Final Report

Development of National Strategies to Manage Citrus Gall Wasp

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NSW Department of Primary Industries

Project code:

CT15006

Project:

Development of National Strategies to Manage Citrus Gall Wasp – CT15006

Disclaimer:

Funding statement:

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

Publishing details:

ISBN 978 0 7341 4486 7

Published and distributed by: Hort Innovation

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www.horticulture.com.au

Content

Summary

Citrus Gall Wasp (CGW) is an endemic pest of citrus in Australia. Severe infestation causes yield loss and reduction of fruit size. Traditionally a pest in southeast Queensland and northeast NSW, CGW is now widespread in all southern citrus production centres, threatening the viability of citrus production in these regions. Until recently, methidathion was the only registered insecticide for CGW control. This insecticide has been under constant review and its future availability is uncertain. New management options were urgently needed to address the escalating problem of CGW in the southern citrus production centres. The project investigated cultural control, biological control, repellents, and alternative insecticides. Phenology models have been developed to guide control timing.

Removing galls by pruning/hedging will reduce local CGW populations. To ensure no adult wasps emerge from pruned galls, pruning should be done at least 56 days before expected adult wasp emergence if pruned galls are left in the shade, or at least 28 days before emergence if pruned galls are left in the open. Otherwise pruned galls should be burned or mulched. Pruning encourages growth of young shoots, which are the preferred oviposition sites for CGW. Shoots produced following pruning between July and October are all susceptible to CGW attack. Excessive growth of young shoots can be treated with a registered systemic insecticide.

The prospect of using trap trees to manage CGW is not promising. CGW females do not appear to favour one citrus variety over another for oviposition. They also do not appear to be attracted to odours from particular citrus varieties. Even if they are attracted to certain citrus varieties, the females appear to be content staying around trees of their emergence. The observed heavier infestations in certain citrus varieties (e.g. grapefruit, lemon) are likely due to reasons other than CGW's innate preferences for these varieties. One suspected reason is that these varieties have more or larger shoots for CGW to lay eggs in than other varieties at the time of CGW oviposition.

Two parasitoid species attack CGW. Following repeated releases, both parasitoid species are now widely established in the southern citrus regions. Parasitism level is generally low (<5%), however, 'hot-spots' of 20% or higher parasitism have been detected in the regions. These hot-spots can be used as local sources of parasitoids for future releases. The parasitoids are able to tolerate up to 5-hours of heat stress (40°C) if they have access to water. Light watering before releases on a hot day will enhance the survival of released parasitoids. As a first step toward mass rearing the parasitoids, a mother culture of the parasitoids has been established.

Several foliar insecticides, including the registered methidathion, readily kill CGW adults; however, they are highly disruptive to beneficial arthropods and do not always provide satisfactory control. An alternative option targeting the adult wasps is the use of repellents. A kaolin-based product was found to be highly repellent to the adult wasps. It is a potential future option for CGW control in heavily infested citrus blocks. Two soil-applied systemic insecticides provided moderate to high levels of control of CGW larvae in late spring and both are now permitted to use for CGW control/suppression. One soil-applied systemic insecticide and a foliar-applied systemic insecticide demonstrated moderate to high level of control of CGW larvae in the autumn. They are potential options for larval control in Valencia trees, for which spring application of systemic insecticides is not feasible due to presence of mature fruit. Frequent use of the kaolin-based repellent or systemic insecticides may cause flare-ups of scale populations.

To help growers time their controls of CGW, degree-day models have been developed for adult emergence and egg hatch. Degree-day is a measure of heat units. Insects require certain numbers of degree-days to complete development. In the southern citrus regions, peak adult emergence is predicted when 723 degree-days above 8°C have been accumulated since August 1 and peak egg hatch is predicted when 1327 degree-days above 2°C have been accumulated since October 1. An interactive, online tool based on the phenology models has been developed allowing growers to use local weather station data to predict when adult wasps are likely to emerge and when eggs laid by the wasps are likely to hatch. The online tool can be accessed at https://citrusgallwasp.shinyapps.io/predict/.

Integrated pest management (IPM) strategies based on monitoring, cultural control, biological control, and judicious use of chemicals are needed for sustainable management of CGW. In new incursion areas where galls are present in isolated trees, any galls found should be removed to delay the build-up of damaging CGW populations. Pruning (skirting/hedging) is also important to manage moderate to high infestations as it removes large numbers of individuals from local CGW populations. Further reductions of local CGW populations can be achieved with the use of registered chemicals. Where infestation is severe, multiple strategies including the use of repellents and systemic insecticides may be required. All chemicals are disruptive to beneficial arthropods. Frequent use of chemicals will suppress CGW parasitoid populations and may cause flare-ups of secondary pests. Setting aside an area of the orchard for minimal chemical use will help build up a local repository for the CGW parasitoids.

Future R&D is needed to (1) estimate the impacts of CGW infestation on yield, (2) investigate cost-effective ways to rear CGW parasitoids, (3) collect further efficacy/residue data to support the registration of the kaolin based repellent, (4) find potential chemical options for CGW control in nursery trees, (5) collect CGW phenology data in WA and QLD to expand the application range of the phenology models, and (6) find safe and effective ways to manage CGW in peri-urban environment in Western Australia to slow/stop the spread of the wasp into commercial orchards.

Public summary

Citrus Gall Wasp (CGW) is an endemic pest of citrus in Australia. Severe infestation causes yield loss and reduction of fruit size. Traditionally a pest in southeast Queensland and northeast NSW, CGW is now widespread in all southern citrus production centres, threatening the viability of citrus production in these regions. This project investigated cultural, biological, and chemical options to manage CGW, and developed phenology models to guide control timing.

Removing galls by pruning/hedging will reduce local CGW populations. To ensure no adult wasps emerge from pruned galls, pruning should be done at least 56 days before expected adult wasp emergence if pruned galls are left in the shade, or at least 28 days before expected adult wasp emergence if pruned galls are left in the open. Otherwise pruned galls should be burned or mulched.

CGW females do not appear to favour one citrus variety over another for oviposition and tend to stay around trees of their emergence. As such, use of trap trees to lure CGW to a target area is unlikely to succeed.

CGW is attacked by two parasitic wasps. Both are now established in the southern citrus regions, however, parasitism rate is currently low (<5%) in most places. 'Hot-spots' of 20% or higher parasitism have been detected in the Riverina and Sunraysia. