus industry research and development levy, contributions from the Australian Government, and co-investment from NSW DPIRD, UQ, and USQ. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

The project is a collaboration between NSW DPIRD Jianhua Mo, Meena Thakur, Andrew Creek, Steven Falivene, and Scott Munro; University of Queensland (UQ) Michael Furlong and Pieter Scott; University of Southern Queensland (UniSQ) Alison McCarthy and Derek Long; Cesar Australia Lisa Kirkland, Kathy Overton, Olivia Reynolds; and Riverina IPM Rob Weppler.

Corteva Agriscience provided chemical samples of spinosad and spinetoram. Keith Danckwerts of Biological Ag and Bree Wilson of UniSQ provided samples of entomopathogens.

Michael Bennett from Narromine, NSW, provided the site for the investigation of lucerne interplanting. Tony Naimo, Marchello Mallamace, Bob Morris, and Giuseppe Iannelli from Leeton, NSW, provided the monitoring sites for FRW monitoring. Tony Naimo and Marchello Mallamace provided the trial sites for the investigation of nematodes. Marchello Mallamace provided the trial site for the investigation of an experimental chemical product for the control of Fuller's rose weevil. Mal Wallis from Munduberra, Queensland, and Helen Newman from Perth, Western Australia, collected data on gall wasp emergence. Mal Wallis also helped with data collection in the PhD study. Jim Hillyer and Ray Durkin from Leeton, NSW, provided sites for demonstration trials.

Appendices

Appendix 1: FRW monitoring using the Tedders trap

Appendix 2 (**CONFIDENTIAL)**: Automated monitoring of citrus gall wasp and Fuller's rose weevil with machine vision

Appendix 3: Potentials of entomopathogenic fungi and nematodes for controlling the Fuller's rose weevil

Appendix 4: New chemical options for the control of the citrus gall wasp

Appendix 5: Optimal timing for the application of a non-systemic insecticide to control the citrus gall wasp

Appendix 6: New chemical options for managing the Fuller's rose weevil

Appendix 7: PhD thesis

Appendix 8: Do parasitic wasps of the lucerne seed wasp parasitise the citrus gall wasp?

Appendix 9: Modelling the future trend of the citrus gall wasp population in southern Australia

Appendix 1: FRW monitoring using the Tedders trap

Dr Jianhua Mo and Mr Scott Munro
NSW Department of Primary Industries and Regional Development

Summary

A Tedders trap offers an attractive alternative to branch shaking for FRW monitoring. We compared monitoring data of FRW collected using the two methods in the Riverina during 2021–2024. We found that Tedders traps provided a more consistent indication of when the local FRW population had peaked than branch shaking. A Tedders trap is also more convenient to use than branch shaking. Baiting the trap with reported attractants of FRW did not enhance weevil trapping. When the FRW population is moderate or high, catches from the Tedders trap can be used to indicate local hotspots of FRW egg contamination. With some modification, a Tedders trap can also be used as a management tool to remove a significant proportion of adult weevils from the local population.

Introduction

Branch shaking is the standard method for FRW (*Asynonychus cervinus*) monitoring. This method is easy to implement, but the results are highly variable and difficult to standardise, as the number of weevils detected is affected by branch selection, strength, and frequency of beating. Improved monitoring techniques are needed to time FRW controls. Several types of traps have been used for weevil monitoring, including pitfall traps (Rieske et al. 1993), trunk traps (Mo and Stevens, 2013), and Tedders traps (Tedders et al. 1994). Pitfall traps collect water and can be flooded during heavy rain and overhead irrigation. Branch traps need to be set up in the tree trunk/branch so that all passing weevils can be funnelled into the collection vial, which can be a challenge when the trunk/branch is irregularly shaped. By contrast, Tedders traps are free-standing and thus easy to set up. The traps exploit the negative geotaxis behaviour of FRW adults and can be used in any weather. Tedders traps have been used to monitor the pecan weevil (*Curculio caryae*) and diaprepes root weevil (*Diaprepes abbreviatus*; Stansly et al. 1997; Ducan et al. 2001).

Trap efficiency might be improved by adding pheromones or attractants to the traps. FRW populations in Australia are all females, and hence, there are no sex pheromones. Aggregation pheromones have been found in several weevil species (e.g. Schmuff et al. 1984; Eller et al. 1996) but not yet for FRW. However, laboratory bioassays suggest FRW adults are attracted to a synthetic blend consisting of green leaf volatiles over a range of concentrations and to clover leaves (Wee et al. 2008). Plant-based attractants might enhance FRW trapping.

In this project, we evaluated the efficiency of Tedder traps for FRW monitoring and investigated whether they can be improved with plant-based attractants.

Materials and Methods

Monitoring sites

Five sites in Leeton in southwest NSW known to have FRW populations were selected for the monitoring (Table A1-1). Tree varieties were 'Valencia', 'Grapefruit', 'Navel', and 'Late Lane'. Tree age ranged from 13 years to over 93 years. All five sites were drip irrigated.

Table A1-1. Description of monitoring sites.

Site	Variety	Rootstock	Spacing (m)	Age
IM	Valencia	Trifoliata	6 × 2.5	32
MaW	Grapefruit	Trifoliata	6 × 4.0	13
MoW	Valencia	Rough Lemon	6 × 7.0	>93
TY	Late Lane	Citrange	5 × 3.0	36
TM	Valencia/Navel	Trifoliata	5 × 3.0	40

Trap design and deployment

The trap consisted of two interlocked, trapezium-shaped plywood sheets (600 × 520 × 50 mm) and a plastic collecting device sitting at the top (Figure A1-1). Ten Tedders traps were used at each site. The traps were placed along the central tree line to avoid damage from machinery. Neighbouring traps were placed at least 2 trees apart. The number of Tedders traps per site was reduced to 7 on 16 June 2022. The removed traps were used for field trials of entomopathogenic nematodes and insecticides. They were put back later.

Figure A1-1. A Tedders trap.

Data collection

Monitoring data was collected twice weekly to fortnightly from January 2021 to March 2024. On each monitoring occasion, the number of adult FRW caught in each Tedders trap was emptied and recorded. For comparison, branch shaking data and fruit contamination data were also collected. Branch shaking data were collected from 5 random branches at each site on each monitoring occasion. Fruit contamination data were collected monthly by examining 100–500 random fruit from each site for the presence of FRW eggs.

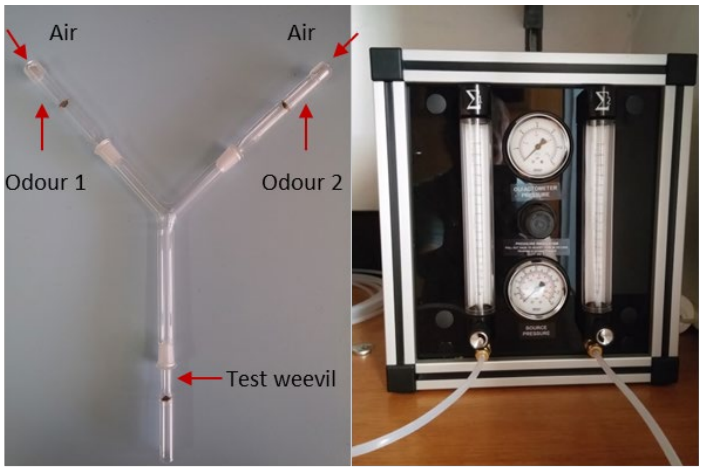
Attractants

Plant volatiles (volatiles) and colours were assessed for their effects on the efficiency of Tedders traps.

The orientation of adult FRW was tested on volatiles from leaves of orange, rose, and clover, their essential oils, and adult FRW. The essential oils tested were sweet orange and rose otto (Springfields, Kulnura, NSW) and clover (Nature's Note Organics).

Behavioural responses of adult FRW to plant volatiles and colours were tested in binary and 4-choice bioassays and colour response in 4-choice bioassays. Adult FRW used in the bioassays were collected from citrus orchards in Leeton, NSW, and kept in a mesh cage (24.5 × 24.5 × 63.0 cm), where they were fed with freshly collected orange leaves. Test weevils were taken out of the rearing cage and starved for 24 hours before being tested.

Binary-choice bioassays were conducted in a Y-tube olfactometer (Figure A1-2). Purified air flows along the two arms of the Y-tubes at a constant rate. Volatiles from two different sources pass through separate arms of the Y-tube and go out through the stem end. Test adult FRW were introduced individually at the stem end of the Y-tube. The number of test weevils choosing each arm was recorded.



Y-tube Air purification and delivery unit

Figure A1-2. Y-tube olfactometer.

Ten binary-choice bioassays were conducted: two between volatiles from the essential oils and controls, four between volatiles from young leaves and controls, three between the volatiles from young leaves from two different species, and one between the volatiles from live weevils and controls. For bioassays on volatiles from the essential oils, paper discs of 5 mm diameter were used as the dispenser. The essential oils were either tested undiluted with water as the control or diluted with hexene as the control. The paper discs were left air-dried and then placed inside 2-cm plastic straws before being loaded into the volatile chambers of the olfactometer. For bioassays on the volatiles from young leaves, the leaves were cut into 4 mm discs. Paper discs of the same sizes were used as the controls. For bioassays on volatiles from live FRW adults, one adult weevil was placed directly into one of the two volatile chambers of the olfactometer, and the other volatile chamber was left empty as the control.

Multiple-choice bioassays were conducted in a 4-choice olfactometer consisting of a round, plastic tray (270 × 85 mm), four cardboard or PVC tubes (38 × 90 mm), each with 8 rows of small holes (≤1 mm in diameter), a mesh lid on top of each tube, and four 75-mL vials (43 × 53 mm; Figure A1-3). The four perforated tubes were placed on top of the four vials positioned 90° apart along the perimeter of the tray. A 30-mm diameter hole was cut on the bottom of the tray where the four pairs of tubes and vials sit.

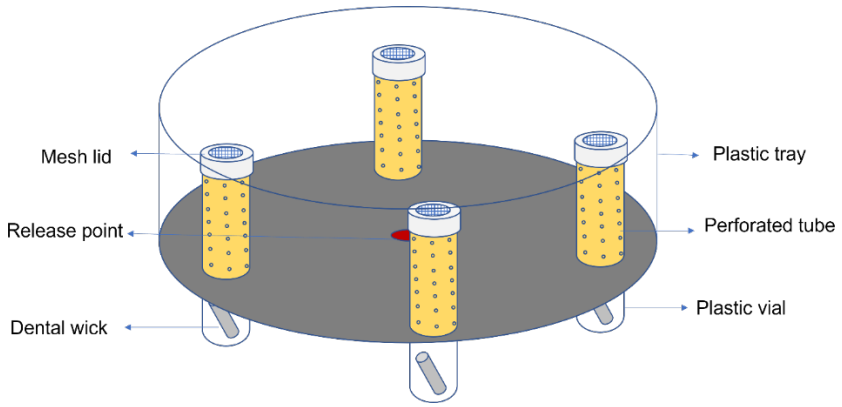


Figure A1-3. 4-choice olfactometer. In bioassays with plant leaves, the dental wick was replaced with leaf/paper discs.

Volatile responses to essential oils were tested in the 4-choice olfactometer with cardboard tubes. The oil was applied to a dental wick (10 × 36 mm), which was then placed inside a vial as the volatile source. A dental wick applied with water was placed in the control vial. Four test weevils were released in the centre of the tray for bioassays 1–6 and 10 for bioassays 7–8. The number of test weevils on each core was recorded every 30 minutes for a minimum of six hours. Eight bioassays were conducted in red light.

Volatile responses to plant leaves were tested in the 4-choice olfactometer with PVC tubes. Two grams of young leaves from each plant were chopped into small pieces before being placed inside the treatment vials. The control vials were left empty. Four test weevils were marked on the elytra with red, yellow, and white paint or nothing before being released in the tray centre. Movement of all test weevils was observed for an hour to record the start and end of each test weevil on each tube. Nine bioassays were conducted in red light.

Volatile responses to live FRW were investigated similarly to the essential oils, with the exception that PVC tubes were used instead of cardboard tubes. Ten live weevils were placed inside the source vial in one of the four choices, with orange leaves, rose leaves and blank control serving as the other three choices. Two bioassays were conducted.

Trap enhancement by attractants

Finally, clover oil and green leaf volatile ((Z)-3-hexenyl acetate, 70%, and (Z)-3-hexenol, 30%), which were reported as attractive to FRW by Wee *et al*. (2008), were investigated to see if they enhance trapping of FRW by Tedders traps. Three field trials were conducted, each designed as randomised blocks, with the five monitoring sites as individual blocks. At each site, nine Tedders traps were used, three each for clover oil, green leaf volatile, and nothing (control). Clover oil and green leaf volatile were loaded in 7-mL McCartney bottles and attached to the traps. The first trial investigated the essential oils at 100% with the loaded bottles placed at the top part of the trap. The second trial investigated the essential oils at 50% with the loaded bottles placed at the top. The third trial investigated the essential oils at 50% with the loaded bottles placed at the bottom. The trials lasted 3–4 weeks each and the number of FRW caught in traps was recorded weekly.

Data analysis

Correlation in weekly catches of FRW between individual traps and individual branches was analysed by community-wide synchrony via Monte Carlo randomisations (Loreau and de Mazancourt, 2008; Purves and Law, 2002). Correlations in weekly catches between the two monitoring methods were analysed by Pearson's product moment correlation (Turney, 2024). The sample size was estimated by the correlation between weekly catches estimated from a subset of traps/branches and those estimated from all traps/branches.

The correlation between the seasonal pattern of the proportion of fruit contamination by FRW eggs and that of mean trap catch over a 1–3-week period before the fruit assessment date (lead period) was analysed by Pearson's product moment correlation. The correlation between the overall proportion of FRW-contaminated fruit at a given site in a given year and the corresponding overall mean trap catch was analysed by Spearman's rank sum test (Spearman, 1904).

Data from Y-tube bioassays were analysed by exact binomial test. Data from multiple choice bioassays were analysed by chi-squared test. Field data of trap enhancement by clover oil and green leaf volatile were analysed by general linear models (GLM) followed by an analysis of variance (ANOVA; Venables and Ripley, 2002). Compounding effects of factors other than the treatment were removed before the analyses. Where a significant treatment effect was detected by ANOVA, treatment means were separated by Fisher's LSD test (Steel and Dickey, 1997).

All statistical analyses were done in R (R Core Team 2021).

Results and Discussion

Trap performance

Tedders traps were efficient in catching FRW adults, with a single trap catching as many as over 40 weevils (Figure A1-4, left). In comparison, less than 20 weevils were detected by individual branch shaking (Figure A1-4, right). Weekly catches of individual traps appear to increase and decrease in unison, whereas those from individual branch shaking appear more erratic (Figure A1-4). Similar results were seen in 2022 and 2023. At most sites and in most years, individual trap catches were more synchronised than individual branch catches (Table A1-2). Tedders traps also produced slightly more synchronised catches across monitoring sites than branch shaking (Table A1-2).

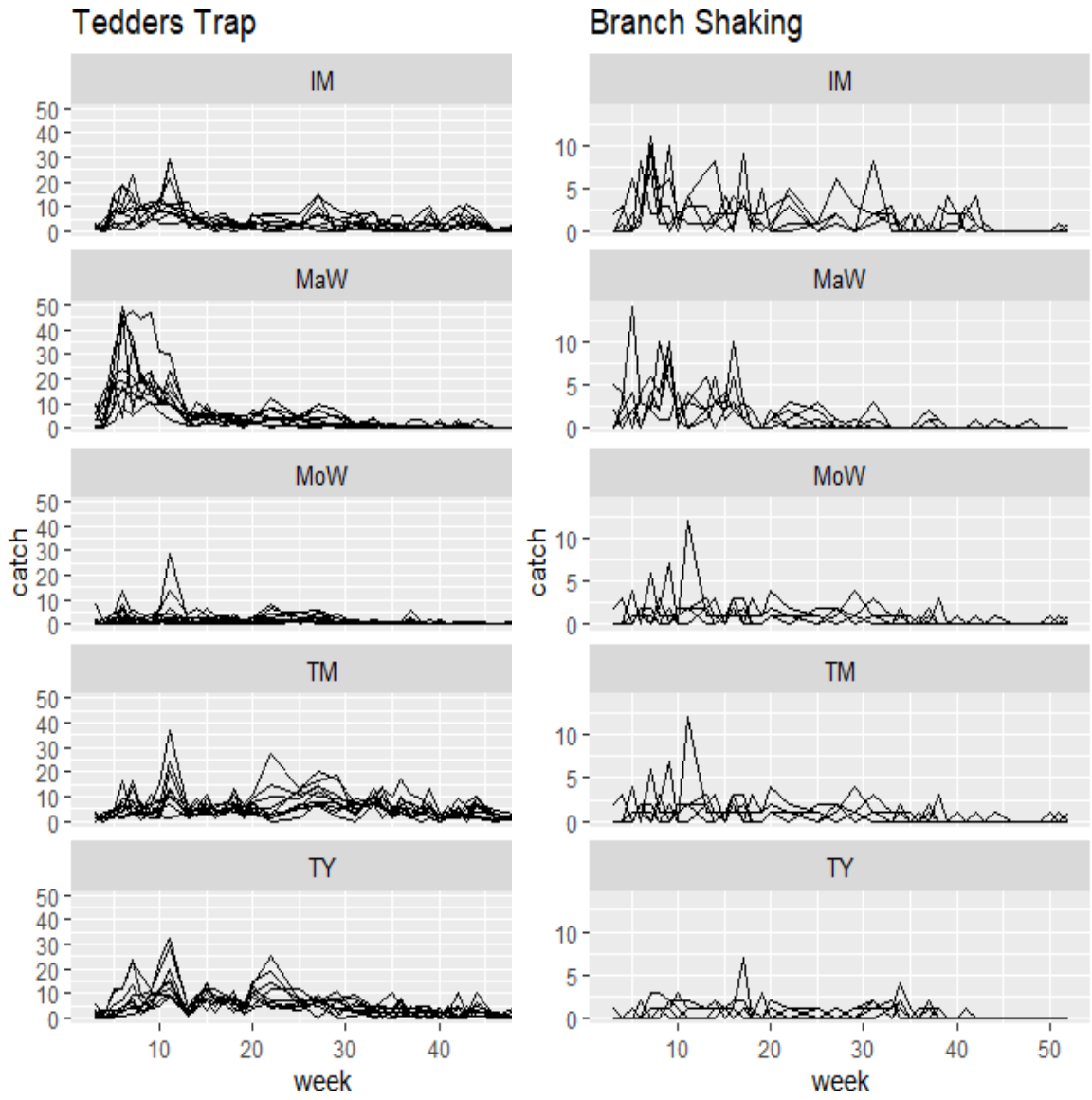


Figure A1-4. Weekly Tedder trap catches of adult FRW at five monitoring sites in the Riverina over in 2021. Different lines indicate different traps. Catch data have been statistically smoothed for visual clarity.

Despite the differences, average weekly catches from the two monitoring methods followed similar patterns at most sites and in most years (Table A1-3). However, there were noticeable differences in the level of correlation with respect to site and year, with the highest correlation observed at sites 'MaW' and 'MoW' in 2021 and an insignificant correlation at sites 'IM' and 'TM' in 2023. The overall catches across different sites between the two monitoring methods were also significantly correlated ($P < 0.05$).

Assuming weekly catches estimated from 5 traps and 5 branches as the respective standards of FRW activity at individual sites in individual years by the two monitoring methods, weekly catches estimated from Tedders traps approached a full correlation with the standards quicker than those estimated from branch shaking as the sample size increased from 1 to 5 (Figure A1-5), suggesting that Tedders trap is more efficient than branch shaking for FRW monitoring. For a minimal correlation of 0.9 with the weekly catches estimated from all 10 traps, the minimal required number of traps is 5.

Table A1-2. Synchrony in weekly catches between individual Tedders traps and individual branch shaking.

Year	Site	Trap	Branch
2021	IM	0.54	0.39
	MaW	0.81	0.46
	MoW	0.39	0.14
	TM	0.36	0.14
	TY	0.66	0.22
	All	0.61	0.59
2022	IM	0.55	0.41
	MaW	0.49	0.30
	MoW	0.18	0.16
	TM	0.32	0.16
	TY	0.43	0.26
	All	0.49	0.40
2023	IM	0.15	0.12
	MaW	0.51	0.30
	MoW	0.20	0.27
	TM	0.20	0.28
	TY	0.39	0.25
	All	0.46	0.35

* Community-wide synchrony via Monte Carlo randomisations (*n = 1000*). All synchrony levels were significantly different from random (*P < 0.05*).

Table A1-3. Correlation between catches from the Tedders trap and catches from branch shaking.

Site	2021	2022	2023
IM	0.64	0.32	-0.08
MaW	0.71	0.30	0.42
MoW	0.74	0.55	0.35
TM	0.49	0.48	0.28
TY	0.50	0.70	0.38
All	0.78	0.75	0.48

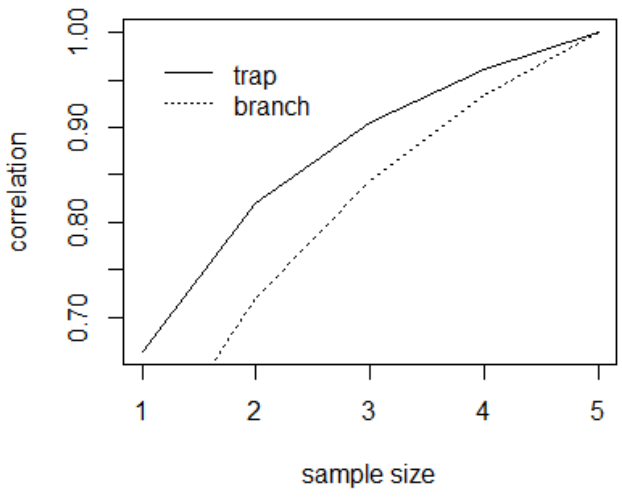


Figure A1-5. Correlation between overall weekly catches estimated from 5 Tedders traps (solid line) or 5 branches (dotted line) and weekly catches estimated from sub-samples of 1–5.

Seasonal patterns

Tedders traps and branch shaking showed similar seasonal patterns of FRW activity at the monitoring sites (Figure A1-6). While local peaks of the two seasonal patterns differed in size and were rarely aligned, both monitored methods detected a period of relatively high FRW activity between weeks 5 and 15 (February–April) at most sites in most years. Both monitoring methods also showed a second peak period between weeks 25 and 35 (June-August) at sites 'IM' in 2021 and 2022, and sites 'MoW' and 'TM' in 2023. The second peak period was also detected by Tedders traps at site 'TM' in 2021 and 2022, and at site 'TY' in 2022 and 2023. However, it was absent in the branch-shaking data for the respective sites and years. Another common feature detected by both monitoring methods was a period of relatively low FRW activity towards the end of the year at most sites and in most years. There were noticeable differences in the seasonal patterns produced by the two monitoring methods at sites 'TM' and 'TY' in 2021. In both cases, Tedders traps registered a flurry of FRW activity throughout the year, however, branch shaking failed to detect such activities.

Both monitoring methods produced similar seasonal monthly patterns of FRW activity to those observed in Hort Innovation project CT07045, with relatively high FRW activity from February to July, relatively low activity from August to January, and the highest activity during March and April (Figure A1-7).

Figure A1-6. Seasonal patterns of average weekly catches from Tedders traps and branch shaking by site and year. Catches by branch shaking have been scaled up by 2 folds for visual comparisons.

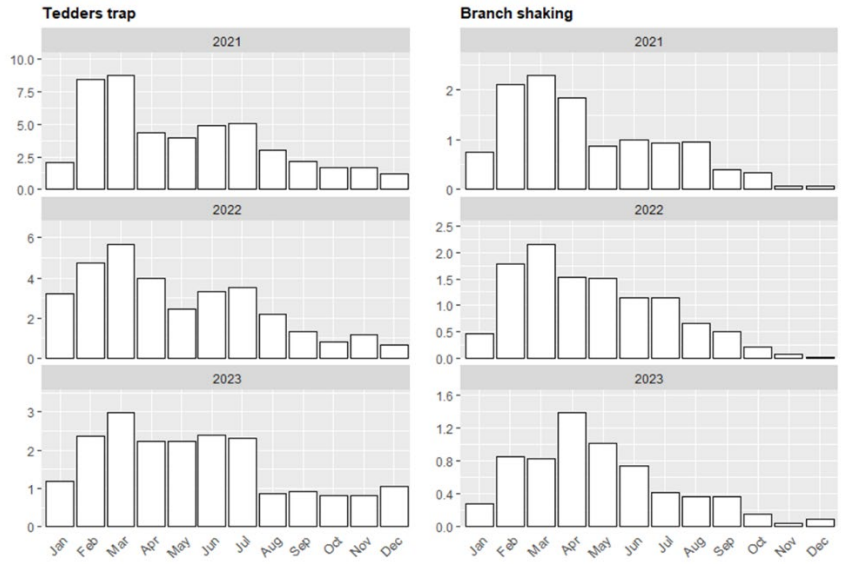


Figure A1-7. Average monthly catches of FRW across the five monitoring sites by Tedders traps (left) and branch shaking (right) in the three monitoring seasons.

Egg contamination in fruit

FRW egg contamination in citrus fruit at the monitoring sites was checked on 25 occasions during 2021 and 2024. Most fruit samples were collected in 2022 and 2023, with 10 sample dates each year. Seasonal patterns of fruit contamination matched closely with that of trap catch data at site 'MaW' in 2022 (Figure A1-8). The two seasonal patterns were significantly correlated ($P < 0.05$). However, no such correlation was detected at the other sites in 2022 or at any sites in 2023 ($P > 0.1$).

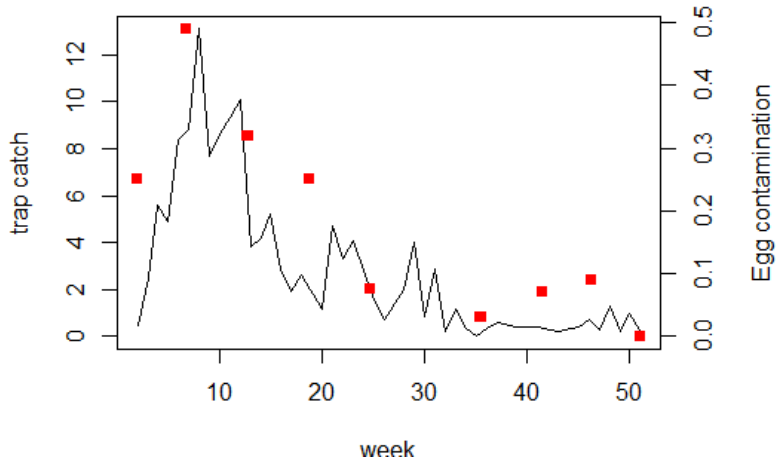


Figure A1-8. Seasonal patterns of trap catch (black line) and proportion of FRW contaminated fruit (red squares) at site 'MaW' in 2022.

There was a weak correlation between the mean proportion of fruit contamination at a site and the corresponding mean trap catches in 2022 ($P < 0.1$), however, no such correlation was detected for the 2023 data ($P > 0.1$; Figure A1-9).

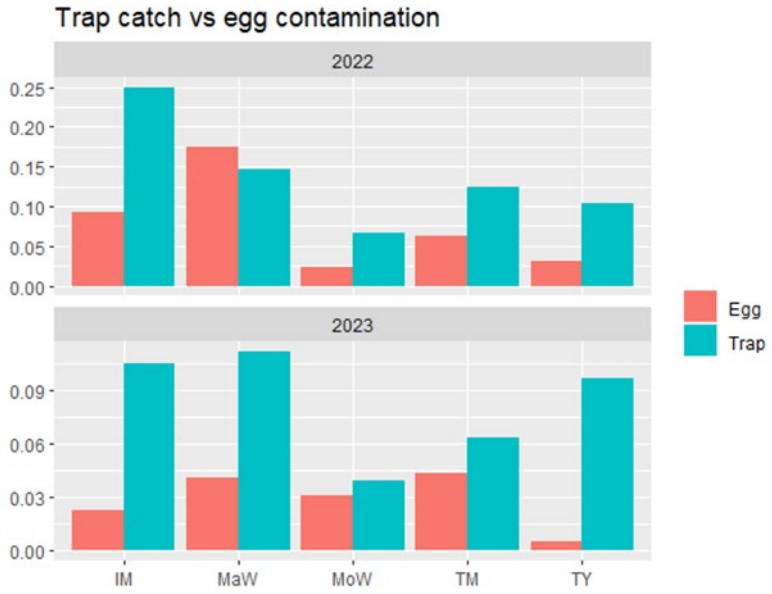


Figure A1-9. Mean proportions of FRW contaminated fruit and mean trap catch at individual monitoring sites. To enhance visual comparisons, trap catch has been scaled down to a maximum of 0.25.

Overall, high trap catches can be used to indicate when and where FRW egg contamination is high, however, the predictability will be low when the FRW population is low. The poor relationship between the two indices might have been due to several factors, including the variability in the fecundity and oviposition behaviour of individual females. When the FRW population is low, the effects of the individual variability on fruit contamination by the eggs will be more easily seen.

Trap enhancement

FRW adults were not attracted to volatiles from essential oils of sweet orange and clover, young orange, clover and rose leaves, or live FRW adults in Y-tube bioassays (*P > 0.1*; Table A1-4).

Table A1-4. Orientation of test weevils with respect to volatiles from orange, rose, and clover plants and their essential oils, and volatile from adult FRW in binary-choice bioassays.

Volatile type	Volatile source	N_t	N_c	P
Essential oil	Orange	16	11	> 0.1
Essential oil	Clover	5	4	> 0.1
Leaf	Orange	8	8	> 0.1
Leaf	Clover	12	6	> 0.1
Leaf	Orange	8	7	> 0.1
Leaf	Rose	8	6	> 0.1
Leaf	Rose	3	10	> 0.1
Leaf	Clover	11	7	> 0.1
Leaf	Rose	6	4	> 0.1
Live insect	FRW	3	5	> 0.1

N_t and N_c were the numbers of weevils entering the arm of the Y-tube loaded with the volatile source and those entering the control arm, respectively. P is the probability from binomial tests that the test weevils do not prefer the test volatile over the bank control.

Of the eight 4-choice bioassays comparing responses of test weevils to volatiles from essential oils of orange, clover, rose and control, significant differences in volatile selection were detected only in four of eight bioassays conducted (*P < 0.01*; Table A1-5). No significant difference was detected in the pooled data (*P > 0.1*).

Table A1-5. Frequencies of test weevils found on essential oil treatments of orange, clover, rose and water in 4-choice olfactometer bioassays. The frequency was estimated as the sum of test weevils found on a treatment at individual checkpoints.

Bioassay	Orange	Clover	Rose	Control	P
1	0	13	15	1	< 0.0001
2	16	0	3	16	< 0.0001
3	19	2	12	7	< 0.01
4	2	4	2	0	NA[2]
5	1	7	2	2	NA
6	4	0	1	2	NA
7	20	19	17	30	> 0.1
8	14	35	13	13	< 0.001
Pooled	76	80	65	71	> 0.1

[1] P is the probability from chi-square tests that the test weevils do not prefer volatile from a particular treatment. [2] NA – insufficient sample size for analysis.

The first selection by the test weevils was mostly clover leaves, followed by rose leaves, with orange leaves and the control being the least selected of the four choices (Figure A1-10, top). Clover was also the choice where most test weevils stayed the longest time, with orange being the choice of the shortest stay (Figure A1-10, bottom). However, differences in neither the first choice (*Chi-square = 1.47, df = 3, P = 0.69*) nor the choice of the longest stay were statistically significant *(Chi-square = 1.73, df = 3, P = 0.63*).

Test weevils were found on rose and orange leaves at similar frequencies in the two bioassays (Bioassay 1: *Chi-square = 3.11, df = 3, P = 0.37;* Bioassay 2: *Chi-square = 2.00, df = 3, P = 0.57*). The pooled data also showed no significant differences (*Chi-square = 0.78, df = 3, P = 0.85*).

Tedders traps baited with the clover oil and the green leaf volatile caught similar numbers of FRW as unbaited traps in all three trials (Trial 1: *F = 1.17, df = 2, 173, P = 0.3111*; Trial 2: *F = 1.08, df = 2, 173, P = 0.3432*; *F = 0.93, df = 2, 128, P = 0.3966*; Figure A1-11).

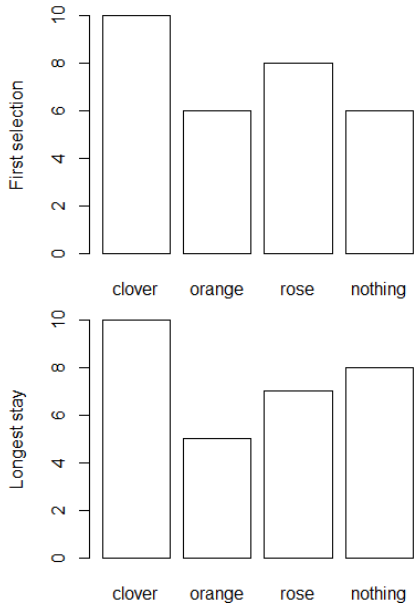


Figure A1-10. First selection frequency and frequency of longest stay of test weevils with respect to types of plant leaves in 4-choice olfactometer bioassays.

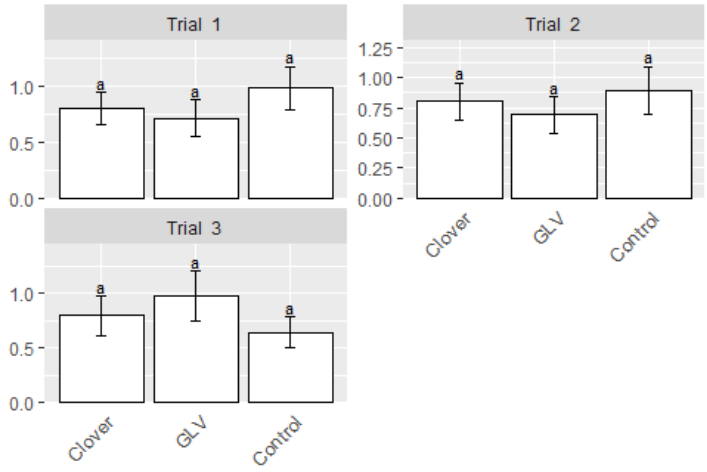


Figure A1-11. Number of FRW caught per trap per week by Tedders traps baited with clover oil, green leaf volatile (GLV), or nothing (Control). Bars labelled with a common letter are not significantly different (P > 0.05).

In conclusion, our results do not support the finding of Wee *et al.* (2008), who reported the two essential oils as being attractive to FRW. FRW is a highly polyphagous insect with a wide host range. As such, they might not have a strong preference for any specific plant odours.

Appendix 3: Potentials of entomopathogenic fungi and nematodes for controlling the Fuller's rose weevil

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Summary

We investigated the potential of entomopathogenic nematodes for controlling FRW larvae in the field and entomopathogenic fungi for controlling FRW adults in the laboratory. We did not find conclusive evidence of efficacy in any of the tested strains. FRW larvae live in the soil and soil moisture is critical for microorganisms such as nematodes and fungi. Although citrus orchards are irrigated, maintaining adequate soil moisture for the microorganisms is challenging in the semi-arid regions where most of Australia's oranges are grown. The prospect of entomopathogenic nematodes or fungi being used for FRW control in the main orange production regions of Australia appears to be low.

Introduction

Entomopathogenic nematodes (EPN) are a group of nematodes that infect and kill insects in the soil. Nematodes in the genera *Steinernema* and *Heterorhabditis* have emerged as excellent biological control agents for many insect pests (Grewal and Georgis 1999). Entomopathogenic fungi (EPF) are the most abundant type of microorganisms that infect insects, accounting for approximately 60% of insect diseases (Faria and Wraight, 2007). *Beauveria bassiana* and *Metarhizium anisopliae* are two of the most widely used entomopathogenic fungi for pest control (Li et al. 2010). As the natural pathogens of a variety of insects, EPF can be environmentally friendly alternatives to chemical insecticides for biological pest control. Some EPF and EPN have demonstrated potential for controlling the Fuller's rose weevil (McCoy and Boucias 1989; Edwards 1996). Several strains of EPN and EPF are available in Australia and have shown potential against several Coleopteran pests (Keith Danckwerts, Biological Ag. Personal communication). It is of interest to see if they can provide effective control of FRW.

This proposed proof-of-concept study is intended to verify the potential of EPN and EPF and provide independent advice on their use for FRW and CGW control.

Materials and Methods

EPN on FRW larvae

Two commercial EPN products, one each based on the species of *Heterorhabditis bacteriophora* and *Steinernema feltiae*, were sourced from Biological Ag. The nematodes were supplied as dried powder, containing 50 million infective juveniles (IJ) per gram. Two field trials were conducted in citrus orchards in Leeton in the Riverina that were known to have FRW infestation.

Field trial I

The first trial was conducted in a block of mature Valencia orange trees during January and May 2021. Five treatments were compared in the trial: single and double applications of *H. bacteriophora,* single and double applications of *H. feltiae,* and a water-only control. Both nematode products were tested at the rate of 30 live IJ/cm^2 (3 x 10^9 live IJ/ha). The trial was designed as complete randomised blocks with five replicates. A plot was a single row of 5 consecutive trees. Neighbouring plots were separated by a 2-tree in-row buffer and a buffer row. The first application was made on 7 January 2021 and the second on 19 January 2021. Both applications were made with a watering can along two 0.5 m-wide strips in each plot centred along the drip lines. The water rate was 18 L/plot (9 L/strip). Before the treatments were applied, each plot was pre-watered with 18 L of water. The abundance of adult FRW in each plot was monitored by monthly branch beating and fruit checking, and fortnightly by emergence traps starting on the day after the first

treatment application. Branch shaking and fruit checks were made on the three central trees in each plot. On each monitoring occasion, two branch shakes were made and 10 fruit were checked for the presence of FRW eggs from each of the three central trees. The total number of adult weevils dislodged by the shaking and the number of fruits found with FRW eggs were recorded for each plot.

Field trial II

The second trial was conducted in a block of mature grapefruit trees during June 2022 and March 2023. The trial investigated the nematode products at the high rate of 100 live IJ/cm^2 (1 x 10^{10} live IJ/ha). The trial was designed as complete randomised blocks with five replicates. A plot was a single row of 3 consecutive trees. Neighbouring plots were separated by a 2-tree in-row buffer and a buffer row. The nematodes were applied with an 8-L manual pump sprayer at the water rate of 8 L/plot on 8 June 2022. FRW population level in the trial area before the treatment application was made was assessed by shaking four branches per plot and a sample of 20 random fruit for checking of FRW egg contamination. After treatment application, the FRW population was checked weekly with a Tedders trap and an emergence trap in each plot.

EPN on FRW adults

Experiment I

This experiment investigated the efficacy of the nematodes under ambient humidity, along with a commercial entomopathogenic fungal product, Myco-Force. Myco-Force contains a mixture of three entomopathogenic fungal species: *B. bassiana*, *M. anisopliae* and *Lecanicillium lecanii.* The two nematode species were both tested at 800 IJs/mL and the Myco-Force at 10 g/L. The experiment had two stages: a 1-d infection stage and a 15-d post-infection stage. The infection stage was conducted in 9-cm plastic petri dishes lined with two Whatman #2 filter papers and the post-infection stage was in 70-mL clear plastic vials with mesh lids. Treatments were applied by pipetting 3 mL of a treatment solution to the filter paper in each petri dish. Ten weevils were introduced to each petri dish. The petri dishes were then closed, wrapped in cling wrap and placed in an incubator with the temperature set at 25 °C. After 24 hours, the test weevils were transferred to the 70-mL vials with a fresh citrus leaf and a wet cotton ball in each vial. The vials were placed in an incubator with the temperature set at 25 °C and light period at 12L:12D. Leaves and cotton balls were replaced daily. Starting at 72-h post-treatment, test weevils were checked every three days and the number of immobile weevils in each vial was counted.

Experiment II

This experiment investigated the efficacy of *H. bacteriophora* and *S. feltiae* on FRW adults on two humidity levels: high humidity and ambient humidity. As in the previous experiment, both nematode species were tested at 800 IJs/mL. The experiment was conducted in 70-mL clear plastic vials with a dental wick threaded through the bottom of the vials for water provision (Figure A3-1). For the high-humidity treatments, the bottom vial was filled with water. For the low-humidity treatments, the bottom vial was left empty. The nematodes were applied directly on adult weevils using a hand-held atomiser in a 1.7-L plastic bucket. After the treatment applications, the weevils were immediately transferred to the 70-mL plastic vials in an incubator with the temperature set a 25 °C and the light period at 12-h light and 12-h dark. A fresh citrus leaf was provided to the test weevil daily. Starting at 72-h post-treatment, test weevils were checked every three days and the number of immobile weevils in each vial was counted.

Both experiments were designed as completely randomised blocks with 5 replicates of 10 weevils each.

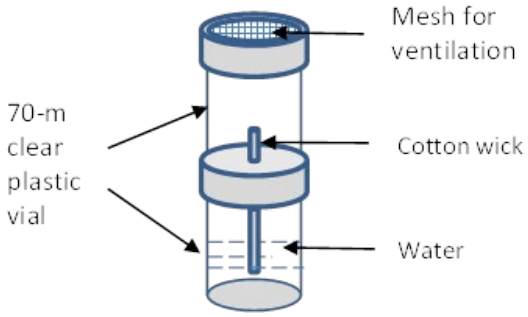

Figure A3-1. An illustration of the test arena for lab investigation of EPN for controlling FRW adults.

EPF on FRW adults

Three strains of *Beauveria bassiana*, B67, GHA, KS1, and three strains of *Metarhrisium anisopliae*, ECS1, NTC, and M16, were screened. All strains were sourced from Biological Ag and tested at 1 x 10^6 conidia/mL. The experiment was designed as completely randomised blocks with 7 treatments and 5 replicates. Each replicate consisted of 10 adult FRW in a 100-mL clear plastic container with ventilation holes on the lid. Treatments were applied using a handheld atomiser in a 1-L plastic container, with the amount of treatment solution applied in each replicate controlled as the output from a full squeeze of the atomiser. After treatment application, the weevils were transferred back to the 100-mL plastic vials with a fresh citrus leaf as food. The vials were then placed in a Controlled temperature rooms with the temperature set at 25 °C and the light period at 15-h light and 9-h dark. A bucket of wet sand was placed on the floor in the CT room to increase humidity. More water was added when required.

Statistical analysis

Data were analysed by general linear models (GLM) followed by analysis of variance (ANOVA; Venables and Ripley, 2002). Confounding effects of factors other than the treatment were removed before estimating the treatment effect. Proportional data (mortality, proportion of large galls) were analysed using the Binomial distribution with a logit link. Where a significant treatment effect was detected, treatment means were separated by Tukey's multiple comparison test or Fisher's LSD test (Steel and Dickey, 1997).

All statistical analyses were performed in R (R Core Team 2012).

Results

EPN on FRW larvae

Field trial I

Before the application of the nematodes, all treatment plots had a similar number of adult FRW, as revealed by branch-beat data ($F = 2.13$, $df = 4,16$, $P > 0.1$). No FRW egg-contaminated fruit was found in any treatments in the pre-treatment check.

After the treatment application, Tedder traps caught similar numbers of adult FRW among the four nematode treatments and the control on each of the seven occasions when the data were collected (Figure A3-2). However, the overall catch during the entire monitoring period was significantly lower in the two *S. feltiae* treatments than in the control (Figure A3-2). The overall catch was also lower in the two *H. bacteriophora* treatments than in the control, however, the difference was not significant ($P > 0.05$).

Branch-beat data showed a significant drop in adult FRW in the double-application treatments of both nematode species than in the control at two weeks after the second application (Figure A3-3). Significant treatment effects were also

detected on the last two monitoring occasions and in the overall data, but the differences were within nematode treatments, not between a nematode treatment and the control (Figure A3-3).

The proportion of FRW-contaminated fruit increased gradually over the monitoring period, from less than 5% on the first post-treatment monitoring occasion to over 20% on the last monitoring occasion. No treatment effects on the proportion were detected on any of the five monitoring occasions, nor in the overall data (*P > 0.05*).

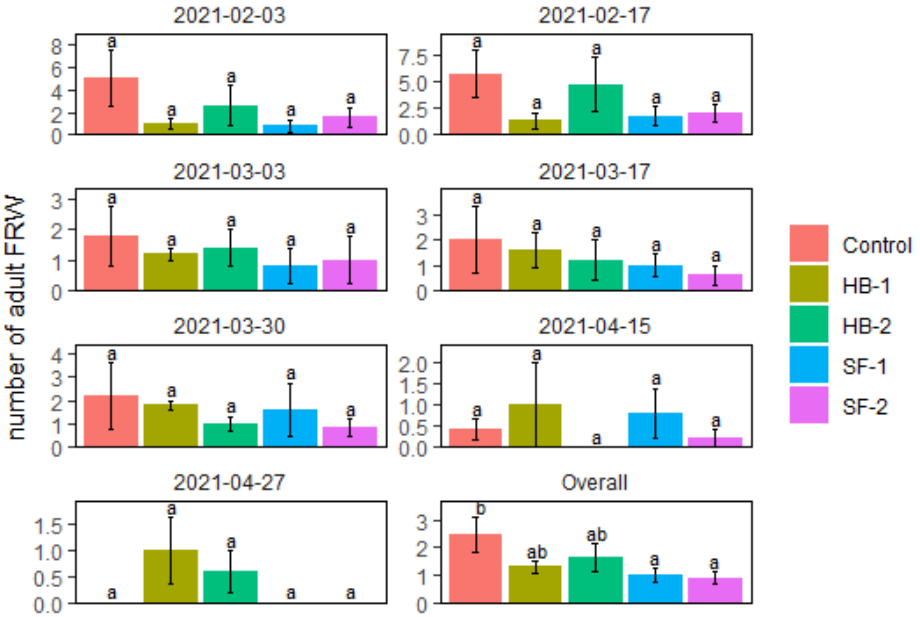

Figure A3-2. Average numbers of adult FRW caught by Tedders traps in plots treated with single or double applications of *Heterorhabditis bacteriophora* (HB-1, HB-2), single or double application of *Steinernema feltiae* (HB-1, HB-2), or water only (control) on individual assessment dates and over the entire monitoring period. Wire bars show the standard errors. Bars not sharing a common letter are significantly different at P = 0.05.

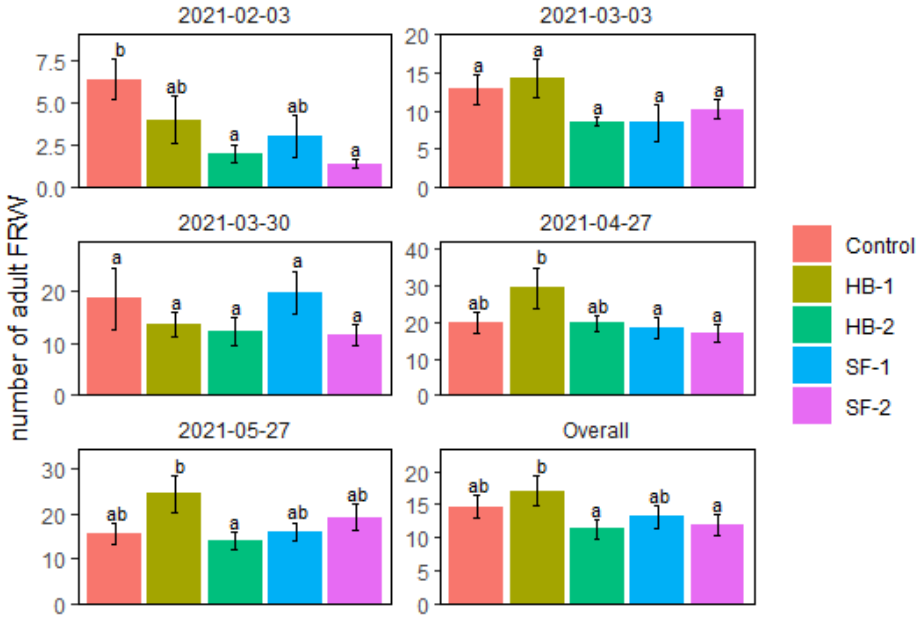

Figure A3-3. Average number of adult FRW caught by branch beating in plots treated with single or double application of *Heterorhabditis bacteriophora* (HB-1, HB-2), single or double applications of *Steinernema feltiae* (SF-1, SF-2), or

water only (control) on individual assessment dates and over the entire monitoring period. Wire bars show the standard errors. Bars not sharing a common letter are significantly different at P = 0.05.

Field trial II

Before the application of the nematodes, all treatment plots had similar levels of FRW egg contamination in fruit (*Chisq = 3.23, df =2, P = 0.1987*) and similar numbers of FRW adults as revealed by branch shaking data (*F = 4.24, df = 2,8, P = 0.0555*). In the 9 months following the nematode application, both emergence traps and Tedders traps caught similar numbers of adult FRW in plots treated with one of the nematode products and untreated plots (Figure A3-4).

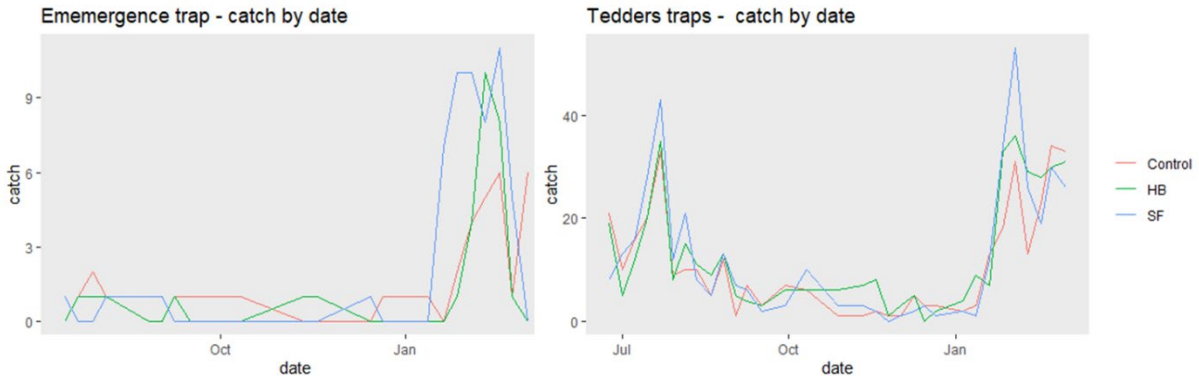

Figure A3-4. Numbers of FRW adults caught by the emergence traps (left) and Tedders traps (right) in plots treated with the entomopathogenic nematode *Heterorhabditis bacteriophora* (HB), *Steinernema feltiae* (SF), or nothing (Control) after the application of the nematode products.

EPN on FRW adults

Experiment I

H. bacteriophora showed efficacy against the test weevils on all five post-treatment assessment dates and *S. feltiae* on all but the first assessment date (Figure A3-5). On the last two assessment dates, mortality associated with the two nematode species was similar. Weevils treated with the two nematode species also produced significantly less frass than untreated weevils in the experiment (Figure A3-6). Myco-Force did not show any effect on the test larvae.

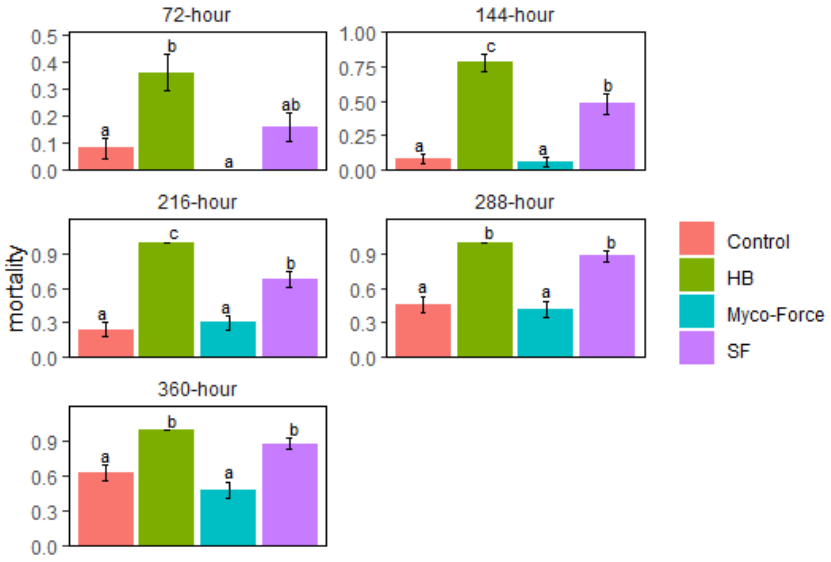

Figure A3-5. Mortality of adult FRW treated with *Heterorhabditis bacteriophora* (HB), *Steinernema feltiae* (SF), Myco-Force, or nothing (Control) at 72, 144, 216, 288, and 360-hour post-treatment application. Wire bars show the standard errors. Bars not sharing a common letter are significantly different at P = 0.05.

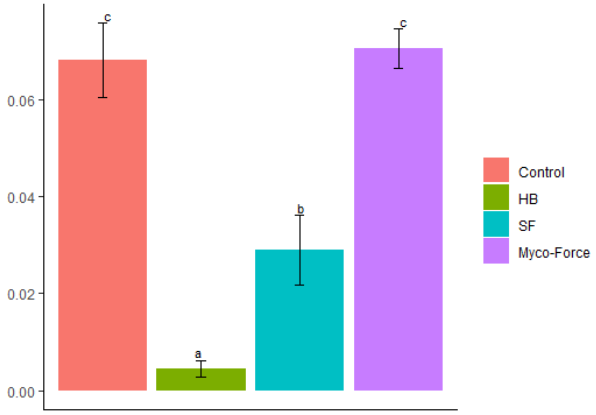

Figure A3-6. Total frass weight (g) produced by test weevils treated with *Heterorhabditis bacteriophora* (HB), *Steinernema feltiae* (SF), Myco-Force, or nothing (Control). Wire bars show the standard errors. Bars not sharing a common letter are significant are significantly different at P = 0.05.

Experiment II

Neither *H. bacteriophora* nor *S. feltiae* showed significant efficacy against the test weevils on any of the five assessment dates, regardless of humidity levels (Figure 3-7). The test weevils also produced a similar amount of frass during the experiment across the treatments (*F = 2.4770, df = 4, 16, P > 0.05*).

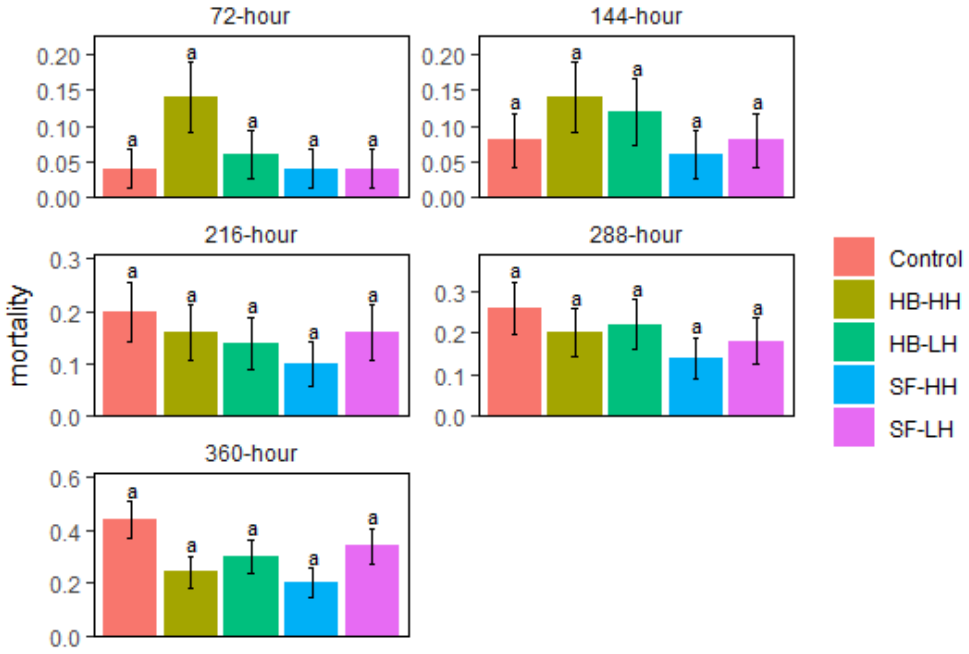

Figure 3-7. Mortality of adult FRW treated with *Heterorhabditis bacteriophora* at high humidity (HB-HH) or ambient humidity (HB-LH), *Steinernema feltiae* at high humidity (SF-HH) or ambient humidity (SF-LH), or nothing (Control) at 72, 144, 216, 288, and 3360-hour post-treatment. Wire bars show the standard errors. Bars not sharing a common letter are significantly different at P = 0.05.

EPF on FRW adults

Of the six fungal strains tested, only GHA killed significantly more adult weevils than the untreated control (Figure A3-8). The effect was seen from 7 days post-treatment. GHA-treated weevils also produced significantly less frass and fewer eggs than untreated weevils (Figure A3-9). Significant reductions in frass and eggs relative to the control were also observed in the strains KS1, NTC, and ECS1; however, the size of the reductions was considerably lower than that of GHA.

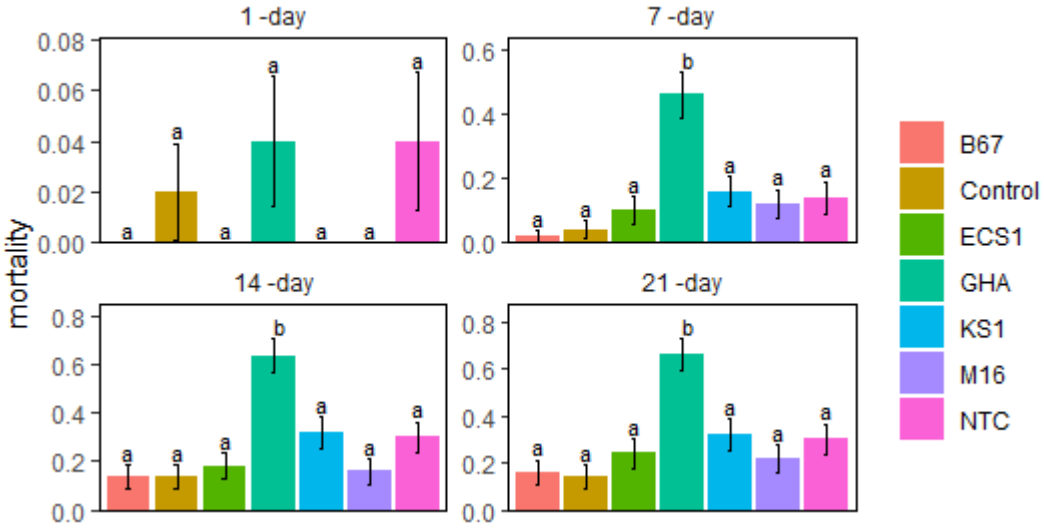

Figure A3-8. Mortality of adult FRW treated with one of three strains of Beauveria bassiana (B67, GHA, KS1), one of three strains of *Metarhizium anisopliae* (ECS1, NTC, M16), or nothing (Control) at 1, 7, 14 and 21-day post-treatment application. Wire bars show the standard errors. Bars not sharing a common letter are significantly different at *P = 0.05*.

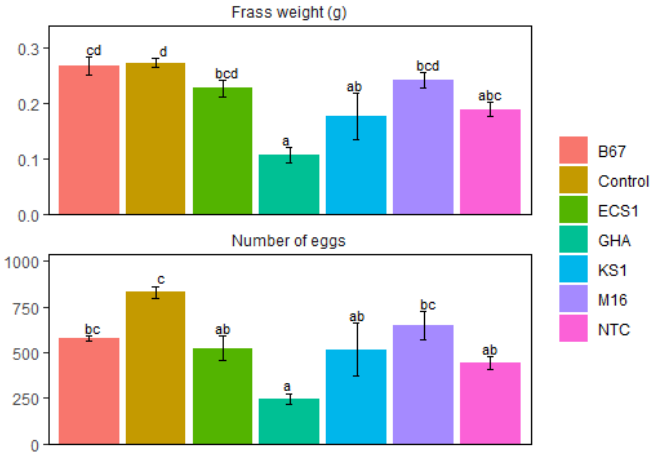

Figure A3-9. Frass weight (g) and number of eggs produced by test weevils 21 days after being treated with one of three strains of *Beauveria bassiana* (B67, GHA, KS1), one of three strains of *Metarhizium anisopliae* (ECS1, NTC, M16), or nothing (control). Wire bars show the standard errors. Bars not sharing a common letter are significantly different at P = 0.05.

Discussion

Two commercial products of entomopathogenic nematodes, one each from the species of *Heterorhabditis bacteriophora* and *Steinernema feltiae*, were investigated for controlling FRW larvae in the field and FRW adults in the

laboratory. Two field trials were conducted. Neither trial provided conclusive evidence confirming the efficacy of the two nematode products against the FRW larvae in the soil. Results from one of the two trials suggest that the nematodes might slightly reduce the survival of FRW larvae in the soil, but the amount of the reduction might not be sufficient for a satisfactory control of FRW infestation. The two nematode products showed some potential for controlling the adult FRW in the laboratory under an artificially high-humidity environment.

Five experimental strains of the entomopathogenic fungi *Beauveria bassiana* and *Metarhrizium anisopliae*, and a commercial mix of *B. bassiana, M. anisopliae, and Lecanicillium lecanii* were investigated in the laboratory for controlling FRW adults. One *B. bassiana* strain showed evidence of disabling/killing the adult weevils. The commercial product showed no efficacy against the adult weevils.

FRW larvae live in the soil and soil moisture is critical for microorganisms such as nematodes and fungi. Although citrus orchards are irrigated, maintaining adequate soil moisture for the microorganisms is challenging in the semi-arid regions where most of Australia's oranges are grown. The prospect of entomopathogenic nematodes or fungi being used for FRW control in the main orange production regions of Australia appears to be low.

Appendix 4: New chemical options for the control of the citrus gall wasp

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*Published in Austral Entomology 2025, April edition 64(2), e70005. https://doi.org/10.1111/aen.70005

Summary

In this study, we searched for new chemical options to manage the citrus gall wasp, focusing on those with relatively short withholding periods. Ten unregistered chemicals were screened to control adult gall wasp. Spinosad and spinetoram showed excellent direct and residual contact activity. Spinetoram appears more effective against adult wasps than spinosad in terms of residual activity and minimal effective rate. A field trial confirmed the efficacy of the two chemicals. Both spinosad and spinetoram showed low toxicity to predatory ladybird beetles and lacewings but high toxicity to parasitic *Aphytis* and predatory mites. Spinosad and spinetoram complement currently registered chemical options as they can be used in all citrus varieties, including Valencia, because of their relatively short withholding period. More than one spray might be needed to achieve satisfactory control. Where a single spray is preferred, the spray should be put on just before the peak emergence of the gall wasp to maximise its effect. Caution should be exercised when using the two insecticides in orchards with a history of red scale or mite problems, considering their negative effects on *Aphytis* and predatory mites.

Introduction

Currently, there are two registered chemicals for CGW control: clothianidin and calcined kaolin. While effective against CGW (Mo et al. 2019), both options have their limitations. Clothianidin is a neonicotinoid with a long withholding period. It is recommended to be used in the spring. As such, it cannot be used to control CGW in Valencia orange trees because the latter has mature fruit at the recommended time. In addition, clothianidin is a neonicotinoid, a group of insecticides that has been recently banned in Europe and some states in the USA. Calcined kaolin is a repellent against CGW and can reduce CGW galls by over 90% (Mo et al. 2019). Despite its effectiveness, calcined kaolin is not yet widely used by growers for CGW control because of its high cost and the concern that it might cause outbreaks of red scale, which is another important pest of citrus in Australia. For sustainable management of CGW, alternative chemical options are needed.

CGW spends most of its life inside the galls, making systemic insecticides, to which clothianidin belongs, an attractive group of insecticides for CGW control. However, CGW can also be controlled by foliar-applied, non-systemic insecticides. Although present only for a short period of time each year, the adult wasps are exposed and can be directly reached by droplets of foliar sprays. Several foliar insecticides were investigated for controlling adult CGW in CT15006. All were found to be efficacious against the adult wasps, which is not surprising, considering that CGW has not been exposed to insecticides for long in Australia. An obvious limitation of a foliar insecticide is that more than one application might be needed if the insecticide has a short residual activity. Despite the limitation, the availability of a foliar insecticide provides citrus growers with a means to control CGW in Valencia trees.

Here we report our investigation of foliar chemical options for CGW control, including their efficacy, minimal effective application rates and effects on beneficial arthropods in citrus.

Materials and Methods

Chemicals

Following a literature review and subsequent consultations with chemical companies and Hort Innovation Australia, 10 unregistered chemicals were selected in the investigation (Table A4.1).

Spinosad and spinetoram are derived from a group of naturally occurring chemicals called spinosyns, which originate from a soil-inhibiting microorganism (*Saccharopolyspora spinosa*; Sparks et al. 2021). Spinosad is a mixture of naturally occurring spinosyns, and spinetoram is a semi-synthetic spinosyn product. Both are widely used in the control of

agricultural pests, including the citrus leaf-miner (*Phyllocnistis citrella*) and the light brown apple moth (*Epiphyas postvittana*). Indoxacarb is an oxadiazine insecticide originally developed for controlling lepidopteran pests. It was considered one of the safest for beneficial arthropods in citrus in the US (Michaud and Grant 2003). Indoxacarb is registered in Australia for controlling FRW in apples and pears but not in citrus. Cyclaniliprole is a novel diamide insecticide. It is currently registered in Australia for controlling coddling moth (*Cydia pomonella*) in apples. Biopest and Vicol oil are mineral oils registered in Australia for controlling a range of horticultural pests. DC-154, DC-195, DC-196, and EXP-A are experimental products, the actives of which, at the request of the suppliers, are kept as 'undisclosed'.

Table A4.1. Insecticides and rates tested for CGW control*

Product	Active	Group	Rate
Entrust Organic	240 g/L spinosad	5	40 mL/100 L
Success Neo	120 g/L spinetoram	5	40 mL/100 L
Avatar eVo	303 g/kg indoxacarb	22A	37.5 g/100 L
EXP-A	Undisclosed	–	50 mL/100 L
Teppan 50SL	50 g/L cyclaniliprole	28	80 mL/100 L
DC-154	Undisclosed	–	80 mL/100 L
DC-195	Undisclosed	–	21 mL/100 L
DC-196	Undisclosed	–	15 mL/100 L
Biopest	Horticultural oil	–	1000 mL/100 L
Vicol oil	Horticultural oil	–	1000 mL/100 L
Control	Water only	–	NA

* Oils DC-195 and DC-196 were not tested in leaf bioassays. The DC formulation of EXP-A was used.

Spinosad, spinetoram, indoxacarb, cyclaniliprole, and the two oil products have no or low systemic effects. DC-154, DC-195, and DC-196 contain actives that are systemic.

Test insects

Test gall wasps were sourced from CGW galls collected in early spring before any adult CGW had emerged from citrus orchards at the Yanco Agricultural Institute. The galls were placed in 1.9 L plastic containers with 10% honey solution or water provided via a dental wick. Emergence of adult CGW in the containers was monitored daily. To ensure all test wasps were <24 hours old, emerged wasps were killed daily until the date when sufficient wasps were available for the bioassays.

Screening

Candidate chemicals were screened at the recommended rates indicated in Table A4.1 in the laboratory to identify promising chemicals. Direct contact activity of the chemicals was investigated by a Potter Tower bioassay and indirect (residual) contact activity by a leaf bioassay.

Direct contact activity

The bioassay was designed as completely randomised blocks, with 11 treatments (10 insecticides plus a control), 10 replicates (blocks), and six wasps per treatment replicate, giving a total of 660 wasps. To increase randomisation, wasps in each Petri dish were sourced from at least three different groups of galls.

The Petri dishes containing the test wasps were sprayed with a Potter Tower calibrated to deliver a spray deposit density of 2 mg/cm^2, equivalent to a spray rate of 200 L/ha. Mortality of the test wasps was checked at 1, 24, 48, 72, and 96 hours after treatment (HAT). A microscope was used to establish the ratio of males to females in each petri dish, with mortality recorded against sex in each petri dish.

Indirect contact activity

All except the two oil products (listed in Table A4.1) were used in the leaf bioassay. The residual activity of the chemicals was investigated at 0, 3, and 7 days after spray (DAS). Chemicals found effective at 7 DAS were further tested at 14 and 21 DAS. The bioassay was designed as randomised blocks with 5 replicates (blocks).

Potted orange trees (age > 20 years, variety 'Late Lane') were sprayed with the target insecticides to run-off with a handheld sprayer. Sprayed trees were left in a glasshouse with the temperature set at 25 °C during the day and 15 °C

during the night and natural light only. On 0, 3, 7, 14, and 21 DAS, leaves were removed from sprayed trees and placed in the upper vial of the double-vial test unit. The test unit consists of two 70-mL clear plastic vials (43 mm diameter x 55 mm) on top of each other with a cotton wick inserted through the floor of the upper vial and the lid of the bottom vial for water supply. Depending on the size of the leaves, 1–2 leaves were placed in each test unit to ensure contact of the wasps to treated leaves inside the test unit. Ten test wasps were introduced to each test unit, which were then placed in a 25 °C incubator, where the mortality of the test wasps was checked daily until all had died. Dead CGW were checked under a microscope to determine their sexes.

Systemic activity

DC-154, DC-195 and DC-196 were investigated for controlling immature CGW along with the industry standard clothianidin (Samurai®). Test rates for DC-154, DC-195 and DC-196 are shown in Table A4.1. Samurai was tested at the rate of 1 g/tree. Fifty potted lemon trees ('Eureka' on 'Citrange' rootstock) were placed in a glasshouse (26 °C during the day and 15 °C during the night, with natural light). Over 200 adult CGW were introduced daily for 7 days to the glasshouse. After all the introduced wasps had died, the 50 trees were randomly assigned to the 5 treatments (4 insecticides plus a water-only control). All treatments except Samurai were applied with a hand-held sprayer at the rate of 36 mL/tree. Samurai was applied directly to the soil with a measuring cup/cylinder at the rate of 91 mL/tree. Two assessments were made. The first assessment was made on 10 March 2021. Five trees were randomly selected from each treatment for the assessment. All galls in the selected trees were removed and counted for numbers in four size categories (<2, 2-5, 5-10, >100 mm long). The total weight of the galls was recorded. All galls were then dissected to count the number of live, dead, and missing larvae. The second assessment was made in early Spring 2021, just before gall wasp emergence.

Minimal effective rates

Four chemicals, spinosad, spinetoram, EXP-A, and DC-154, were found to have the potential for CGW control in the screening bioassays. They were further tested at a series of rates (Table A4.2) in the laboratory to estimate their minimal effective rates.

Table A4.2. Test rates in rate-response leaf bioassays conducted during November-December 2021

Insecticide	Test rate (mL/100 L)
Spinosad	40, 20, 10, 5, 2.5, 1.25, 0
Spinetoram	40, 20, 10, 5, 2.5, 1.25, 0
EXP-A	50, 25, 12.5, 6.25, 3.13, 1.56, 0
DC-154	80, 40, 20, 10, 5, 2.5, 0

* The DC formulation of EXP-A was used. The highest rate for each insecticide was used in the screening bioassays in 2020. A zero rate was added for each insecticide as a control.

Direct contact activity

Each chemical was tested individually, with different replications for each bioassay according to wasp availability. The delay between the time of emergence and testing in the bioassay was <24 hours for spinetoram but was extended to 48 hours for the other three chemicals to allow sufficient wasps to emerge. Wasps from galls sent from Yanco, NSW, were used to test spinetoram and spinosad, while galls collected from Pascoe Vale, Victoria, were used to test EXP-A and DC-154. Test wasps were placed in Petri dishes before being sprayed in a Potter Tower.

Indirect contact activity

The insecticides were mixed with non-ionic surfactant polyoxyethylene alkyl ether (Agral®) at 25 mL/100 L and sprayed to run-off (about 6 L/tree) to 2-m tall navel orange trees at the Yanco Agricultural Institute, Yanco, NSW, using a battery-powered backpack sprayer. Each rate was sprayed on a separate tree with a 2-tree buffer between neighbouring test trees.

After the spray droplets had dried up, mature leaves were collected from the sprayed trees and used for the bioassay. The same double-vial test unit described earlier was used. One sprayed leaf was placed in the upper vial of the test unit.

Twenty newly emerged wasps (<24 h old) of mixed sexes were then introduced to each test unit. The test units were placed on the bench of a laboratory with temperature control (22–25 °C).

Test wasps were checked daily for survival for three days. Dead CGW were checked under a microscope to determine their sexes. Sprayed leaves in the test units were replaced daily with freshly collected treated leaves at the same rate of treatment.

Each rate of each insecticide was tested in 5-6 test units (replicates) of 20 wasps each. Test wasps were randomly assigned to the test rates.

Field efficacy

A field trial of spinosad, spinetoram, and DC-154 was conducted in a block of mature Valencia orange trees at the Yanco Agricultural Institute between October 2023 and March 2024. The trial had six treatments (Table A4.3). All three chemicals were tested at their label rates and sprayed on 23 October 2024. To assess the effect of spray frequency and timing, spinetoram was also tested in a double-spray treatment with the second spray applied 7 days after the first spray, and a single, late spray treatment applied 11 days after the first spray. The first and second spray dates were before the CGW peak emergence and the third just after the peak emergence, according to the CGW emergence timing tool (https://citrusgallwasp.shinyapps.io/predict/). All sprays were made with a hand-held spray wand at the water rate of 6 L/tree.

Table A4.3. Treatments tested in the field trial of promising chemicals for CGW control.

Treatment	Rate
Spinosad – single early spray – 23 October	40 mL/100 L
Spinetoram – single early spray – 23 October	40 mL/100 L
Spinetoram – double sprays – 23 and 30 October	40 mL/100 L
Spinetoram – single late spray – 3 November	40 mL/100 L
DC-154 – single early spray – 23 October	80 mL/100 L
Control	–

The trial was designed as completely randomised blocks with five replicates for each treatment. A plot consisted of a rectangular area of 3 rows by 4 trees. Neighbouring plots were separated by at least one buffer row and one buffer tree within the row.

Pre-treatment data of CGW infestation were collected on 3 and 7 November 2023, before the new-season galls had formed using a 50 x 50 x 50 cm wireframe. Two frame counts were made in each plot, one each in the two central trees. The wireframe was randomly inserted into the canopy and the numbers of current-season galls <2, 2-5, 5–10, and >10m mm long were counted. Post-treatment data of new-season galls were collected on 27 March 2024. The wireframe was randomly inserted into the tree canopy and all galls inside the wireframe were removed. Two wireframes of galls were collected from the two central trees in each plot. In the laboratory, the collected galls were measured individually for length and the total weight of galls from the same plot was recorded.

Effect on beneficial arthropods

Spinosad, spinetoram, DC-154 and EXP-A were investigated for their toxicity at their respective label/recommended rates (Table A4.1) to four important beneficial arthropods of citrus in Australia: the red scale parasitoid *Aphytis lingnanensis*, the ladybird beetle *Cryptolaemus montrouzieri*, the green lacewing *Mallada signatus*, and the predatory mite *Neoseiulus californicus*. The beneficial insects were sourced from Bugs for Bugs™ (Toowoomba, Queensland) and Biological Services (Loxton, SA). The DC formulation of EXP-A was used in the tests.

Petri dish bioassays

The protocols for testing acute toxicity of insecticides to beneficial arthropods published by the International Organisation for Biological Control (IOBC; Sterk et al. 1999) were used in the effect on beneficial arthropods study. Exposure to residues, rather than direct exposure, is generally considered to be the most field-relevant test for insecticide toxicity testing for beneficial arthropods (Hassan, 1985).

C. montrouzieri was tested both as adults and larvae. *Mallada signatus* was tested only as larvae. *Neoseiulus californicus* was tested as adults. Fifteen to thirty individuals of each species were exposed to fresh, dry chemical residue in a petri dish, and mortality was recorded at 24, 48, and 72 hours post-exposure (except mites, which were completed at 48 hours). In addition to the four target insecticides, the bioassays included a negative control (demineralised water) and a positive control (Lorsban® at 100 mL/100 L).

Mortality values were averaged across replicates and were used to assess the toxicity of each chemical dose as low, medium, high, or very high, as per the IOBC standard for evaluations under laboratory conditions (Sterk et al. 1999), and have been colour coded accordingly. Generally, assessments after 48 hours of exposure to the field rate are considered the most appropriate for predatory mites, while 72 hours is most appropriate for ladybird beetles and lacewings.

Leaf bioassays

The insecticides were mixed with Agral® at 25 mL/100 L and sprayed to run-off to potted navel orange trees ('Late Lane') using a handheld sprayer. Leaves were collected from the sprayed trees and tested for residual toxicity to the beneficial arthropods. *A. lingnanensis, C. montrouzieri* and *N. californicus* were tested as adults and *M. signatus* as larvae. Each insecticide was tested for its toxicity at 1 and 7 days after spray (DAS). A water only treatment was included as the control in each bioassay.

The toxicity of the insecticides to *C. montrouzieri* and *M. signatus* was studied using the double-vial test unit. Ten *C. montrouzieri* were tested in each test unit. The number of *M signatus* larvae introduced to each test unit varied from 6 to 16 due to the variability in the supply of lacewing larvae.

The toxicity of the insecticides to *A. lingnanensis* at 1 DAS was investigated using the double-vial test unit and at 7 DAS using a modified test unit. Observation during the 1-DAS investigation showed some test wasps drowned on the wet dental wick. To eliminate drowning, the water-filled bottom vial and the dental wick of the double-vial test unit were removed. The number of wasps introduced to each test vial varied from 7 to 45 as it was difficult to introduce *Aphytis* individually without damaging them due to their small size.

The toxicity of the insecticides to *N. californicus* was studied using a method similar to that described by Duso et al. (2008) and Dennehy et al. (1993). The test unit was a PVC ring (40 mm diam, 1 cm deep) sandwiched between two treated leaves. To attach the leaves, a thin strip of BluTack (Bostik Australia Pty Ltd, Thomastown, Victoria, Australia) was pressed onto the opening edges of the PVC ring. Care was taken to ensure that there were no gaps between the PVC ring and the leaf. Two bioassays were conducted. In the first, around 0.5 g of the mite mix from the supplier, which included *N. californicus*, *Lepidoglyphus destructor* (prey mite), and vermiculite, was placed inside each test unit. In the second bioassay, the amount of mite mix placed inside each test unit was reduced to around 0.3 g to reduce the number of mites introduced. One day after mite introduction, the test unit was opened, and the number of live *N. californicus* was counted under a stereo microscope. After the counting, the content inside the test unit, including counted live mites, was emptied into a petri dish containing 70% ethanol. Finally, the total number of *N. californicus* in the petri dish was counted.

Statistical analysis

Data were analysed by general linear models (GLM) followed by analysis of variance (ANOVA; Venables and Ripley, 2002). Confounding effects of factors other than the treatment were removed before estimating the treatment effect. Proportional data (mortality, proportion of large galls) were analysed using the binomial distribution with a logit link. Where a significant treatment effect was detected by ANOVA, treatment means were separated by Tukey's multiple comparison test or Fisher's LSD test (Steel and Dickey 1997). Minimal rates for 50, 90 and 95% mortality (LC50, LC90, and LC95) and their 95% confidence intervals were estimated using the 2-parameter log-logistic model in the R package 'drc'.

All statistical analyses were performed in R (R Core Team 2012).

Results

Screening

Direct contact activity

Over 80% of the test wasps treated with spinosad, spinetoram, EXP-A, and DC-154 died at 24 HAT, significantly higher than that in the control (zero mortality; $P < 0.05$; Figure A4.1). These four insecticides remained top-performing treatments at 48, 72, and 96 HAT (Figure A4.1). Cyclaniliprole, DC-196 and indoxacarb also showed significant efficacy at 48, 72, and 96 HAT, but the mortality rate was low (<50%).

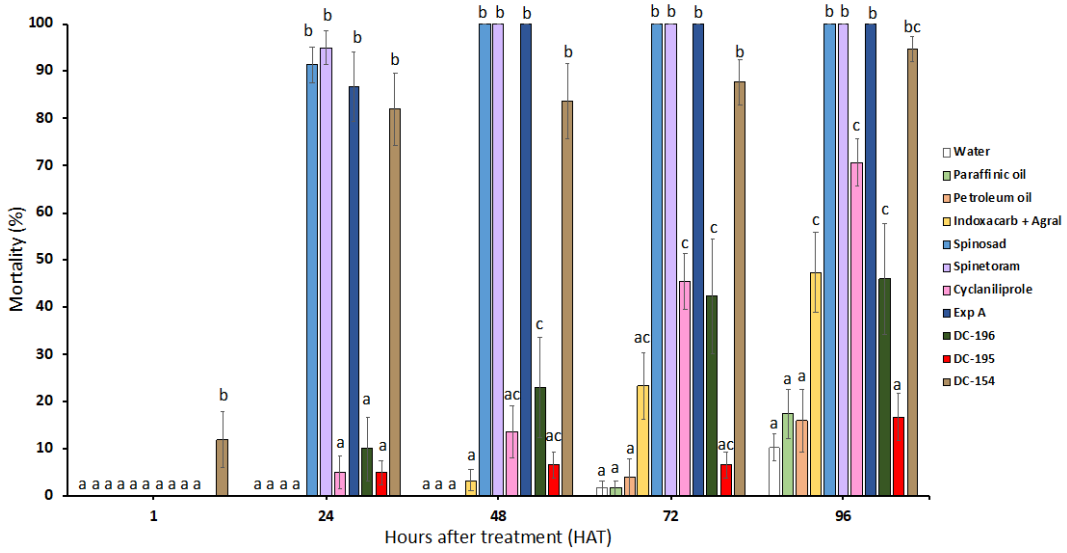

Figure A4.1. Mean citrus gall wasp mortality (% ± SE) after direct contact sprays were applied at 1, 24, 48, 72 and 96 hours after treatment (HAT). Letters above bars within the same scoring period (i.e. 1, 24, 48, 72, or 96 HAT) denote significant differences detected at α = 0.05.

Indirect contact activity

Out of six insecticides tested, spinetoram and spinosad were the most effective against CGW adults. Both killed significantly more wasps than the untreated control at all five post-spray times ($P < 0.05$). On leaves sprayed the same day (0 DAS), they achieved 100% mortality on the first day. Even 21 days after spraying, they still caused around 60% mortality, well above the control ($P < 0.05$). Two other products, EXP-A and DC-154, showed some activity but were much less effective. Mortality rates after 3 days of exposure showed similar patterns of treatment effects to those of 1-day exposure (Figure A4.2), indicating that most test wasps died during the first day.

Systemic activity

Results from the first assessment showed that Samurai was the only treatment that significantly reduced all five CGW infestation indices measured relative to the control (total gall weight, total number of galls, number of galls over 50 mm long, and number of live larvae per cm of gall length ($P < 0.05$; Figure A4.3). DC-154 and DC-196 significantly reduced the number of galls over 5 cm long relative to the control ($P < 0.05$), but they show no effects on the other CGW infestation indices.

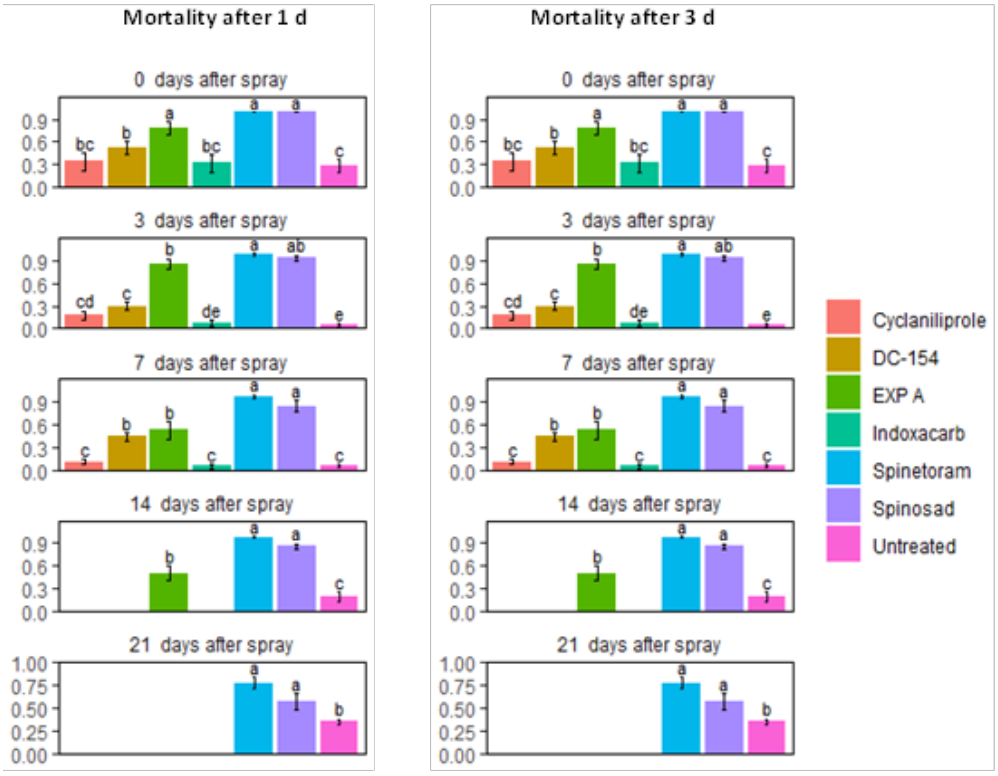

Figure A4.2. Mortality of citrus gall wasps exposed to treated leaves collected at 0, 3, 7, 14, and 21 days after the spray (DAS; mean± SE). Bars not sharing a common letter are significantly different at P = 0.05. All treatments targeted in this bioassay were tested at 0, 3, and 7 DAS, but only subsets of the treatments were tested at 14 and 21 DAS based on results obtained at earlier post-spray dates.

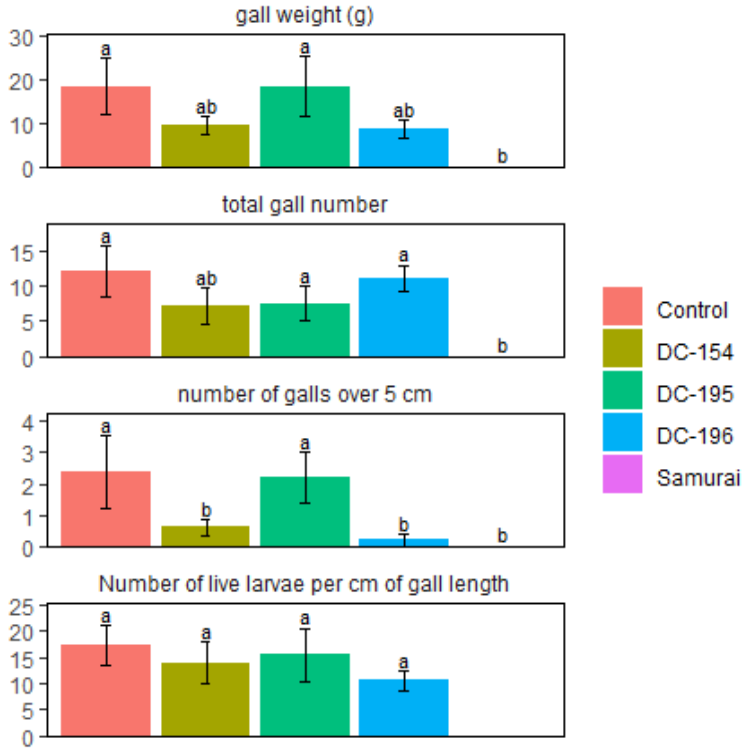

Figure A4.3. Total gall weight (g), total number of galls, number of galls over 5 cm long, and number of live larvae per cm of gall length (mean± SE). Bars not sharing a common letter are significantly different at P = 0.05. The number of live larvae per cm of gall length cannot be estimated for Samurai as there were no galls in the treatment.

Minimal effective rates

Direct contact activity

These bioassays generally resulted in very high mortality observed within 24 hours of exposure, regardless of the rate tested. For spinetoram, all rates from 1.25 to 40 mL/100 L resulted in ≥98% mortality by 24-HAT, which was significantly higher than the water control (16%; Table A4.4).

Likewise, by 24-HAT, Spinosad had caused 100% mortality to all wasps, compared with 0% mortality for the water controls (Table A4.5).

Table A4.4. Average citrus gall wasp mortality (%) at 1, 24, and 48 hours after treatment with spinetoram, and results from one-way ANOVAs comparing treatments. Different letters indicate significantly different means at each time (at the *P < 0.05* level, Tukey's-b post hoc test).

Treatment	Rate	1-HAT		24-HAT		48- HAT	
Spinetorum	0 mL/100L	8	a	16	A	16	A
	1.25 mL/100L	8.5	a	98	b	100	B
	2.5 mL/100L	6	a	100	b	100	B
	5 mL/100L	2	a	100	b	100	B
	10 mL/100L	14.5	a	98	B	100	B
	20 mL/100L	6	a	100	b	100	B
	40 mL/100L	18	a	100	b	100	B
P value		0.125		<0.0001		<0.0001	
F statistics		1.746		106.8		153.8	
df		6		6		6	

Table A4.5. Average citrus gall wasp mortality (%) at 1 and 24 hours after treatment with spinosad and results from one-way ANOVAs comparing treatments. Different letters indicate significantly different means at each time (at the *P < 0.05* level, Tukey's-*b* post hoc test).

Treatment	Rate	1-HAT		24-HAT	
Spinosad	0 mL/100L	0	a	0	a
	1.25 mL/100L	0	a	100	b
	2.5 mL/100L	0	a	100	b
	5 mL/100L	0	a	100	b
	10 mL/100L	0	a	100	B
	20 mL/100L	8.3	a	100	b
	40 mL/100L	0	a	100	b
P value		0.045		<0.0001	
F statistics		2.5		8.083e+29	
df		6		6	

Chemical EXP-A caused 100% mortality to wasps within 24-HAT at all rates except the lowest rate (1.56 mL/100 L), which caused 80% mortality, which increased to 100% by 48-HAT (Table A4.6).

A dose-response was evident for DC-154 after only 1-HAT, whereby treatment caused 31% mortality at the lowest rate of 2.5 mL/100 L, and the highest rate (80 mL/100 L) caused 100% mortality (compared to 3% for the water controls; Table A4.7). After the initial knockdown effect of DC-154, mortality was somewhat slower to increase, and increasing background mortality in the water controls in this bioassay quickly caught up with that caused by the lowest rate. The rate of 20 mL/100 L (i.e., ¼ of the proposed field rate) caused >90% mortality within 1-HAT, while lower rates did not meet this threshold even by 96-HAT.

Table A4. 6. Average citrus gall wasp mortality (%) at 1, 24, and 48 hours after treatment with EXP-A, and results from one-way ANOVAs comparing treatments. Different letters indicate significantly different means at each time (at the *P < 0.05* level, Tukey's-*b* post hoc test).

Treatment	Rate	1-HAT		24-HAT		48-HAT	
EXP A	0 mL/100L	0	a	14.3	a	35.7	a
	1.56 mL/100L	3.1	a	80.2	b	100	b
	3.13 mL/100L	0	a	100	c	100	b
	6.25 mL/100L	0	a	100	c	100	b
	12.5 mL/100L	3.1	a	100	c	100	b
	25 mL/100L	0	a	100	c	100	b
	50 mL/100L	0	a	100	c	100	b
***P* value**		0.567		<0.0001		<0.0001	
F statistics		0.81		54.41		363.5	
df		6		6		6	

Table A4.7. Average citrus gall wasp mortality (%) at 1, 24, 48, 72, and 96 hours after treatment with DC-154, and results from one-way ANOVAs comparing treatments. Different letters indicate significantly different means at each time (at the *P < 0.05* level, Tukey's-*b* post hoc test).

Treatment	Rate	1-HAT		24-HAT		48-HAT		72-HAT		96-HAT	
DC- 154	0 mL/100L	3.1	a	22.9	a	47.9	a	60.4	a	79.2	a
	2.5 mL/100L	31.3	b	40.6	ab	56.3	a	65.6	a	81.3	a
	5 mL/100L	46.9	b	59.4	b	75.0	ab	81.3	ab	96.9	a
	10 mL/100L	59.4	b	65.6	b	75.0	ab	78.1	ab	84.4	a
	20 mL/100L	93.8	c	100	c	100	b	100	b	100	a
	40 mL/100L	96.4	c	100	c	100	b	100	b	100	a
	80 mL/100L	100	c	100	c	100	b	100	b	100	a
***P* value**		0.567		<0.0001		<0.0001		<0.0001		0.061	
F statistics		26.68		22.7		9.11		5.25		2.18	
df		6		6		6		6		6	

Indirect contact activity

The minimal effective rate for spinosad was 10 mL/100 L for 1 to 3-day mortality (Figure A4.4). The three application rates higher than or equal to the minimal effective rate all achieved over 90% mortality after 3 days, however, none achieved >90% mortality in the first day. The top test rate, 40 mL/100 L, was below the required rates for 90 or 95% mortality based on the estimated LC90, and LC95 values after only one day of exposure of the test wasps to the insecticide but above the required rates after 2-3 days of exposures (Table A4.8). The 20 mL/100 L rate is above the required rates after three days of exposure.

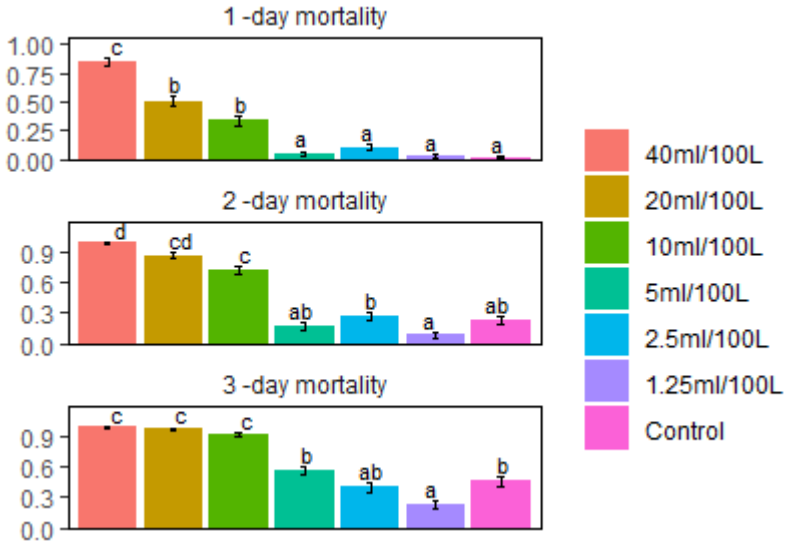

Figure A4.4. One, two, and three-day mortality of adult citrus gall wasp following exposure to leaves sprayed with six rates of spinosad. Bars not labelled with a common letter were significantly different at P = 0.05 by Tukey's multiple comparison tests following the detection of significant treatment effects by the general linear model (GLM) for the binomial distribution.

Table A4.8. Estimated LC50, LC90, and LC95 (95% confidence intervals; mL/100 L) for the toxicity of spinosad, spinetoram and EXP-A to the adult citrus gall wasp after 1, 2, and 3 days of exposure.

	1-day	2-day	3-day
		Spinosad	
LC50	16.71 (13.40 – 20.02)	6.52 (4.16 – 8.89)	3.18 (2.56 – 3.81)
LC90	72.75 (37.70 – 107.80)	24.10 (16.40 – 31.81)	11.96 (8.11 – 15.82)
LC95	119.98 (45.35 – 194.62)	37.59 (22.97 – 52.21)	18.76 (11.49 – 26.03)
		Spinetoram	
LC50	4.57 (3.52 – 5.63)	0.72 (0.50 – 0.95)	0.55 (0.27 – 0.82)
LC90	40.24 (15.59 – 64.90)	3.20 (2.52 – 3.87)	1.38 (1.24 – 1.53)
LC95	84.31 (18.21 – 150.40)	5.30 (3.49 – 7.11)	1.90 (1.42 – 2.38)
		EXP-A	
LC50	40.66 (23.88 – 57.45)	2.84 (2.18 – 3.94)	1.43 (0.92 – 1.93)
LC90	242.60 (18.72 – 466.48)	48.68 (10.52 – 86.85)	19.44 (7.92 – 30.96)
LC95	NA	NA	47.28 (6.95 – 87.61)

The minimal effective rate for spinetoram was 1.25 mL/100 L, the lowest tested rate for this insecticide (Figure A4.5). However, only the 40 mL/100 L rate achieved over 90% mortality on the first day. The other tested rates achieved close to 90% mortality 2 or three days later. Based on the estimated LC90 and LC95 values, 40 mL/100 L was about the same as that required for 90% first-day mortality but below that required for 95% first-day mortality (Table A4.8). The top three test rates, 40, 20, and 10 mL/100 L, were all above the required for 95% mortality after 2 or 3 days (Table A4.8).

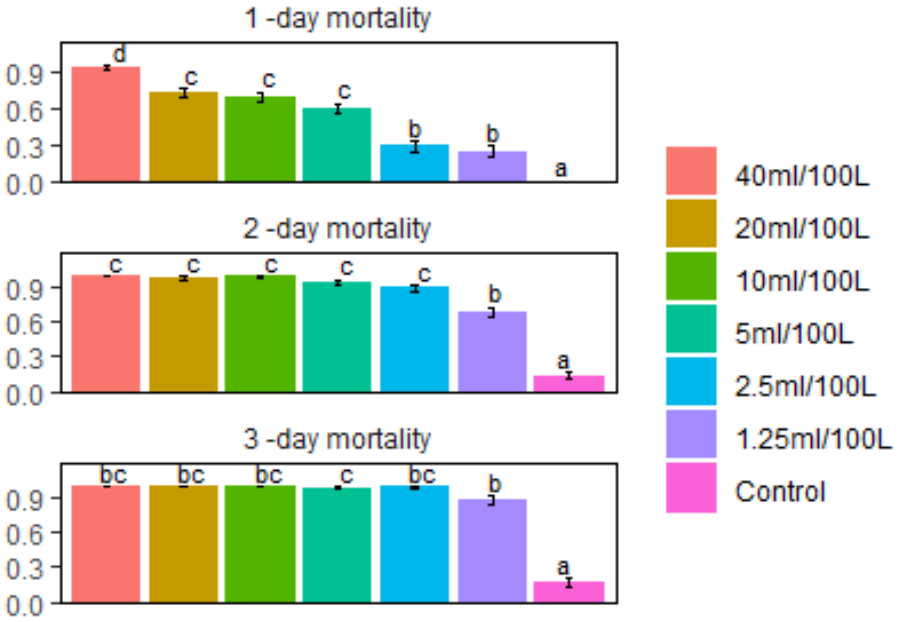

Figure A4.5. One, two, and three-day mortality of adult citrus gall wasp following exposure to leaves sprayed with six rates of spinetoram. Bars not labelled with a common letter were significantly different at P = 0.05 by Tukey's multiple comparison tests following the detection of significant treatment effects by the general linear model (GLM) for the binomial distribution.

The minimal effective rate for EXP-A was 25 mL/100 L for 1-day mortality and 1.56 mL/100 L for 2 or 3-day mortality (Figure A4.6). None of the six test rates achieved 90% or over mortality on the first day. The first-day mortality was less than 70%, even at the top rate of 50 mL/100 L. The top rate was above the rate required for 90% or higher mortality on the second day, and the 25 mL/100 L rate was above the rate required for 90% or higher on the third day (Table A4.8).

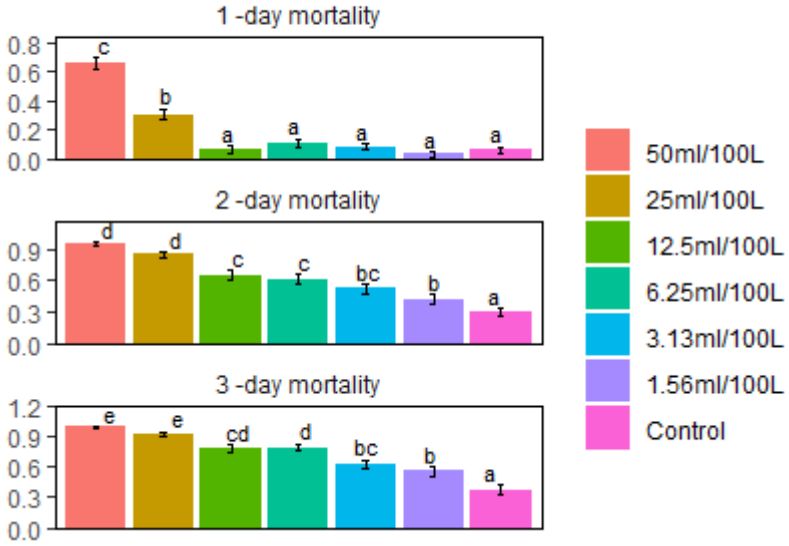

Figure A4.6. One, two, and three-day mortality of adult citrus gall wasp following exposure to leaves sprayed with six rates of EXP-A. Bars not labelled with a common letter were significantly different (*P < 0.05*) by Tukey's multiple comparison tests following the detection of significant treatment effects by the general linear model (GLM) for the binomial distribution.

Field efficacy

All treatments had similar numbers of previous-season galls (*F = 0.72, df = 5, 20, P = 0.6163*). The numbers of large galls (≥ 50 mm long) from the previous season were also similar between the treatments (*F = 1.48, df = 5, 20, P = 0.2418*). Post-treatment data of new-season galls showed significant differences between the treatments in total gall length (*F = 3.59, df = 5, 20, P = 0.0176*), total gall weight (*F = 3.02, df = 5, 20, P = 0.0344*), and numbers of large galls (*F = 3.22, df = 5, 20, P = 0.0272*). Compared with the control, the four chemical treatments reduced the total gall length by 50–60%, total gall weight by 53–66%, and number of large galls by 56–86% (Figure A4.7). There were no significant differences in any of the three gall indices within the four chemical treatments, however, the reduction rate of large galls was noticeably lower in the late spinetoram treatment (Figure A4.7).

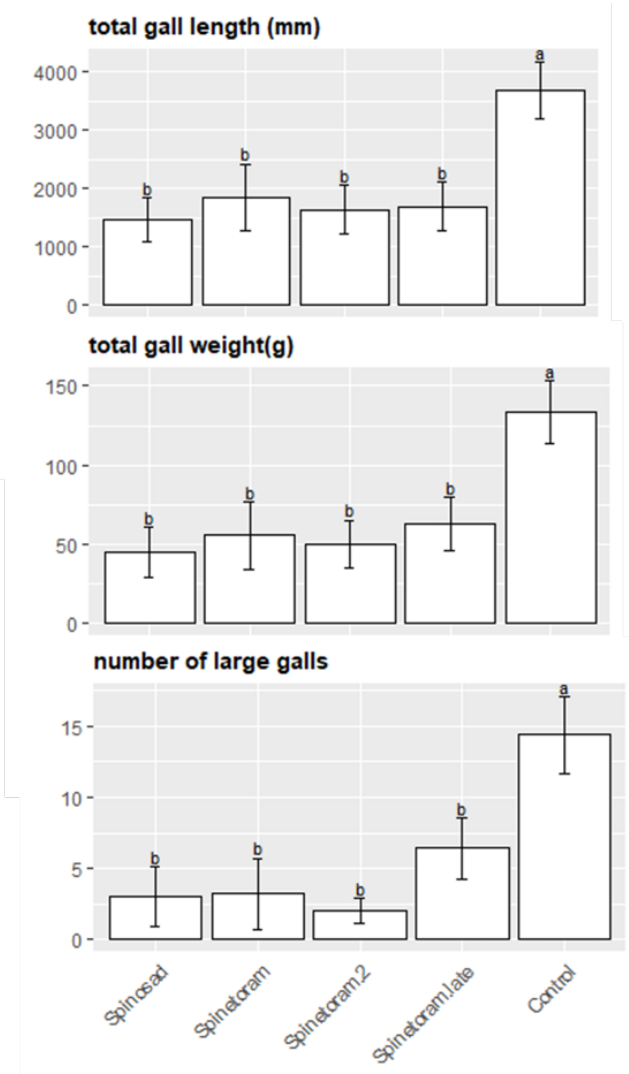

Figure A4.7. Total gall length, total gall weight, and number of large galls (≥ 50 mm long) in new-season galls following the application of spinosad and spinetoram in the previous spring. Wire bars show the standard errors. Bars not labelled with a common letter were significantly different (*P < 0.05*) by Fisher's LSD tests following the detection of significant treatment effects by a general linear model (GLM).

Effect on beneficial arthropods

Petri dish bioassays

Spinetoram was observed to have low acute toxicity to both larval and adult ladybirds (*C. montrouzieri*) at 72 hours. For lacewings (*M. signatus*), spinetoram resulted in low toxicity after 72 hours at the 10% and 50% rates and medium acute

toxicity at the proposed maximum field rate. Spinetoram was shown to be harmful to predatory mites (*N. californicus*), with 100% mortality at 48 hours at the proposed maximum field rate and high mortality (93.3%) at half this rate (Table A4.9).

Table A4.9. Acute toxicity data for beneficial arthropod predators following exposure to freshly dried residue of spinetoram.

Spinetoram	Beneficial arthropod	Product rate (mL/100L)	% Mortality		
			24 hrs	48 hrs	72 hrs
	Ladybird adult	4	10.0	10.0	20.0
		20	3.3	10.0	20.0
		40	3.3	6.7	13.3
	Ladybird larvae	4	0.0	0.0	3.3
		20	0.0	0.0	0.0
		40	3.3	3.3	3.3
	Lacewing	4	3.3	3.3	3.3
		20	3.3	3.3	10.0
		40	3.3	10.0	36.7
	Predatory mite	4	6.7	6.7	-
		20	46.7	93.3	-
		40	53.3	100.0	-

IOBS toxicity category		% Mortality
Low	Green	<30%
Medium	Yellow	30-79%
High	Light red	80-99%
Very High	Dark Red	>99%

Spinosad showed similar results to spinetoram; it was observed to have low acute toxicity to larval and adult ladybirds, as well as lacewings at 72 hours. For predatory mites, spinosad showed high to very high toxicity after 48 hours of exposure, even at 10% of the proposed maximum field rate (Table A4.10).

Table A4.10. Acute toxicity data for beneficial arthropod predators following exposure to freshly dried residue of spinosad.

Spinosad	Beneficial arthropod	Product rate (mL/100L)	% Mortality		
			24 hrs	48 hrs	72 hrs
	Ladybird adult	4	0.0	6.7	10.0
		20	0.0	6.7	10.0
		40	3.3	10.0	13.3
	Ladybird larvae	4	0.0	3.3	3.3
		20	0.0	3.3	6.7
		40	0.0	0.0	3.3
	Lacewing	4	0.0	0.0	3.3
		20	0.0	0.0	3.3
		40	0.0	0.0	0.0
	Predatory mite	4	80.0	93.3	-
		20	65.0	100.0	-
		40	66.7	93.3	-

IOBS toxicity category		% Mortality
Low	Green	<30%
Medium	Yellow	30-79%
High	Light red	80-99%
Very High	Dark Red	>99%

EXP-A was the most toxic product tested in our assays, with 100% mortality observed in predatory mites after just 24 hours of exposure, even at only 10% (5 mL/100 L) of the proposed maximum field rate. High to very high toxicity was also noted in both larval and adult ladybirds at 72 hours across all tested concentrations. However, adult ladybird beetles were generally less susceptible to insecticides than larvae. Notably, EXP-A was the only chemical that resulted in a toxicity rating higher than low for adult ladybirds, showing high acute toxicity even at 10% of the proposed maximum field rate after 72 hours. EXP-A exhibited medium acute toxicity to lacewings at the half (25 mL/100 L) and full (50 mL/100 L) application rates, while low toxicity was observed at 10% of the proposed maximum field rate after 72 hours of exposure (Table A4.11).

Table A4.11. Acute toxicity data for beneficial arthropod predators following exposure to freshly dried residue of EXP-A.

EXP A	Beneficial arthropod	Product rate (mL/100L)	% Mortality		
			24 hrs	48 hrs	72 hrs
	Ladybird adult	5	3.3	16.7	80.0
		25	0.0	63.3	100.0
		50	0.0	76.7	100.0
	Ladybird larvae	5	33.3	100	100.0
		25	80.0	96.7	100.0
		50	100.0	100.0	100.0
	Lacewing	5	0.0	0.0	0.0
		25	25.0	51.7	55.0
		50	26.7	43.3	63.3
	Predatory mite	5	100	100	-
		25	100	100	-
		50	100	100	-

IOBS toxicity category		% Mortality
Low	Green	<30%
Medium	Yellow	30-79%
High	Light red	80-99%
Very High	Dark Red	>99%

DC-154 was observed to have low acute toxicity to predatory mites and adult ladybird beetles at the proposed maximum field rate after 48 hours and 72 hours of exposure, respectively (Table A4.12). However, DC-154 showed high toxicity to lacewings and ladybird beetle larvae at the proposed maximum field rate after 48 hours and 72 hours of exposure, respectively.

Table A4.12. Acute toxicity data for beneficial arthropod predators following exposure to freshly dried residue of DC-154.

DC-154	Beneficial arthropod	Product rate (mL/100L)	% Mortality		
			24 hrs	48 hrs	72 hrs
	Ladybird adult	8	0.0	3.3	3.3
		24	3.3	6.7	20.0
		80	3.3	3.3	16.7
	Ladybird larvae	8	3.3	20.0	23.3
		40	26.7	36.7	66.7
		80	56.7	76.7	90.0
	Lacewing	8	3.3	10.0	20.0
		40	36.7	85.0	91.7
		80	33.3	83.3	90.0
	Predatory mite	8	0.0	6.7	-
		40	0.0	0.0	-
		80	0.0	0.0	-

IOBS toxicity category		% Mortality
Low	Green	<30%
Medium	Yellow	30-79%
High	Light red	80-99%
Very High	Dark Red	>99%

Leaf bioassays

Spinosad, spinetoram and DC-154 were all highly toxic to the *Aphytis* wasp, with a residual toxicity of 100% at 1 DAS and >98% at 7 DAS (Figure A4.8). The effect was highly significant (*P < 0.0000*) for both post-spray dates.

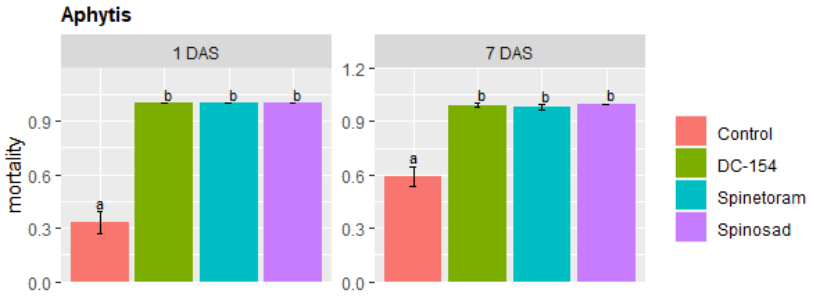

Figure A4.8. Mortality of the *Aphytis* wasp after being exposed to sprayed leaves. Bars not labelled with a common letter were significantly different (*P* < 0.05) by Tukey's multiple comparison tests following the detection of significant treatment effects by a general linear model (GLM) under binomial distribution.

Mortality of the adult ladybird beetles was less than 20%, regardless of treatments. There were noticeable differences in the mortality rates in different treatments at both 1 and 7 DAS (Figure A4.9), however, the differences were not significant (*P > 0.05*).

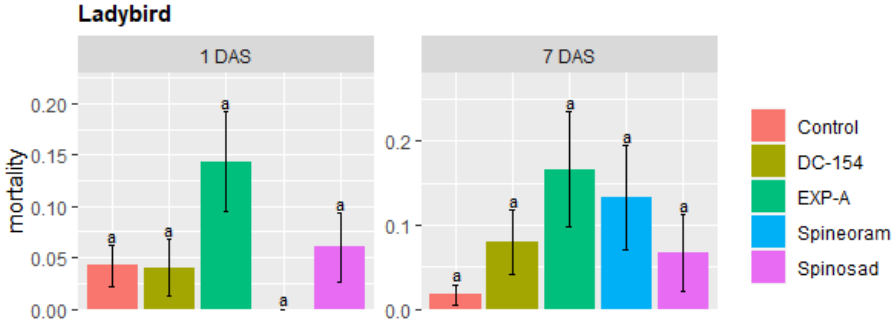

Figure A4.9. Mortality of the ladybird adult after being exposed to sprayed leaves. Bars not labelled with a common letter were significantly different (*P < 0.05*) by Tukey's multiple comparison tests following the detection of significant treatment effects by a general linear model (GLM) under binomial distribution.

DC-154 caused the highest mortality in lacewing larvae at both 1 and 7 DAS (Figure A4.10). However, the mortality rate was not significantly different from that of control at both post-spray dates (*P > 0.05*). Spinosad and spinetoram also cannot be separated from the control in terms of mortality rates in the lacewing larvae. EXP-A can be separated from the untreated control at 1 DAS, however, the separation was due to EXP-A causing higher mortality to the lacewing larvae than the control (Figure A4.10). The anomaly was likely due to random errors associated with small sample sizes.

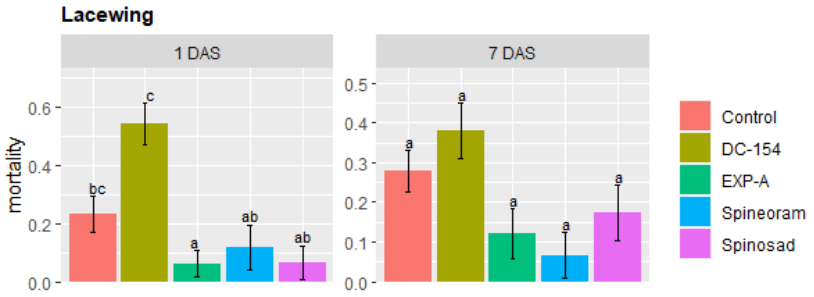

Figure A4.10. Mortality of the lacewing larvae after being exposed to sprayed leaves. Bars not labelled with a common letter were significantly different (*P < 0.05*) by Tukey's multiple comparison tests following the detection of significant treatment effects by a general linear model (GLM) under binomial distribution.

At 1 day after spraying, DC-154 was the only insecticide that showed significantly higher toxicity to predatory mites compared to the untreated control (*P < 0.05*, Figure A4.11). However, by 7 DAS, spinosad and spinetoram but not DC-154 were significantly more toxic to predatory mites than the untreated control (P < 0.05; Figure A4.11).

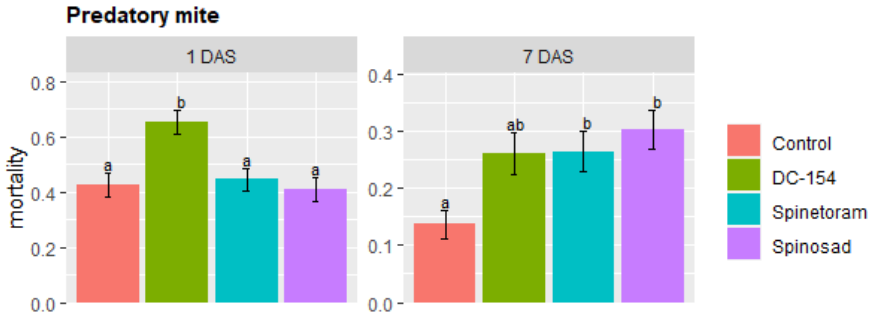

Figure A4.11. Mortality of the predatory mites after being exposed to sprayed leaves. Bars not labelled with a common letter were significantly different (*P < 0.05*) by Tukey's multiple comparison tests following the detection of significant treatment effects by a general linear model (GLM) under binomial distribution.

Discussion

Of the 10 new chemical options screened, spinosad and spinetoram showed the highest toxicity to adult CGW, both via direct contact and indirect contact through sprayed leaves. The two chemicals also demonstrated excellent residual activity, with sprayed leaves remaining toxic to adult CGW 21 days after the spray. Note: the residual activity was estimated in the laboratory and the sprayed trees were kept in a shade-house. The residual activity in the field is likely to be much shorter due to rain or UV.

Spinetoram appeared to be more toxic to the adult CGW than spinosad. The top tested rate for both chemicals was 40 mL/100 L, which is the recommended rate on the labels of registered insecticides containing the two chemicals. This rate was much lower than the required rate for spinosad to kill 90% or more adult CGW in 1 day but was about right for spinetoram to achieve this level of efficacy in 1 day. The required rate for 90% mortality could be greatly reduced for both insecticides if the mortality data were expanded to include those that died after 2–3 days. Considering that adult CGW only lives for about 5 days and that they can mate and lay eggs on the same day (Final report, CT10021), the decision to select an application rate should be based on 1-day mortality rather than 2 or 3-day mortality.

The field trial confirmed the efficacy of spinosad and spinetoram. A single application of either chemical just before the peak emergence of adult CGW reduced the total weight of the next season's galls by over 44%. Treated trees had a similar number of galls as the untreated trees, but the proportion of large galls (length ≥50 mm) was significantly lower. Of the 5 treatments compared, the double-spray treatment of spinetoram achieved the best control of CGW, reducing

the total gall weight by 66% and the proportion of large galls by 84%, compared with the untreated control. While the differences between the single and double-spray treatments of spinetoram in the two indices were not significant, the results suggest that a second spray might be needed for a satisfactory control of CGW with spinetoram. The field trials also highlighted the importance of timing for controlling adult CGW with a contact, non-systemic insecticide. In this trial, the late-application treatment of spinetoram was applied just after the peak adult CGW emergence. This treatment did not reduce next-season galls.

Spinosad, spinetoram, and DC-154 are all highly toxic to the red scale parasitoid *A. lingnanensis*, with a residual toxicity period of at least 7 days. Spinosad and spinetoram generally have low toxic effects on the ladybird, *C. montrouzieri* and the green lacewing *M. signatus*, but they are highly toxic to the predatory mite *N. californicus* at application rates that are potentially relevant to the citrus industry. They also showed significant residual toxicity to the predatory mite 7 days after spray. DC-154 showed a quite different pattern of toxicity. It showed low to moderate acute toxicity to the predatory mite, but high toxicity to ladybird larvae and lacewing larvae. EXP-A showed a range of toxic effects on all species; it resulted in very high toxicity to predatory mites and ladybirds (both life stages) and medium toxicity to lacewings. Given its efficacy against Fuller's rose weevil in a separate study of this project, it appears that EXP-A has a large effect on Coleoptera. Considering that laboratory bioassays of insecticide toxicity often reflect worst-case laboratory conditions, caution should be taken when extending the findings of this study to field conditions. Previous studies have shown that chemicals found to be harmful in the laboratory do not always show harmful toxic effects in semi-field trials (e.g. Candolfi et al. 1999). Direct 'in-field' assessments on beneficial arthropods are thus warranted. Further studies should also consider the potential sub-lethal chemical effects, such as physiological and behavioural effects, that can disrupt beneficial populations (Desneux et al. 2007).

We also investigated three experimental products for controlling CGW larvae in a potted-tree trial. None of the three showed potential for this purpose.

In summary, we searched for new chemical options to manage the citrus gall wasp, with a focus on those that have relatively short withholding periods. Ten unregistered chemicals were screened for controlling the adult gall wasp. Spinosad and spinetoram showed excellent direct and residual contact activity. Spinetoram appears more efficacious against adult wasps than spinosad in terms of residual activity and minimal effective rate. A field trial confirmed the efficacy of the two chemicals. Both spinosad and spinetoram showed low toxicity to predatory ladybird beetles and lacewings but high toxicity to parasitic *Aphytis* and predatory mites. Spinosad and spinetoram complement currently registered chemical options in that they can be used in all citrus varieties, including Valencia, because of their relatively short withholding period. More than one spray might be needed to achieve satisfactory control. Where a single spray is preferred, the spray should be put out just before the peak emergence of the gall wasp to maximise its effect. Caution should be exercised when using the two insecticides in orchards with a history of red scale or mite problems, considering their negative effects on *Aphytis* and predatory mites.

Disclaimer

Spinosad and spinetoram are not yet registered for the control of the citrus gall wasp and so cannot be used on commercial properties for this purpose.

Appendix 5: Optimal timing for applying a non-systemic insecticide to control the citrus gall wasp

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* Published in Crop Protection 174(2023) 106428 https://doi.org/10.1016/j.cropro.2023.106428

Summary

The citrus gall wasp (CGW), *Bruchophagus fellis* (Girault, Hymenoptera: Eurytomidae), is a serious pest of citrus in southern Australia. Severe infestations can result in yield loss and a reduction in fruit size. Several parasitoid species attack CGW, with *Megastigmus brevivalvus* (Girault, Hymenoptera: Torymidae: MBV) being the dominant species. Both CGW and MBV have one generation per year and adult wasps emerge from galls in the spring. The median emergence of MBV lags behind that of CGW by 2–3 weeks. We modelled the effect of spray timing for foliar-applied, non-systemic insecticides on the relative exposures of CGW and MBV based on their emergence distributions and estimated the optimal spray timing that maximises the control of CGW while minimising the effect on MBV. Three temporal emergence distribution scenarios for the two species and 28 periods of residual insecticide activity were considered in the investigation. The results showed that the optimal spray timing was always before the peak emergence of CGW. Depending on the residual activity of the insecticide, the optimal spray date pre-dated the peak emergence of CGW by 1–24 days, with longer lead times for insecticides with longer residual activity. By contrast, the optimal spray date varied little with the different emergence scenarios, suggesting that the results apply over a wide range of locations and seasons. A single insecticide application at the optimal timing might not guarantee satisfactory control of CGW. When the residual activity of the insecticide is short, multiple applications with a combined residual activity of 16 days are needed to ensure insecticide contact with ≥90% of all CGW that emerge in a season.

Introduction

The citrus gall wasp (CGW), *Bruchophagus fellis* (Girault, Hymenoptera: Eurytomidae), is an endemic pest of citrus in Australia (Noble, 1936). Heavily infested trees are covered with galls of various sizes, ranging from less than 0.5 cm to over 50 cm long. Severe infestations can result in yield loss and a reduction in fruit size. CGW was originally confined to central and northern New South Wales (NSW) and central and southern Queensland, but over the last 10 years, it has become a serious pest in southern NSW, north-western Victoria, and north-east South Australia, where most of Australia's export oranges are produced (Citrus Australia, 2023).

Adult CGW emerge from galls in the spring (Mo and Stevens, 2014). They mate immediately and lay eggs in new spring shoots, fruit stems, and leaf petioles (Noble, 1936, 1938; Mo, 2012, 2018). After hatching, the larvae burrow into the soft bark tissue, where they feed in individually constructed cells until pupation. The area of the shoot where feeding larvae are concentrated swells as the season progresses, and eventually, characteristic galls become visible. The lifecycle is completed in one year. Except for the adults, all life stages of CGW are spent inside the galls and are thus protected from predation. The key natural enemies of CGW are the parasitoids *Megastigmus brevivalvus* (Girault, Hymenoptera: Torymidae; MBV) and *M. trisulcus* (Girault; Noble, 1936), with MBV being the dominant species and accounting for over 90% of all parasitism (Mo, 2018).

Biological control is an important component in most successful IPM programs (Ehler, 2006; Zalucki et al. 2009; Colmenarez et al. 2018). Modelling shows that parasitism is the key factor determining the future trends of CGW populations in the southern Australian orange production regions (Mo J, unpublished). After repeated augmentative releases, CGW parasitoids have now been established in most citrus orchards in these regions, but their incidence remains low (< 5% parasitism; Cannard, 2007; Flett, 2011; Mo, 2018). For meaningful biological control in the future, it

is important to conserve these parasitoids, allowing them to increase in numbers and, therefore, have a greater effect on CGW populations. Theoretically, this can be achieved by setting up insecticide-free blocks of citrus and/or the use of selective insecticides that reduce effects on adult parasitoids. However, growers might be reluctant to set up insecticide-free blocks due to concerns of short-term economic losses and the presence of a constant source of CGW reinfestation. Selective insecticides that kill CGW but not its parasitoids are yet to be found. Taking advantage of the fact that adult MBV emerges 2–3 weeks after adult CGW (Mo, 2012), we investigated optimal spray timing for a foliar-applied, non-systemic insecticide that will maximise the control of adult CGW but result in minimal losses of MBV.

Methods

Fifteen sets of CGW and MBV emergence data were used in our study. The data were collected using sticky cup traps (Mo and Stevens, 2021) from commercial orchards of Valencia oranges, navel oranges, lemons, and grapefruit in southern New South Wales and northwest Victoria in 2015 and 2018, and in central Queensland in 2020. The traps were made from clear plastic cups (480 mL capacity, 85 mm diameter at rim) with a thin layer of Tangle-Trap® (The Tanglefoot Company, Grand Rapids, MI, USA) coated on the interior surface. The traps were wrapped around individual galls to capture emerging wasps. Ten to 20 traps were used at each site in each season at a density of 1 trap/tree. Data were collected weekly during the early and later part of the emergence period and twice weekly around the time of peak emergence, starting before any CGW had emerged and finishing after three consecutive weeks of zero captures of either CGW or MBV.

Emergence patterns at individual sites and seasons

For each dataset, calendar dates were converted to days after the winter solstice in the year of collection (Julian date) and the cumulative proportions of the emergence of CGW and MBV were tallied for each date. The cumulative emergence data for each of the two species was then fitted separately to the Weibull distribution function (Equation 1).

$$F(t) = 1 - \exp\left(-(t/\lambda)^k\right) \qquad (1)$$

where λ and k are the scale and shape parameters of the Weibull distribution.

As each parasitised CGW produces a single parasitoid, parasitism rates were estimated from the total numbers of adult CGW and parasitoids that emerged. The estimates might vary slightly from the true parasitism rates considering the likely different juvenile mortalities of the two species, however, they provide the only viable measure of field parasitism rates across sites and seasons.

Pooled distribution of emergence

To remove the effects of site and year, the Julian dates in each dataset were adjusted by subtracting the corresponding median emergence date of CGW estimated from the fitted Weibull parameters. The adjusted emergence data were then combined to get the pooled emergence data for CGW and MBV. Finally, the pooled data for the two species were separately fitted to the Weibull distribution function. An offset of 100 days was added to the adjusted Julian dates before the fitting to prevent negative date values, which are not valid under the Weibull distribution.

Abundance of live individuals

The abundance of live individuals on a given day depends not only on the emergence pattern but also on the life span of emerged individuals. If L is the life span, the number of live individuals on a given day is the sum of individuals who emerged from L-1 days ago to that date. Under the Weibull distribution of emergence (Equation 1), the abundance on day t, $N(t)$, can be estimated as:

$$N(t) = M\{F(t) - F(t - L + 1)\} \qquad (2)$$

where M is the total number of individuals who emerged over the entire season, and $F(t) – F(t-L+1)$ is the proportion of

individuals who emerged between day *t* and day *t-L+1*.

When exposed to a foliar spray of a contact insecticide within its effective period (residual activity), individuals are subjected to an extra level of mortality in addition to that caused by ageing. The exposure window starts at the time of spraying and ends when the insecticide is no longer effective. For the convenience of estimating the proportion of exposed individuals, it is assumed that the mortality caused by the insecticide within its residual period is 100%. Let t_0 be the day of spray application and *r* be the period of the residual activity of the insecticide, then the exposure window is from day t_0 to day $t_0 + r$. The number of live individuals on day *t* after a foliar spray, *S(t)*, is (a) zero when *t* is within the exposure window, (b) the same as *N(t)* in Equation 2 when *t* is either before the day of spray application or *r+L–1* day after the spray, or (c) the sum of individuals emerged after the end of the residual activity when *t* is elsewhere, i.e.,

$$S(t) = \begin{cases} M\{F(t) - F(t-L+1)\}, t < t_0 \text{ or } t > t_0 + r + L - 1 \\ M\{F(t) - F(t_0 + r)\}, \quad t_0 + r < t \le t_0 + r + L - 1 \\ 0, \qquad\qquad t_0 \le t \le t_0 + r \end{cases} \tag{3}$$

The areas under the curves of *N(t)* and *S(t)* are the seasonal totals of live insect days under the no-spray and spray scenarios, respectively. Let A_0 be the total insect days under the no-spray scenario and A_1 the total insect days under the spray scenario, then the proportion of insect days exposed to the spray, P, is

$$P = \frac{A_0 - A_1}{A_0} = 1 - \frac{\int_{T_0}^{T_1} S(t)dt}{\int_{T_0}^{T_1} N(t)dt} \tag{4}$$

where T_0 and T_1 are the start and end, respectively, of the entire season when the insect is present. Note that, unlike *N(t)* and *S(t)*, the exposed proportion is not affected by the total number of individuals who emerged over the entire season, M, which is cancelled out by the division.

Effect of spray timing

CGW and MBV will have different exposures to the spray due to their different emergence patterns and life spans. In this study, we have assumed the two species to have the same susceptibility to any given insecticide, considering both are small chalcidoids of similar sizes. The life span of adult CGW was set at 5 days, according to Noble (1936) and Mo (2012) and that of adult MBV at 4 days, according to Noble (1938).

To determine the optimal spray timing, we estimated the exposed proportions of the two species under a series of combinations of spray timings and durations of residual activity of the insecticide. Spray timing was investigated daily from 28 days before peak emergence of adult CGW to 28 days after peak emergence. The residual activity of the insecticide was investigated in the range of 1 to 28 days after foliar application.

The optimal timing is defined as the time at which the exposed proportion of CGW is the highest and that of MBV is the lowest. To provide a unified measure for comparing different timings, we calculated the effect ratio, ER, for each spray timing,

$$ER = \frac{P_{CGW}}{1 + P_{MBV}} \tag{5}$$

where P_{CGW} and P_{MBV} are the exposed proportions of CGW and MBV, respectively. Note that *ER* ranges from 0 to 1.

P_{CGW}, P_{MBV} and *ER* for all combinations of spray application timing and residual insecticide activity period were estimated for three scenarios of CGW and MBV emergence distributions: scenario 1 – pooled emergence distributions of the two species, scenario 2 – emergence distributions of the two species showing the largest overlap from all datasets, and scenario 3 – emergence distributions of the two species showing the least overlap from all datasets.

Data analysis

All data analyses were performed in R (R Development Core Team, 2019). Weibull fitting was done using the 'nlsLM' function from the 'minpack.lm' package. Integration was done numerically using the 'integrate' function from the 'stats' package. R codes will be supplied upon request.

Results

Emergence patterns at individual sites and seasons

The emergence of adult CGW occurred from late August to early November in central Queensland and from mid-October to early January in southern New South Wales and northwest Victoria, with peak emergence occurring in early October and early to mid-November in the two regions, respectively (Table A5.1). The emergence of adult MBV lagged behind that of CGW by 2–5 weeks in the starting dates and by 2–3 weeks in peak emergence (Table A5.1). However, adult MBV emergence was completed on similar dates, or even earlier, than adult CGW, indicating a shorter emergence period for the parasitoid. Estimated parasitism rates were low (0.1–13.6%) at all sites in all years and, except for one site in New South Wales in 2016–2017, MBV was the dominant parasitoid species, accounting for all the parasitism in 12 datasets and 79–86% of the total parasitism in two datasets (Table A5.1). The only other parasitoid species collected was *M. trisulcus*.

Table A5.1. Emergence timings and parasitism rates at individual sites and seasons.

			Emergence period*		Parasitism (%)	
Site	State	Season	CGW	MBV	MBV	All**
GM	QLD	2020–2021	Sep 17–Nov 06 (Oct 05)	Oct 02–Nov 06 (Oct 19)	10.8	13.6
SB	QLD	2020–2021	Aug 31–Nov 06 (Oct 04)	Oct 05–Nov 06 (Oct 19)	2.8	2.8
BG	NSW	2016–2017	Nov 02–Jan 03 (Nov 18)	Nov 29–Dec 30 (Dec 13)	0.1	0.1
BG	NSW	2017–2018	Oct 17–Dec 19 (Nov 14)	Nov 14–Dec 19 (Nov 21)	1.0	1.0
MS	NSW	2016–2017	Nov 08–Dec 23 (Nov 22)	Dec 02–Dec 23 (Dec 13)	0.5	10.5
MS	NSW	2017–2018	Oct 12–Dec 15 (Nov 14)	Nov 17–Dec 19 (Nov 24)	1.7	1.7
CK	NSW	2015–2016	Oct 22–Dec 17 (Nov 02)	Nov 05–Dec 17 (Nov 19)	0.6	0.7
CK	NSW	2016–2017	Oct 19–Dec 22 (Nov 17)	Nov 14–Dec 22 (Dec 09)	1.5	1.5
CK	NSW	2017–2018	Oct 27–Dec 04 (Nov 09)	Nov 09–Dec 07 (Nov 17)	0.8	0.8
NN	VIC	2015–2016	Oct 22–Dec 17 (Nov 02)	Nov 12–Dec 07 (Nov 19)	0.2	0.2
NN	VIC	2016–2017	Oct 12–Dec 10 (Nov 21)	Nov 09–Dec 03 (Dec 12)	0.8	0.8
NN	VIC	2017–2018	Nov 03–Dec 19 (Nov 09)	Nov 13–Dec 04 (Nov 20)	1.1	1.1
NG	VIC	2015–2016	Oct 12–Dec 10 (Nov 02)	Nov 09–Dec 03 (Nov 19)	0.1	0.1
NG	VIC	2016–2017	Nov 03–Dec 22 (Nov 21)	Nov 18–Dec 22 (Dec 09)	0.2	0.2

NG	VIC	2017–2018	Oct 19–Dec 04 (Nov 13)	Nov 09–Dec 04 (Nov 23)	1.6	1.6

* Dates inside brackets were observed at peak emergence dates. ** includes parasitism by *M. trisulcus.*

The 2-parameter Weibull distribution fitted the cumulative emergence well in all datasets, explaining at least 98% of the variations in the observed data (Table A5.2). There were considerable differences in the estimated values of the two Weibull parameters across the 15 datasets, with the shape parameter (*k*) ranging from 20.33 to 74.21 for CGW and 18.88 to 98.81 for MBV and the scale parameter (*λ*) ranging from 122.33 to 172.33 for CGW and 139.53 to 194.34 for MBV. The differences were reflected in the different emergence patterns of the two species (Figure A5.1). Dataset CK2017 showed the highest overlap of the emergence patterns of the two species, whereas dataset NN2016 showed the least overlap. The shape of the emergence patterns also varied between datasets, ranging from concentrated emergence within a relatively short period to spread out emergence over a longer period. Despite the variations, a clear separation of the emergence peaks of the two species was evident in all 15 datasets.

Table A5.2. Fitted Weibull parameters for the emergence of CGW and MBV at individual sites and seasons

			CGW			MBV		
Site	State	Season	*k*	*λ*	R^2	*k*	*Λ*	R^2
GM	QLD	2020–2021	37.88	126.20	0.99	*50.44*	*139.53*	*>0.99*
SB	QLD	2020–2021	20.33	122.33	>0.99	*22.39*	*142.50*	*>0.99*
BG	NSW	2016–2017	43.66	170.84	>0.99	*47.59*	*194.34*	*>0.99*
BG	NSW	2017–2018	47.46	161.14	>0.99	*18.88*	*181.68*	*0.99*
MS	NSW	2016–2017	46.24	172.33	>0.99	*38.15*	*195.34*	*>0.99*
MS	NSW	2017–2018	67.88	163.88	>0.99	*19.04*	*184.95*	*0.98*
CK	NSW	2015–2016	35.89	156.00	=0.99	*30.11*	*173.46*	*>0.99*
CK	NSW	2016–2017	45.06	171.72	>0.99	*54.78*	*192.08*	*>0.99*
CK	NSW	2017–2018	28.96	159.50	>0.99	*31.65*	*173.60*	*0.99*
NN	VIC	2015–2016	36.72	155.21	0.98	*98.81*	*169.78*	*>0.99*
NN	VIC	2016–2017	74.21	171.71	>0.99	*98.36*	*191.97*	*>0.99*
NN	VIC	2017–2018	53.46	161.59	>0.99	*46.93*	*176.38*	*>0.99*
NG	VIC	2015–2016	38.60	151.08	>0.99	*52.86*	*168.98*	*>0.99*
NG	VIC	2016–2017	45.52	169.80	>0.99	*28.23*	*191.26*	*>0.99*
NG	VIC	2017–2018	23.35	160.56	0.98	*36.39*	*176.21*	*0.99*

** k* and *λ* are shape and scale parameters of the Weibull distribution function, respectively. R^2 is the proportion of variations in cumulative emergence that can be accounted for by the fitted Weibull distribution function.

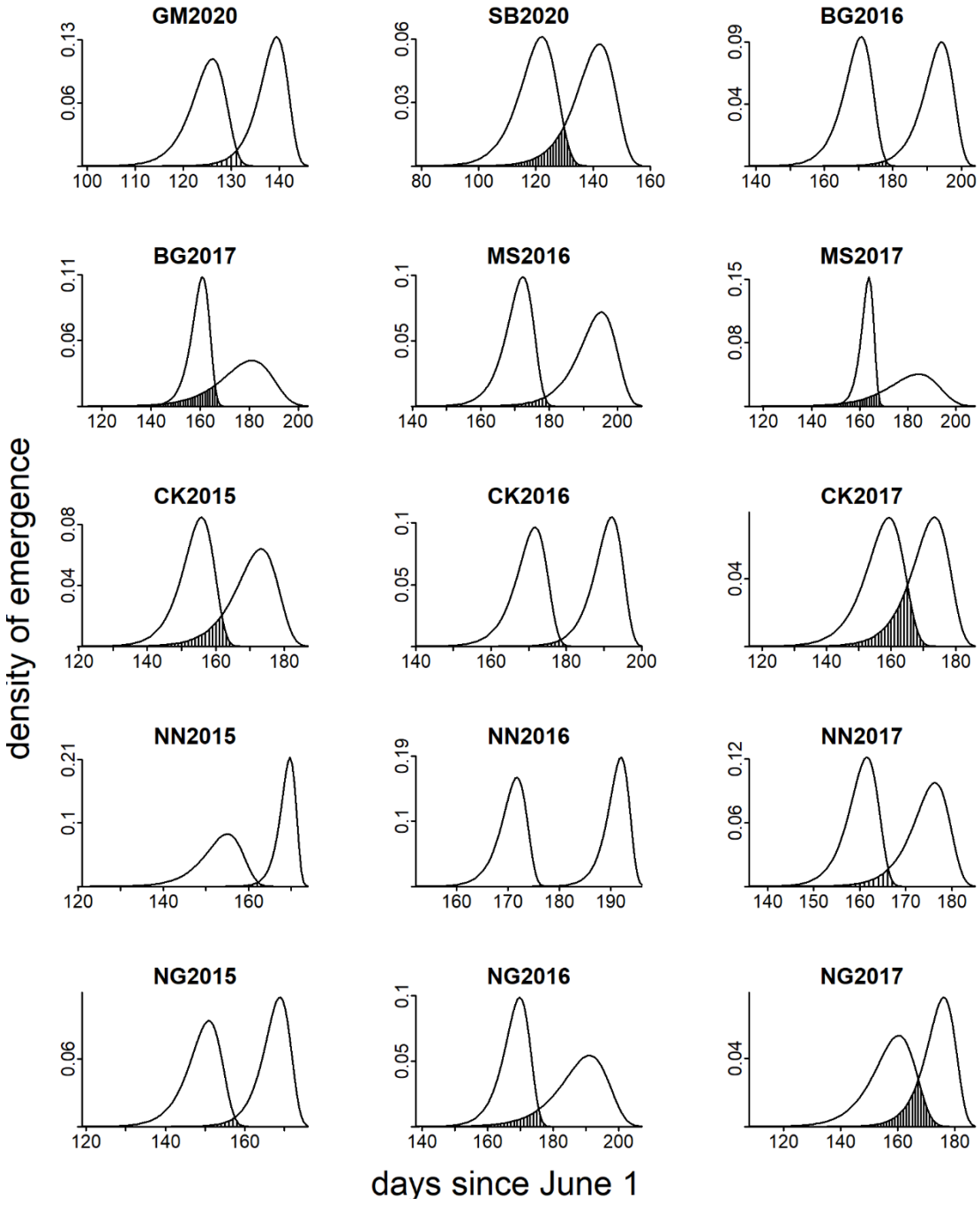

Figure A5.1. Emergence density of CGW and MBV at individual sites and seasons as shown by their respective Weibull density distributions. Hatched areas mark the overlapping areas under the two density curves. In all cases, the earlier peak represents CGW and the later one is MBV.

Pooled distributions of emergence

The Weibull distribution function fitted the pooled data well for both species, explaining 99% and 95% of the variations in the cumulative emergence of CGW and MBV, respectively (Figure A5.2). The estimated parameters and their corresponding 95% confidence intervals are k = 22.13 (21.19–23.13) and λ = 102.10 (101.93–102.27) for CGW, and k = 20.20 (18.33–22.36) and λ = 120.49 (119.98–121.01) for MBV. The estimated median emergence date of MBV lagged behind that of CGW by 18 days.

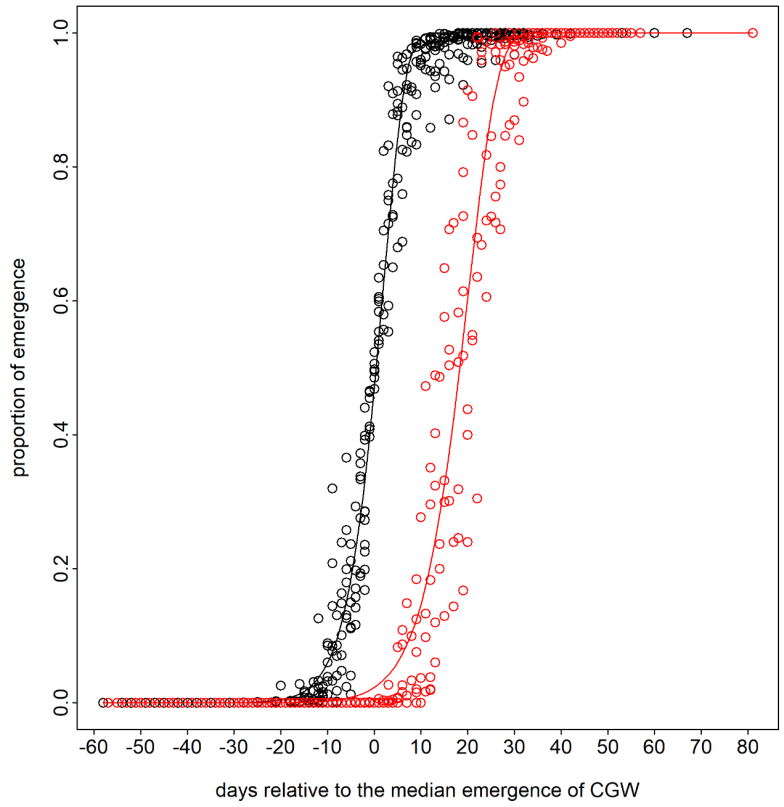

Figure A5.2. Pooled cumulative emergence of CGW and MBV. Black lines and circles show the fitted and observed values for CGW and red lines and circles show the fitted and observed values for MBV.

Optimal spray timing

Figure A5.3 illustrates the effect of spray timing on the abundance of CGW and MBV for an insecticide with a 7-d residual activity under the pooled Weibull distributions of the two species. To highlight the effect on the abundance of both species, the total number of emerged individuals over the entire season was fixed at 100 for each species. As expected, locations of the exposed sections of CGW (black hatched area) and MBV (red hatched area) move with spray timing, from only covering individuals that emerged early to only covering individuals that emerged late. The size of the exposed proportion of CGW increases with spray timing to a maximal value of 0.59 and then decreases, whereas that of MBV increases steadily with spray timing, reaching a maximal value of 0.34 at the last spray timing investigated. According to the effect ratio (ER), the best of the five candidate spray dates investigated is 7 days before the peak emergence of CGW (7 DPPC), the same as that for the maximal CGW exposure. If a 7-d residual insecticide is applied at this time, then 59% of CGW but only 4% of MBV will be exposed.

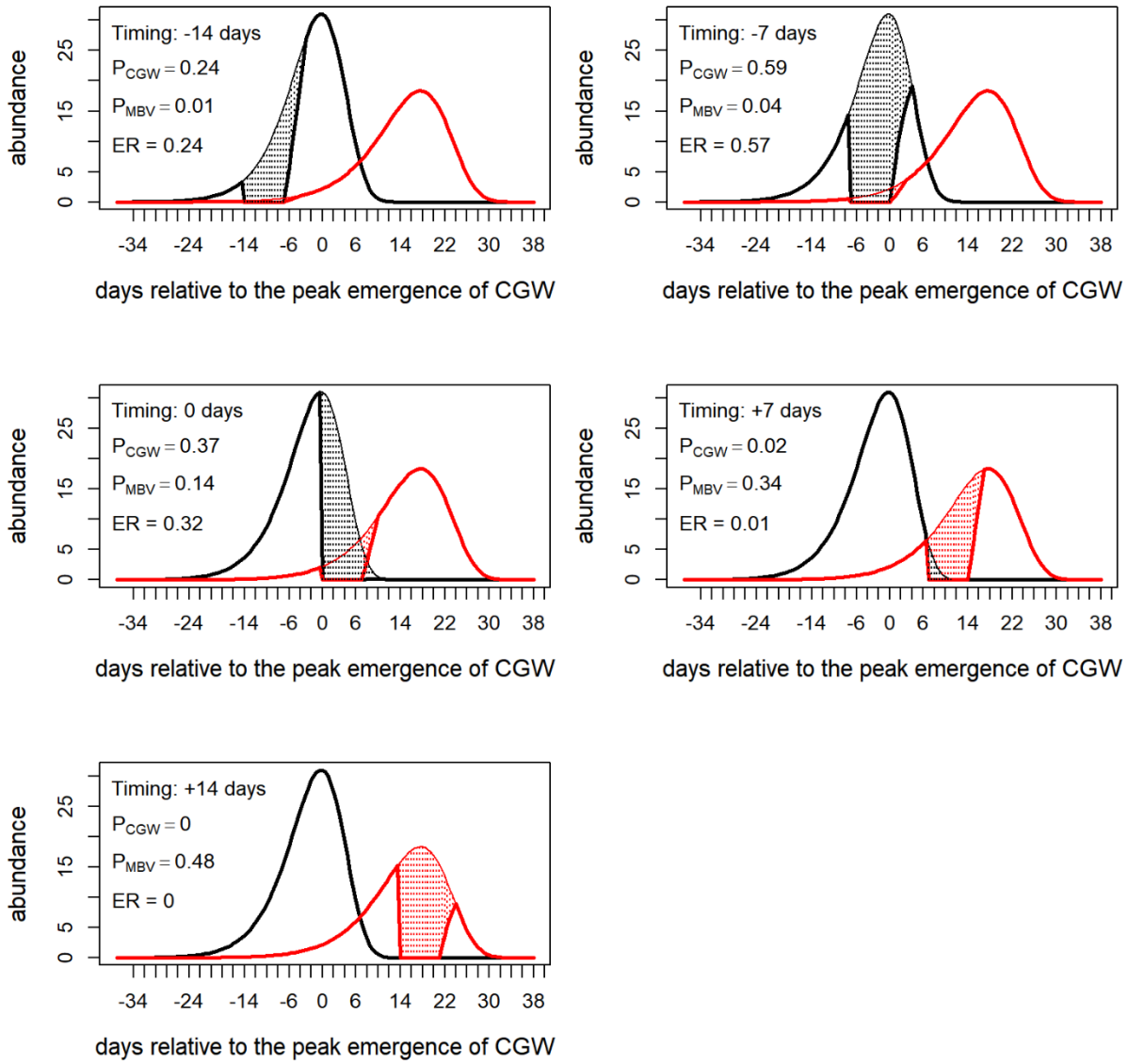

Figure A5.3. Predicted patterns of seasonal abundance of adult CGW (thick black lines) and adult MBV (thick red lines) when a non-systemic insecticide with a 7-day residual activity is applied at five different timings, assuming the emergence of adult CGW and MBV follows the Weibull distributions estimated from the pooled data, and the total number of emerged individuals is 100 for both species. Hatched areas under the abundance curves highlight the spray-exposed sections of CGW (black hatching) and MBV populations (red hatching). PCGW and PMBV are the exposed proportions of CGW and MBV, and ER is the effect ratio.

Optimal spray timings for all durations of residual activity and emergence distributions are shown in Figure A5.4A. Regardless of the emergence distribution scenario, the optimal spray timing becomes progressively earlier from 1 to 24 DPPC as the residual activity of the insecticide increases from 1 to 28 days. There is a maximal 1-day variation in the optimal spray timing among the three emergence distribution scenarios when the residual activity is less than 11 days. Afterwards, the optimal spray timings for all three emergence distribution scenarios converge to the same values.

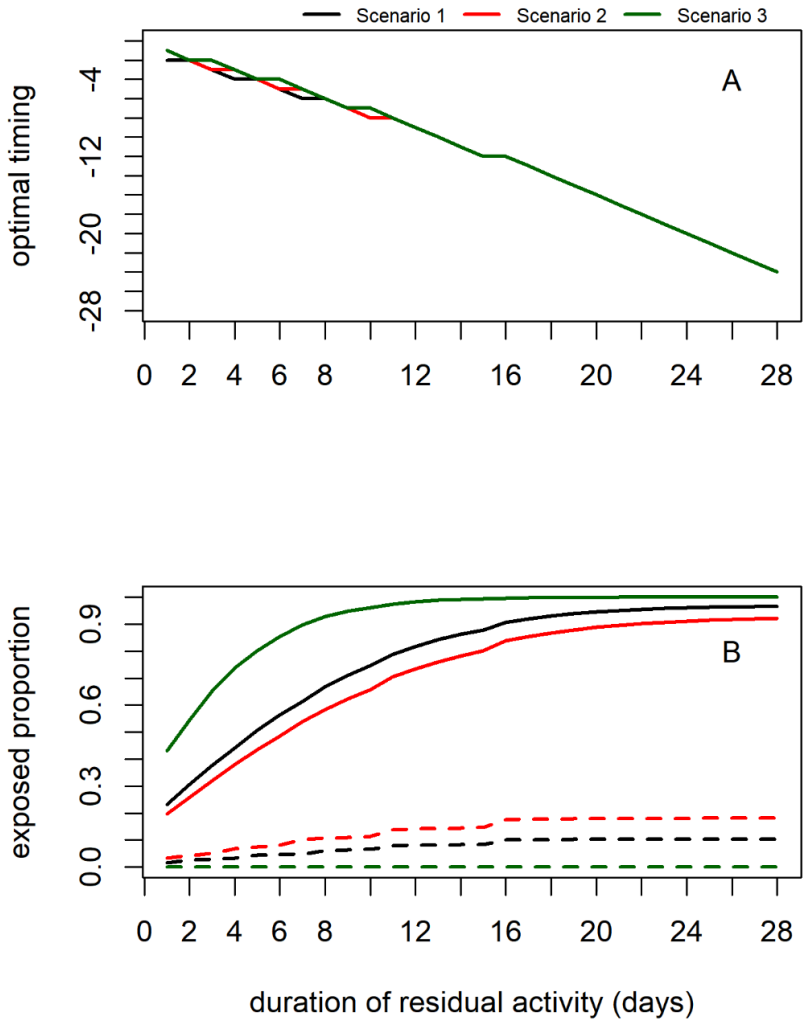

Figure A5.4. (A) Optimal spray timings in days before the peak emergence of CGW and (B) the corresponding exposed proportions of CGW and MBV for a non-systemic insecticide with a residual activity of 1 to 28 days under three emergence distribution scenarios of the two species when that insecticide is applied at its optimal timing. Scenario 1: pooled distributions of the emergence of CGW and MBV; Scenario 2: emergence distributions of CGW and MBV shown by the dataset with the highest overlap; Scenario 3: emergence distributions of CGW and MBV shown by the dataset with the least overlap.

When the insecticide is applied at the optimal spray timing, the proportion of exposed CGW in a season is expected to increase asymptotically to a maximum of 0.92–1.00 with increasing residual activity of the insecticide (Figure A5.4B, solid lines). For the same residual activity, the exposed proportion is highest under scenario 3 and lowest under scenario 2. Differences in the exposed proportion among the three emergence distribution scenarios are higher when residual activity is relatively short than when it is relatively long. The largest difference is seen when the residual activity is 6 days, at which point the exposed proportion for CGW under scenario 3 almost doubles that under scenario 2. Thereafter, the difference gradually decreases, approaching a constant value of 0.07 at the residual activity of 28. The expected exposed proportion of CGW is less than 0.38, 0.32, and 0.66 under scenarios 1, 2 and 3, respectively, when the residual activity is 3 days or shorter, and less than 0.62, 0.54, 0.90, respectively, under the three scenarios when the residual activity is 7 days or shorter.

The corresponding exposed proportion of MBV also increases with increasing residual activity of the insecticide, but

at much lower values than the exposed proportion of CGW, regardless of emergence distribution scenarios (Figure A5.4B, dashed lines). For the same residual activity, the exposed proportion is highest under scenario 2 and lowest under scenario 3. The difference in the exposed proportion among the three emergence distribution scenarios increases with increasing residual activity, reaching a maximum of 0.18 at the residual activity of 28 days. Under all three emergence distribution scenarios, the exposed proportion of MBV is expected to be less than 0.1 when the residual activity is less than 16 days and less than 0.2 at all other durations of residual activities investigated.

Discussion

Spray timing for agricultural insect pests is mostly based on either the phenology of the target pest (Rice et al. 1984; Reissig et al. 1985, 1998; Kudon et al. 1988; Ghidiu and Zehnder et al. 1993; Ahmad, 1995; Fettig et al. 2000; Reddy and Guerrero, 2001) or the host crop (Javid, 1990; Hanula et al. 2002; Myers et al. 2005; Aghaee et al. 2019). The negative effect of insecticides on natural enemies is well known, but rarely considered explicitly in studies of spray timing. In this study, we report our modelling of optimal spray timing for a foliar-applied, non-systemic insecticide for controlling adult CGW based on the phenology of both CGW and its primary parasitoid, MBV. We defined our optimal timing as the one that maximises the control of CGW and minimises the effect on MBV.

Persistence of non-systemic insecticides used in horticultural crops varies from less than 1 day to over 50 days (Fantke et al. 2014), however, most studies reported a maximum residual activity of less than 30 days (Veierov et al. 1988; Ripley et al. 2001; Sharma et al. 2007; Brévault et al. 2009; Chauhan et al. 2012; Visnupriya and Muthukrishnan, 2019). Specific information on the persistence of insecticides currently used against CGW is not publicly available, so to reflect the majority of findings, we chose 1–28 days to represent a broad range of insecticide residual activities. The maximum residual activity investigated here is longer than the reported period for 5–95% emergence of adult CGW in a season (Mo and Stevens, 2021). Although the entire emergence period can be much longer (Table A5.1), emergence outside the 5–95% window is sporadic. There is no need to investigate optimal spray timing for an insecticide when the activity of the insecticide persists for much longer than the period when most CGW emergence occurs because, in this situation, the optimal spray timing would always be sometime before the target pest has started to emerge.

Our study shows that the optimal spray timing is always before the peak emergence date of CGW, regardless of variations in emergence distributions and the residual activity of the insecticide. This is not surprising, as spraying after the peak date is likely to miss more adult CGW than spraying before the peak. Ruppel (1984) modelled the spray timing for a hypothetical insect population and found that the optimal spray date was when the insect population was starting its rapid increase, which occurs before the population peak. We found that the optimal spray date predates the peak emergence date of CGW by 1–24 days, with longer leading days for insecticides with longer residual activity. The number of leading days is equivalent to 0–4 days less than the duration of the residual activity. For the same residual activity, the optimal spray date varies by no more than 1 day among the three emergence scenarios, suggesting that the results are applicable over a wide range of sites and seasons.

A single spray at the optimal timing might not guarantee satisfactory control of CGW, especially if the residual activity of the insecticide is relatively short. To cover 90% or more of all emerged CGW in a season by a single spray, the insecticide needs to be effective for 16, 20, and 8 days under the emergence distribution scenarios 1, 2 and 3, respectively. Scenarios 1 and 2 represent emergence distributions of CGW and MBV with some level of overlap, which was found true for the emergence distributions of the two species at most sites in most seasons (Figure A5.1). Hence, the minimal required residual activity of the insecticide to achieve 90% or more coverage of CGW populations is closer to 16 days. If an insecticide with this level of residual activity cannot be found, then multiple spray applications will be needed.

There are many ways to compare the desirability of different spray timings. In this study, we used an index based on the ratio of proportions of CGW and MBV exposed to the insecticide for the comparison and selected the timing that produced the highest value of the index as the optimal timing. The optimal spray timing based on the index is influenced

more by CGW exposure than MBV exposure. Such a bias is desirable as the focus of the spray application is to reduce the pest population. To add weight to the influence of MBV exposure, we can adjust Equation 5 by adding a multiplier that is greater than 1 to the exposed proportion of MBV. It can be demonstrated that as the value of the multiplier increases, the optimal spray timing would be found gradually earlier, with greater shifts when the emergence distributions of CGW and MBV have a greater overlap. As a result of increasing the weight of the MBV exposure in the index formula, the optimal spray timing becomes more sensitive to the negative effect of the insecticide on MBV, and earlier spray timing would reduce that effect.

Although no foliar-applied insecticides are registered for use against the CGW, a research project is underway to find such insecticides (Mo J, unpublished). Some registered, foliar-applied insecticides in citrus have demonstrated effects against CGW (personal communication, M Wallace, Citri Care, October 2018). The results of our study can be used to develop a timing guide to help growers optimise their use of foliar-applied, non-systemic insecticides to control CGW and, at the same time, protect its primary parasitoid. The peak emergence date of CGW, which is used as the reference point for optimal spray timing, can be easily predicted using an online tool developed by the authors (https://citrusgallwasp.shinyapps.io/predict/). The methodology described in this study can be easily modified to investigate spray timing in other host-parasitoid or host-predator systems, where the host and its natural enemies emerge or arrive over defined periods with asynchronous emergence/arrival peaks.

Appendix 6: New chemical options for managing the Fuller's rose weevil

Dr Jianhua Mo[1], Dr Lisa Kirkland[2], Dr Kathy Overton[2], and Mr Scott Munro[1]

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Summary

In this study, we screened nine new chemicals for FRW control. EXP-A and Indoxacarb were the only two chemicals showing potential for FRW control. Both have shown the potential for reducing FRW egg contamination in citrus fruit, which is a key measure of a successful FRW control strategy. If EXP-A is to be pursued for registration, we recommend it be further investigated for field efficacy and field effects on beneficial arthropods in citrus. Indoxacarb is also a worthwhile candidate for consideration of registration for FRW control when its MRL data become available in Australia's export markets.

Introduction

A major challenge for the Australian Citrus Industry is the growing concern about the potential loss of chemicals currently registered for pest control. Of particular concern are the potential losses of chlorpyrifos and neonicotinoids. Both are currently registered for the control of the Fuller's rose weevil (FRW). For sustainable management of FRW, alternative chemical options are needed.

Here we report our investigation of new chemical options for FRW, including control products and products that are registered for FRW control in other crops but not in citrus. We investigated their efficacy, minimal application rates and field efficacy.

Materials and Methods

Chemicals

Following a literature review and subsequent consultations with chemical companies and Hort Innovation Australia, 9 unregistered chemicals were selected for the investigation.

Table A6.1. Insecticides and rates tested for FRW control

Product	Active	Rate
Entrust Organic	240g/L spinosad	40 mL/100 L
Success Neo	120g/L spinetoram	40 mL/100 L
Avatar eVo	303g/kg Indoxacarb	37.5g/100 L
Exp-A	Confidential	DC formulation: 50 mL/100 L, SC formulation: 5 mL/100 L
Teppan 50SL	50 g/L cyclaniliprole	80 mL/100 L
DC-154	Confidential	80 mL/100 L
DC-196	Confidential	15 mL/100 L
DC-195	Confidential	21 mL/100 L
Exirel (Positive control)	100 g/L cyantraniliprole	75 mL/100 L
Control	Water only	N/A

Spinosad and spinetoram are derived from a group of naturally occurring chemicals called spinosyns, which originated from a soil-inhibiting microorganism (*Saccharopolyspora spinosa*; Sparks et al. 2021). Spinosad is a mixture of naturally occurring spinosyns, and spinetoram is a semi-synthetic spinosyn product. Both are widely used in the control of agricultural pests, including the citrus leafminer (*Phyllocnistis citrella*) and the light brown apple moth (*Epiphyas postvittana*). Indoxacarb is an oxadiazine insecticide originally developed for controlling lepidopteran pests. It was considered one of the safest for beneficial arthropods in citrus in the US (Michaud and Grant, 2003). Indoxacarb is not currently registered in citrus in Australia. Cyclaniliprole is a novel diamide insecticide. It is currently registered in Australia for controlling coddling moth (*Cydia pomonella*) in apples. DC-154, DC-195, DC-196, and EXP-A are experimental products. Exirel® is a registered product for FRW control and it was included as a positive control.

Test weevils

Test weevils were sourced from citrus orchards in Leeton, NSW, and maintained in ventilated containers with fresh citrus leaves at room temperature and natural photoperiod until the commencement of the bioassay.

Screening

EXP-A was screened using the DC formulation at 50 mL/100 L. The other candidate chemicals were screened at the label/recommended rates given in Table A6.1 in the laboratory to identify promising chemicals. Direct contact activity of the chemicals was investigated by a Potter Tower bioassay and indirect (residual) contact activity by a leaf bioassay.

Direct contact activity

The bioassay was designed as completely randomised blocks with 5 replicates and conducted using a Potter Tower, calibrated to deliver a spray deposit of 2 mg/cm^2, equivalent to a spray rate of 200 L/ha.

Test weevils were cooled at 4°C for 10 minutes before treatment to ease handling. Treatments were applied by spraying 10 weevils dorsally at a time. Treated weevils were immediately transferred to a plastic container (280 mL) with ventilation holes in the lid, along with a fresh orange leaf. Test containers were maintained at ambient room temperature and humidity (21 ± 2°C, 55 ± 10% rH) under a natural photoperiod for the duration of the bioassay. Orange leaves were replaced every 1–2 days with fresh leaves.

At 1, 3, 7, and 14 days after treatment (DAT), the number of alive, dead, and incapacitated weevils were recorded. The number of egg batches present and any obvious cessation of feeding (noted by whether the leaf had no chewing damage) was also recorded. Given that this species 'plays dead', incapacitated individuals (with inhibited movement) were identified by poking the ventral surface with the stem of a leaf (or forceps); if the weevil climbed onto the leaf and could move in a coordinated fashion, it was deemed to be 'alive', and if not, it was deemed 'incapacitated'. After the final assessment (14–DAT), the frass in each container was weighed to determine if any treatments had resulted in differences in feeding behaviours with respect to the water control.

Indirect contact activity

All treatments in Table A6.1, except cyantraniliprole, were used in the bioassay. Treatment effects were assessed at 0, 3, 7, 14, and 21 days after spray (DAS), each in a separate sub-bioassay. All sub-bioassays were designed as randomised blocks with 5 replicates (blocks) and 10 weevils per replicate per treatment.

Potted orange trees (age > 20 years, variety: 'Late Lane') were sprayed to run-off with a handheld sprayer. Sprayed trees were left in a glasshouse with temperatures set at 25°C during the day and 15°C during the night and natural light only.

On 0, 3, 7, 14, and 21 DAS, leaves from treated trees were placed individually in 100 mL clear plastic containers with perforated lids. Ten test weevils were introduced to each container. An untreated leaf was added to each container daily. Untreated leaves added on the previous day were removed, but the treated leaves were left inside the containers. The containers were left in the lab (22–25°C).

Mortality of the test weevils was checked daily in the first 3 days and again on the 7th day after the weevils were first exposed to the treated leaves. In the final check, the number of FRW eggs and frass weight (g) in each container were also recorded.

Minimal effective rates

Screening bioassays identified EXP-A and indoxacarb as promising new chemical options for FRW control. Considering that there are no MRLs in the export markets of Australian oranges, only EXP-A was investigated for minimal effective rates. The rate tests were conducted using the DC formulation at 50, 25, 12.5, 6.25, 3.13, and 1.56 mL/100 L.

Direct contact activity

Treatments were applied by spraying 10 weevils dorsally, one at a time, with five replicates per treatment, using a Potter Tower at the deposit density of 2 mg/cm^2. Treated weevils were immediately transferred to a clean plastic container (280 mL) with ventilation holes in the lid, along with a fresh orange leaf. At 1, 3, 7, and 14-DAT (days after treatment), the number of alive, dead, and incapacitated weevils were recorded. The number of egg batches present and any signs of feeding (noted by whether the leaf had chewing damage) were also recorded. Given that this species 'plays dead', incapacitated individuals (with inhibited movement) were identified by poking the ventral surface with the stem of a leaf (or forceps); if the weevil climbed onto the leaf and could move in a coordinated fashion, it was deemed to be 'alive', and if not, it was deemed 'incapacitated'. After the final assessment (14-DAT), the frass in each container was weighed to determine if any treatments had resulted in differences in feeding behaviours with respect to the water control.

Indirect contact activity

The chemical was mixed with Agral® at 25 mL/100 L and sprayed to run-off (about 6 L/tree) to 2-m tall navel orange trees at the Yanco Agricultural Institute, Yanco, NSW, using a battery-powered backpack sprayer. Each rate was sprayed to a separate tree with a 2-tree buffer between neighbouring test trees.

After the spray droplets had dried up, mature leaves were collected from the sprayed trees and placed inside 100-mL clear plastic cups with lids with air holes. One leaf and 10 weevils were placed in each cup. The cups were placed on the bench of a laboratory with temperature control (22–25°C). Test weevils were checked for survival and incapacitation at 1, 3, 7, and 14 days after their exposure to sprayed leaves. Incapacitated but alive individuals were identified by their movement after being poked with the tip of a pair of tweezers. On each assessment date, sprayed leaves in the cups were replaced with freshly collected, treated leaves of the same rate treatment. On the final assessment date, the number of FRW eggs in each cup was counted and the frass in each cup weighed.

Field efficacy

The SC formulation of EXP-A was investigated for FRW control in a field trial in Leeton during April–May 2023. Exirel® (100 g/L cyantraniliprole) and a water-only treatment (Control) were included for comparison. EXP-A (SC) was applied at the recommended rate of 5 mL/100 L plus Agral® at 10 mL/100 L and Exirel® at its label rate of 75 mL/100 L. The application was made using a Ute-mounted boom sprayer at 6 L/tree.

The trial was designed as completely randomised blocks with five replicates for each treatment. A plot consisted of a rectangular area of three rows by four trees. Neighbouring plots were separated by at least one buffer row and two buffer trees within the row.

FRW infestation was assessed by Tedders traps, branch shaking, and fruit check. Tedder traps and branch shaking data were collected at 1-d before and 7, 14, and 21-day after the spray (DAS). One Tedders trap was placed between the 2 central trees in each plot. Two branch shakes were made on each monitoring occasion in the 2 central trees in each

plot. Fruit checks were conducted at 7-day before and 28-d post-treatment application. On each monitoring occasion, 25 random fruits from each of the two central trees in each plot were checked for FRW egg contamination.

Statistical analysis

Data were analysed by general linear models (GLM) followed by analysis of variance (ANOVA; Venables and Ripley 2002). Confounding effects of factors other than the treatment were removed before estimating the treatment effect. Proportional data (mortality, proportion of large galls) were analysed using the Binomial distribution with a logit link. Where a significant treatment effect was detected by ANOVA, treatment means were separated by Tukey's multiple comparison test or Fisher's LSD test (Steel and Dickey 1997).

All statistical analyses were performed in R (R Core Team 2012).

Results

Screening

Direct contact activity

Indoxacarb and Exp-A showed promising efficacy against FRW (Figure A6.1). Indoxacarb caused weevils to become incapacitated from day 1. After 3-DAT, some incapacitated individuals recovered while others died, therefore explaining why mortality decreased over time. However, despite the reduction in mortality, it is likely that incapacitated individuals in the field would have died due to exposure or predation. Exp-A showed efficacy from 3-DAT onwards, tended to cause death rather than incapacitation, and reached 98% mortality by 14-DAT (i.e., 1 weevil out of 50 survived).

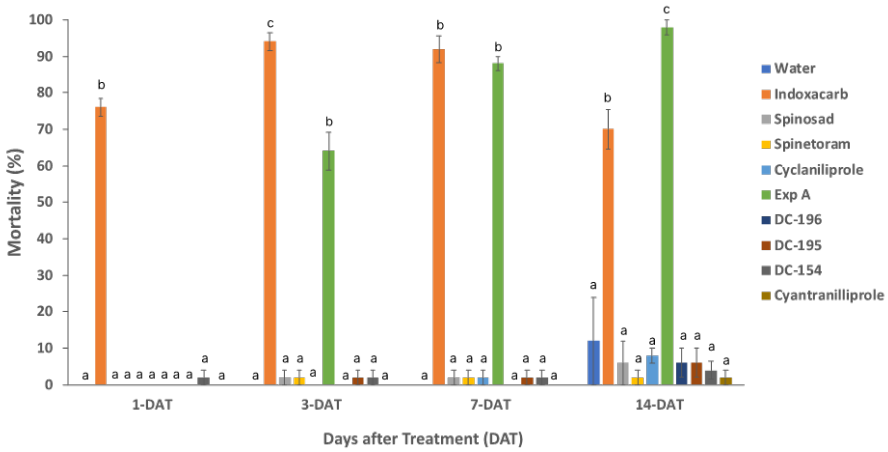

Figure A6.1. Mean Fuller's rose weevil mortality (% ± SE) at 1, 3, 7, and 14 days after treatment (DAT) applied via direct contact sprays. Letters above bars within the same time denote significant differences detected at α = 0.05 (Tukey's post-hoc tests).

Indoxacarb did not prevent chewing damage to leaves except for when all weevils were incapacitated at each given sampling time. Frass weights for indoxacarb-treated weevils were similar to the water control (Figure A6.2, top). Similarly to Exp-A, the average number of egg batches laid by indoxacarb-treated weevils was low relative to the water control (Figure A6.2, bottom). Leaves exposed to weevils treated with Exp-A had almost no chewing damage from 3-DAT onwards. The average frass weight for Exp-A treated weevils was the lowest of all treatments (Figure A6.2, top). The average number of egg batches laid by weevils treated with Exp-A was also substantially lower than the water control in addition to all other treatments (Figure A6.2, bottom), reflecting the high level of mortality observed in this treatment.

Indirect contact activity

Exp-A was the only treatment causing significant mortality in test weevils after seven days of exposure, killing 100% of test weevils at all five post-spray dates (Figure A6.3). Indoxacarb also killed significantly more weevils than the untreated control at 21 DAS, but the mortality rate was only 30%. The other tested treatments showed no effects on weevil mortality.

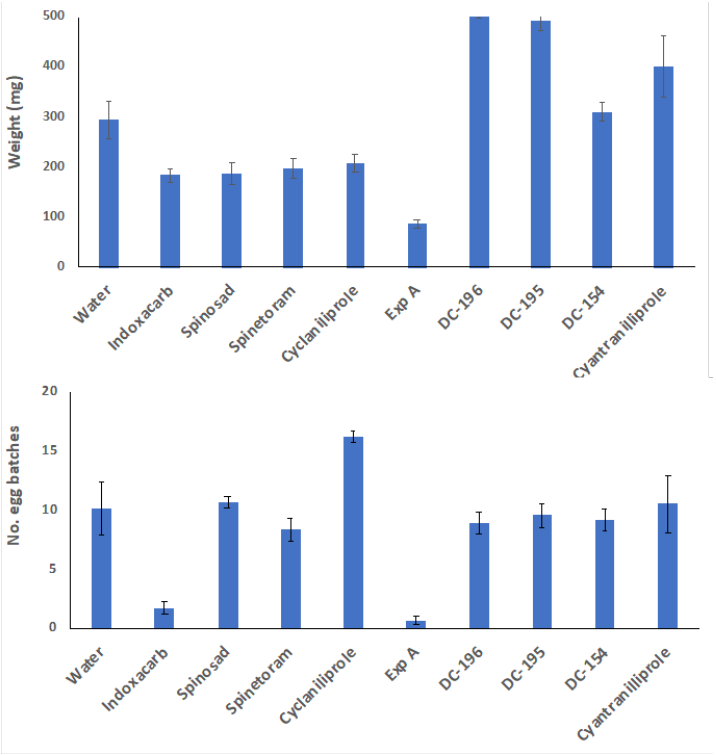

Figure A6.2. Mean frass weights by treatment (top) and mean number of FRW egg batches at 14 days after treatment (bottom).

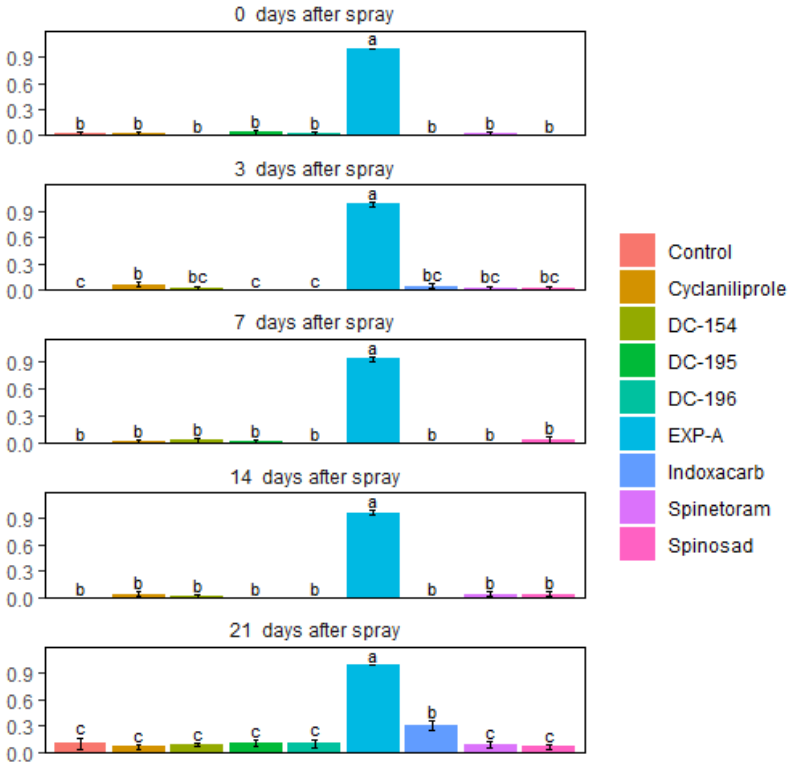

Figure A6.3. Mortality of test weevils exposed to treated leaves collected at 0, 3, 7, 14, and 21 days after the spray (DAS; mean± SE). Bars not sharing a common letter are significantly different at P = 0.05.

The total weight (g) of frass produced by test weevils at 7 DAS was significantly lower in Exp-A and indoxacarb than in the other treatments (*P < 0.05*; Figure A6.4. The reduction rate relative to the control exceeded 95% for Exp-A and 87% for indoxacarb at all five post-spray dates. Moderate but significant reduction of frass weight relative to the control was also observed in cyclaniliprole at all five post-spray dates, DC-154 at 3, 14, and 21 DAS, and DC-196 at 21 DAS.

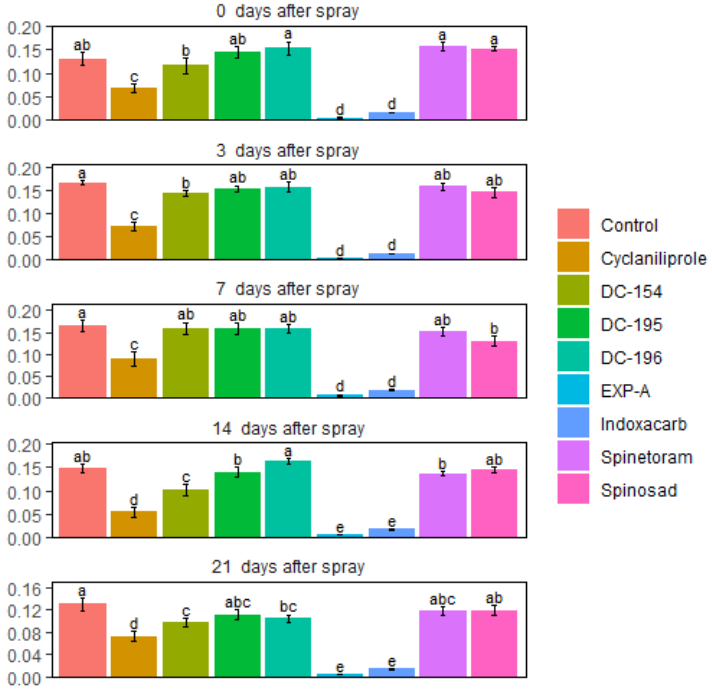

Figure A6.4. Total frass weight (g) of weevils exposed to treated leaves collected at 0, 3, 7, 14, and 21 days after the spray (DAS; mean± SE). Bars not sharing a common letter are significantly different at P = 0.05.

Similar patterns were seen in the number of eggs laid by test weevils, with an almost complete cessation of oviposition in Exp-A and indoxacarb-treated weevils (Figure A6.5). Moderate but significant reduction of eggs relative to the control was also observed in cyclaniliprole at 3, 7 and 14 DAS and DC-154 at 14 and 21 DAS.

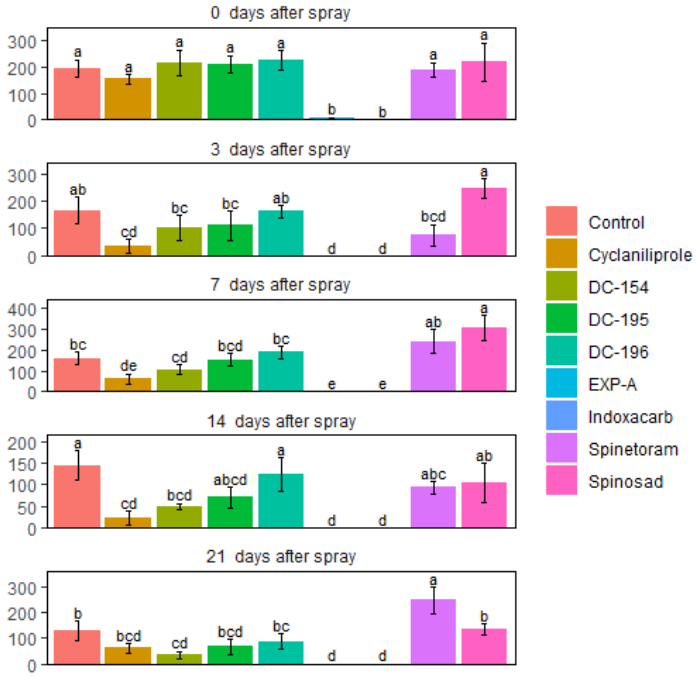

Figure A6.5. Total eggs laid by weevils exposed to treated leaves collected at 0, 3, 7, 14, and 21 days after the spray (DAS; mean± SE). Bars not sharing a common letter are significantly different at P = 0.05.

Minimal effective rate

Direct contact activity

Exp-A showed a range of efficacy levels, correlating with the rate tested (Figure A6.6). At 1-DAT, only the highest rate (50 mL/100 L) had caused >50% mortality, however, by 3-DAT, the half rate of 25 mL/100 L had led to 90% mortality, and 50 mL/100 L caused 98% mortality. By 7-DAT, near complete control was achieved for rates of 25 and 50 mL/100 L (≥98% mortality), while the next lowest rate of 12.5 mL/100 L had caused 62% mortality. This rate caused ≥90% mortality by 14-DAT, but background (control) mortality had reached 20% at this time.

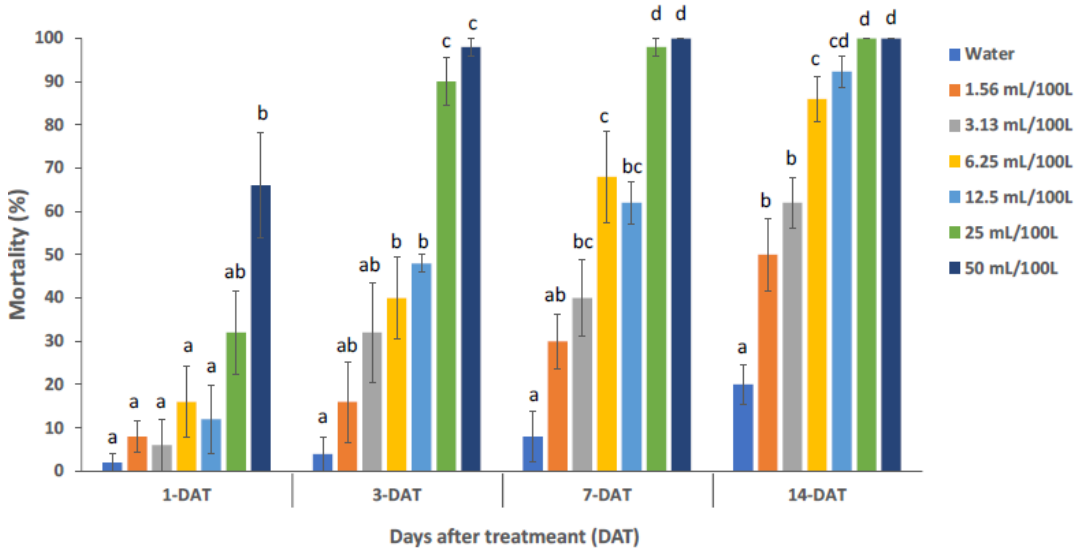

Figure A6.6. Mean Fuller's rose weevil mortality (% ± SE) at 1, 3, 7, and 14 days after treatment (DAT) applied via direct contact sprays of a range of rates of chemical Exp A, displayed with rates grouped by time. Letters above bars within the same time denote significant differences detected at α = 0.05 (Tukey's post-hoc tests).

Topical treatment with Exp-A reduced feeding by Fuller's Rose weevil in a manner which corresponded to the rate of treatment. Mean frass weights at 14-DAT declined with increasing rates of Exp A treatment, indicating less feeding behaviour (Figure A6.7, left). From 3-DAT onwards, little to no chewing marks were observed on orange leaves by weevils treated with 25 and 50 mL/100 L of Exp A. Further, chewing marks were smaller for the lower rates of Exp A compared with the water control (data not shown). Egg laying was also reduced by treatment with Exp A. Compared with control weevils, all rates of Exp A lowered the number of egg batches laid by over 2/3rds, and weevils treated with rates 25 and 50 mL/100 L laid zero eggs (Figure A6.7, right).

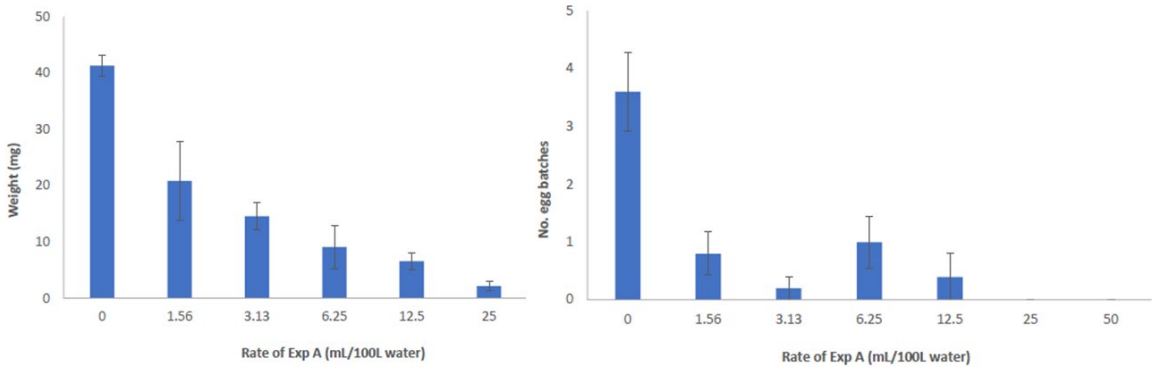

Figure A6.7. Mean frass weight and mean number of egg batches by Exp-A.

The results suggest a minimum effective rate of 25 mL/100 L for the application of Exp-A for FRW control.

Indirect contact activity

The minimal effective rate was 6.25 mL/100 L for 1 or 3-day mortality and 1.56 mL/100 L for 7 or 14-day mortality (Figure A6.8). Note that 1.56 mL/100 L was the lowest rate tested, so the real minimal effective rate for 7-day mortality could be lower than 1.56 mL/100 L. Rates at or above 6.25 mL/100 L achieved 100% mortality after 7 days. None of the test rates achieved 100% mortality after 1 day.

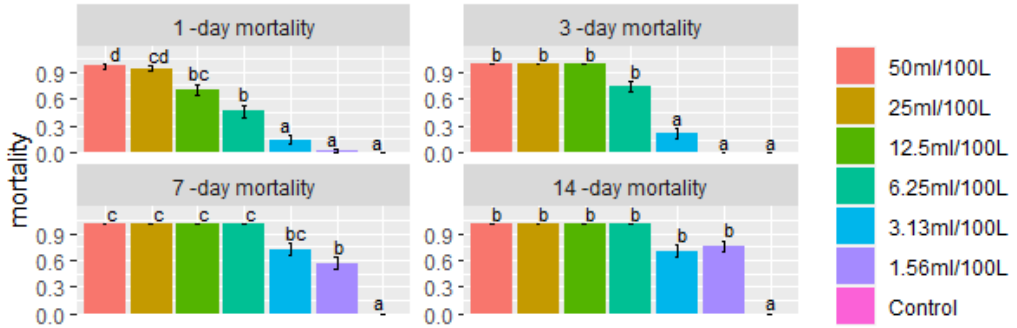

Figure A6.8. The proportion of test weevils that died or were incapacitated after 1, 3, 7, and 14 days of exposure to leaves sprayed with Exp-A at different rates. Bars not labelled with a common letter were significantly different at P = 0.05 by Tukey's multiple comparison tests following the detection of significant treatment effects by the general linear model (GLM) for the binomial distribution.

All test rates reduced frass weight and number of eggs laid by the test weevils (Figure A6.9). The estimated rate for 90% mortality or incapacitation was 22.47, 7.78, 4.06 and 3.84 mL/100 L based on 1, 3, 7, and 14-day mortality, respectively.

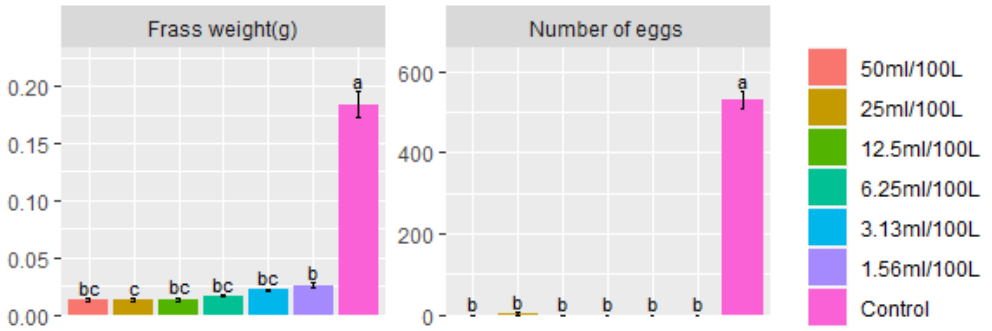

Figure A6.9. Frass weight and number of eggs laid by the test weevils exposed to leaves sprayed with Exp-A at different rates. Bars not labelled with a common letter were significantly different at P = 0.05 by Tukey's multiple comparison tests following the detection of significant treatment effects by the general linear model (GLM) for the binomial distribution.

Field efficacy

Tedders traps caught similar numbers of adult FRW in all three treatments before and after the spray, although the number in Exp-A-treated plots dropped to less than half of that in the control 21 days after the spray (Figure A6.10). A significant reduction of FRW adults in EXP-A-treated plots compared to the control plots was seen in the branch shaking data collected 14 days after the spray ($P < 0.05$; Figure A6.11). The level of reduction was similar to that in plots treated with Exirel®. The proportion of citrus fruit contaminated with FRW eggs in EXP-A-treated plots was similar to that in the control plots before the spray, however, it dropped to less than half of that in the control plots 28 days after the spray (Figure A6.12). A smaller reduction of FRW egg contamination was also seen in plots treated with Exirel® (Figure A6.12), however, as with EXP-A, the difference was not significant ($P > 0.05$)

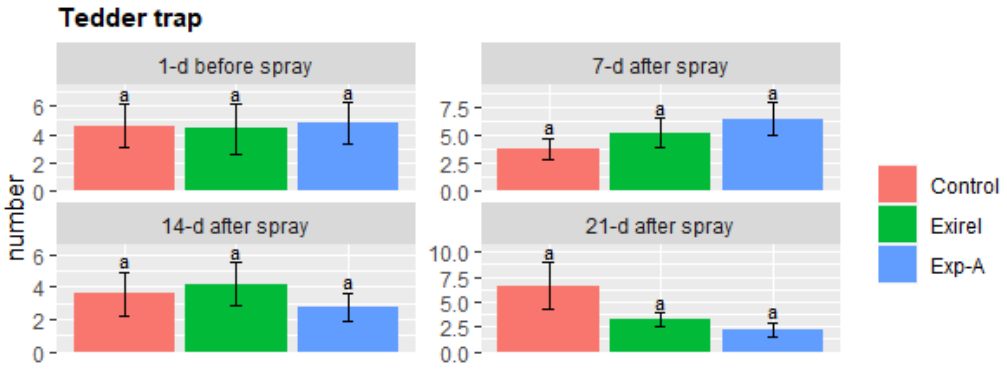

Figure A6.10. Number of FRW adults caught by the Tedders traps in plots sprayed with EXP-A, Exirel, or water only (Control) at 1-day before and 7, 14, and 21-day after the spray. Bars not labelled with a common letter were significantly different at P =0.05 by Fisher's LSD tests following the detection of significant treatment effects by the general linear model (GLM) for the binomial distribution.

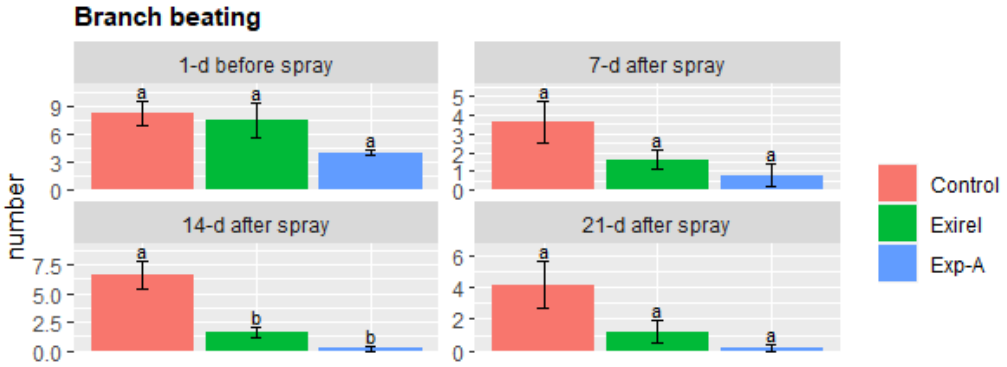

Figure A6.11. Number of FRW adults found by branch shaking in plots sprayed with EXP-A, Exirel, or water only (Control) at 1-day before and 7, 14, and 21-day after the spray. Bars not labelled with a common letter were significantly different at P = 0.05 by Fisher's LSD tests following the detection of significant treatment effects by the general linear model (GLM) for the binomial distribution.

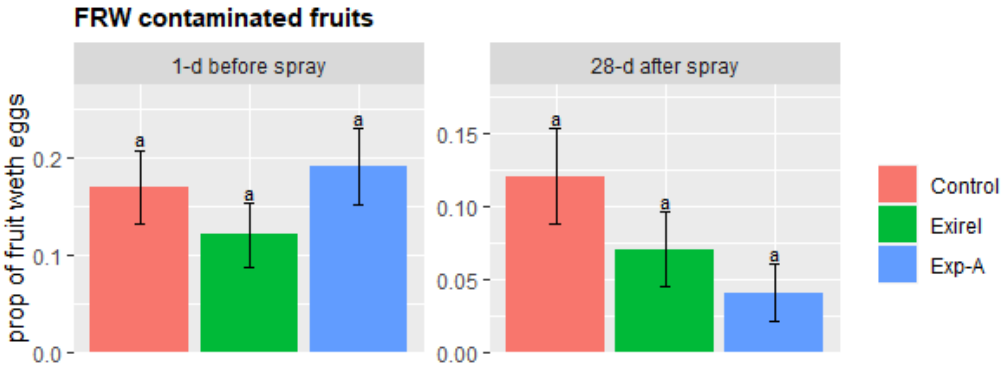

Figure A6.12. The proportion of fruit with FRW eggs in plots sprayed with EXP-A, Exirel, or water only (Control) 1 day before and 28 days after the spray. Bars not labelled with a common letter were significantly different at P = 0.05 by Tukey's multiple comparison tests following the detection of significant treatment effects by the general linear model (GLM) for the binomial distribution.

Discussion

In this study, we screened nine new chemicals for FRW control. EXP-A and indoxacarb were the only two chemicals showing potential for FRW control. In the laboratory, EXP-A demonstrated high toxicity to the adult weevils. Indoxacarb, on the other hand, did not immediately kill the weevils but incapacitated them. Some of the incapacitated weevils later

recovered in the laboratory, but their survival out in the field would be low as they would be more likely eaten by predators than healthy weevils. Despite the difference in their effects, both have shown the potential for reducing FRW egg contamination in citrus fruit, which is a key measure of a successful FRW control strategy. However, due to the lack of MRLs for indoxacarb in any of Australia's citrus export markets, only EXP-A was further investigated for rate response, field efficacy, and effect on beneficial arthropods in citrus.

Based on the rate-response data collected in the laboratory, EXP-A can achieve satisfactory control of FRW at the rate of 25 mL/100 L or higher. According to the supplier, this rate is equivalent to 2.5 g ai/100 L, which is half of the recommended rate for the DC formulation of the chemical or about the same as the recommended rate for the updated SC formulation. To confirm its efficacy, we tested it in a replicated field trial, along with Exirel®, a recently registered chemical for FRW control. We noticed that EXP-A achieved a similar level of control of FRW as Exirel® in the field, however, we were unable to confirm its field efficacy due to large random variations in the data. The field trial used the updated formulation at the rate of 5 mL/100 L, which is equivalent to 2 g ai/100 L. In the investigation of new chemical options for CGW control, we showed that EXP-A might be highly toxic to predatory mites and ladybirds and might also be toxic to lacewings (Appendix 4). However, considering that laboratory bioassays of insecticide toxicity often reflect worst-case laboratory conditions, caution should be taken when extending the findings of this study to field conditions. It has been reported that chemicals found to be harmful in the laboratory do not always show harmful toxic effects in semi-field trials (e.g. Candolfi et al. 1999). Direct 'in-field' assessments on beneficial arthropods are thus warranted. Further studies should also consider the potential sub-lethal chemical effects, such as physiological and behavioural effects, that can disrupt beneficial populations (Desneux et al. 2007).

In summary, EXP-A and indoxacarb are effective against FRW. If EXP-A is to be pursued for registration, we recommend it be further investigated for field efficacy and field effects on beneficial arthropods in citrus. Indoxacarb is also a worthwhile candidate for consideration of registration for FRW control when its MRL data become available in Australia's citrus export markets.

Disclaimer

EXP-A currently has no registration for FRW control in citrus. Pesticide Act 1999 states chemicals must only be used for the purpose described on the product label.

Appendix 7: PhD thesis

Thesis title: Understanding the biology and ecology of the citrus gall wasp (*Bruchophagus fellis*) for improved management

Contents

The draft report has been attached as a separate document with this report.

Appendix 8: Do parasitic wasps of the lucerne seed wasp parasitise the citrus gall wasp?

Dr Jianhua Mo, Mr Scott Munro, and Mr Andrew Creek

NSW Department of Primary Industries and regional Development

Some citrus growers practise Lucerne interplanting (Figure A8.1) to suppress weeds, reduce soil compaction and improve soil fertility. Recently, it was reported that lucerne interplanting improved the control of the citrus gall wasp (CGW). One possible explanation is that lucerne enhanced the biological control of CGW. Lucerne seeds are attacked by a gall-forming wasp closely related to CGW, the lucerne seed wasp (LSW). Adult LSW looks very similar to adult CGW, and, like CGW, LSW is attacked by a suite of parasitic wasps, at least two of which are present in Australia (Figure A8.2). The assumption was that parasitic wasps of LSW also attacked CGW. Does such cross-species parasitism really occur?

Figure A8.1. Lucerne planted between citrus.

To answer the question, we investigated CGW parasitism on a farm in central west NSW, where the benefit of lucerne interplanting for CGW control was reportedly observed. Despite being in a historically high CGW infestation region, this farm had not seen any citrus galls until the summer of 2019, 10 years after its establishment. The farm has 20 ha of 'Hamlin' oranges on the 'Trifoliata' rootstock and 20 ha of 'Pineapple' oranges on the 'Citrange' rootstock. Lucerne was planted in two out of every three rows throughout the farm.

Figure 1Figure A8.2. Top – Citrus gall wasp (left) and its parasitic wasps (middle and right); Bottom – Lucerne seed wasp (left) and its parasitic wasps (middle and right).

A total of 844 galls were randomly collected from citrus foliage in a 14-ha 'Pineapple' block, a 5-ha 'Pineapple' block, and a 13-ha 'Hamlin' block on the farm in late October 2020. A total of 28,612 CGW adults and 3,754 parasitic wasps emerged from these galls. Random dissection of the galls revealed 11,755 un-emerged CGW adults and 466 un-emerged parasitic wasps, bringing the total number of CGW adults to 33,617 and that of the parasitic wasps to 4,220. The overall parasitism level was about 11%. The parasitic wasps were exclusively *Megastigmus brevivalvus*, the primary parasitic wasp species of CGW in Australia. No known parasitic wasps of LSW were recovered.

In addition to parasitic wasps, we checked CGW infestation in the 14-ha 'Pineapple' block. We found similar CGW infestation levels between citrus rows next to lucerne plantings and citrus rows not next to lucerne plantings. We did a sweep-net sampling in the same block at a later date when the seed pods were available. We did not find any known parasitic wasps of either the LSW or CGW. We also collected over 1500 lucerne seed pods and reared them for wasp emergence. No known parasitic wasps of LSW emerged from the seed pods.

In summary, we did not rear any parasitic wasps of LSW from CGW galls, neither did we see reduced CGW infestation in citrus rows next to lucerne plantings. We do not rule out the possibility of parasitic wasps of LSW parasitising CGW, however, the chances of finding such cross-species parasitism are low. Most parasitic wasps only attack their own hosts. In addition, parasitic wasps of LSW and CGW emerge at different times of the year, further reducing the chances of cross-species parasitism.

This article is published in the Australian Citrus News, Autumn 2021, P31–33

Appendix 9: Modelling the future trend of the citrus gall wasp population in southern Australia

Dr Jianhua Mo[1], Michael Furlong[2], and Pieter Scott[2]

[1] NSW Department of Primary Industries and Regional Development, [2] University of Queensland

Introduction

Citrus gall wasp (CGW), *Bruchophagus fellis*, is a native, gall-forming wasp of citrus in Australia (Figure A9.1). It was first reported in the southern citrus production regions in the early 2000s. Since its first detection, CGW has spread rapidly throughout the southern citrus production regions. In some places, the infestation level appears to be steadily rising, with no signs of easing. Will the CGW population continue to rise, or will it come down after some time? To answer the question, I developed a preliminary model for the populations of CGW and its primary parasitoid species, *Megastigmus brevivalvus*. Both CGW and the parasitoid have a single generation per year. The parasitoid lays its eggs inside the CGW eggs. Un-parasitised eggs develop into CGW adults and parasitised eggs develop into the adult parasitoid.

Figure 9.1. CGW galls (top), adult CGW (bottom left) and its primary parasitoid, *M. brevivalvus* (bottom right).

Method

The model is a variation of the Nicholson–Bailey model with the inclusion of density-dependence in the growth of the CGW population. It assumes that (1) the growth of the CGW population follows the Logistic function, (2) the total number of parasitising attempts by a parasitoid is governed by Holling's type II equation, and (3) the number of parasitising attempts per host follows the negative binomial distribution (NBD). A combination of unpublished and published data was used to estimate the fecundity, sex ratio, and stage-specific mortalities of the two species.

Results and Discussion

Natural enemies often arrive sometime after a pest incursion. Assuming the parasitoid arrives 5 years after the CGW, the model predicted an initial phase of exponential growth of the gall wasp population followed by a period of high-

level oscillations (Figure A9.2). Afterwards, the population was predicted to continue oscillating at high levels, drop to a lower level and then quickly stabilise, drop to a lower level and then undulate before stabilising, or fluctuate between very low and very high levels, depending on the value of the aggregation parameter k of the NBD. The size of the stable population decreased with decreasing levels of aggregation (larger k values). According to the model predictions, CGW populations in many orchards in the southern citrus production regions are still in the initial growth phase. It is worth noting that a higher aggregation level of the parasitoid was associated with a lower level of parasitism.

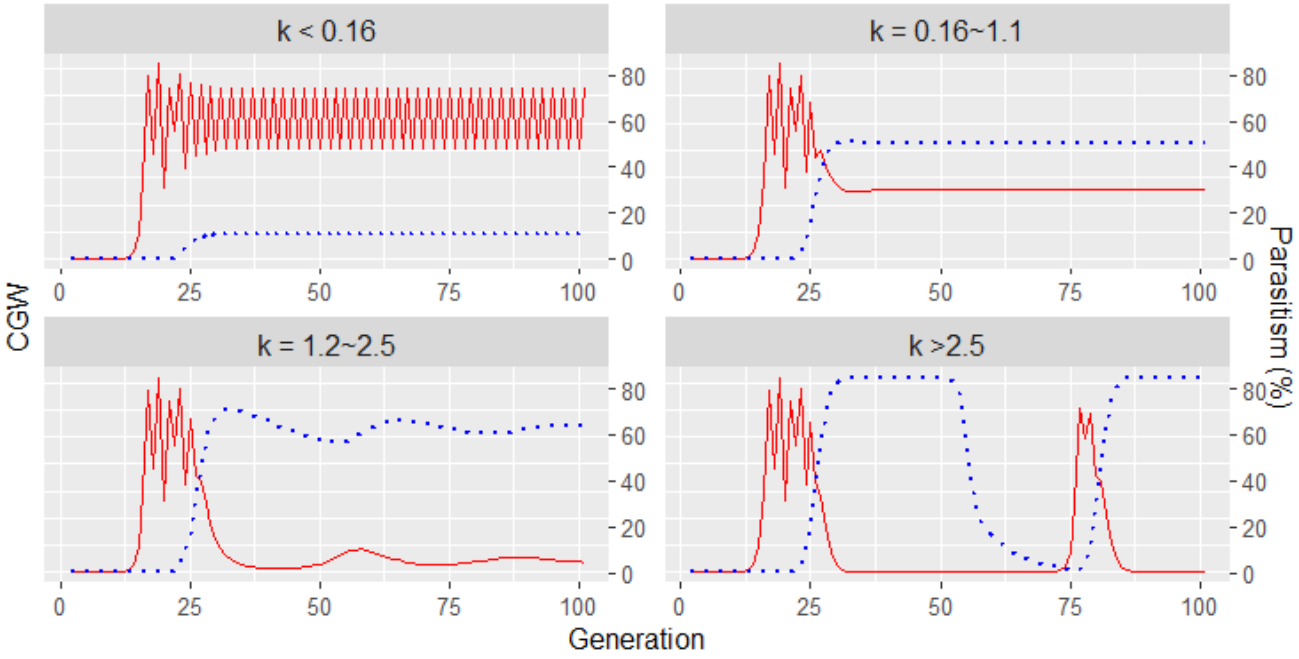

Figure A9.2. Predicted population patterns for the citrus gall wasp (solid red line) and corresponding parasitism (dotted blue line) under different aggregation levels of the parasitoid.

Duration of the initial exponential growth phase of the CGW population increased with the time lag in the arrival of the parasitoid, from less than 10 years at the 1-year time lag to over 20 years at the 20-year time lag (Figure A9.3).

The modelling results highlight the importance of parasitoids. Unlike chemical interventions, a parasitoid population increases as its host population increases. As a result, it is able to change the CGW population trends and limit the future infestation level. One interesting finding of the model is that once the parasitoid population has built up sufficient numbers, further parasitoid releases might not be necessary.

Under a moderate aggregation scenario for the parasitoid (k =2), insecticide applications reduced the maximal population size, with larger reductions at lower spray thresholds (Figure A9.4). However, insecticide applications also increased the median population size in the entire simulation period compared to when no insecticides were applied. The increases in the median population sizes were due to the prolonged initial growth phase or continued population fluctuations after the initial growth phase as a result of insecticide applications. The results suggest that there is no apparent benefit to applying an insecticide when the CGW population is low. Spraying in those years might harm the parasitoids more than it does to the CGW.

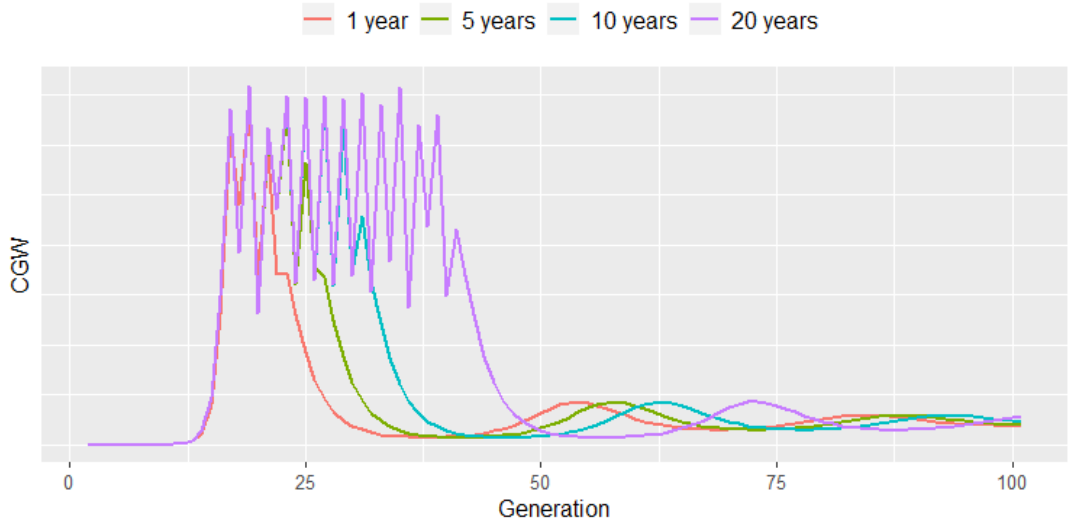

Figure A9.3. Predicted CGW population patterns when the parasitoid arrives 1, 5, 10, and 20 years after the arrival of CGW under the moderate aggregation scenario of the parasitoid (k = 2.0)

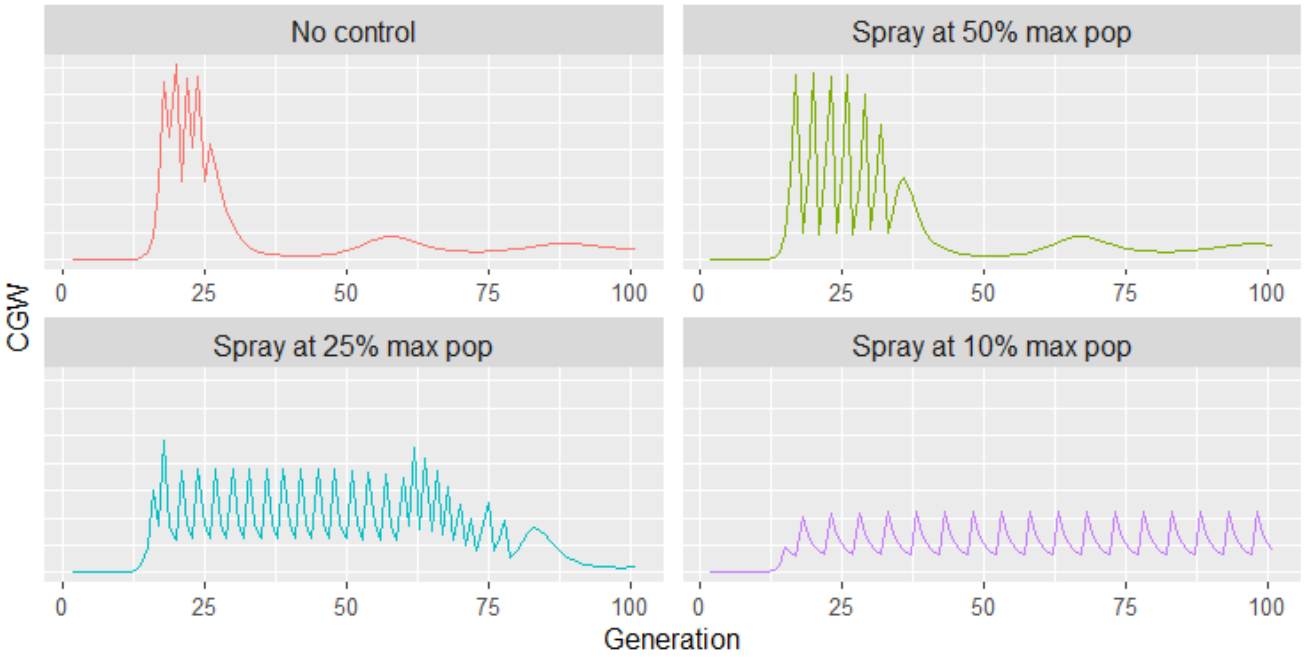

Figure A9.4. Predicted CGW population patterns when not controlled, controlled at 50, 25 or 10% of the maximal population size using a systemic insecticide with a 75% efficacy.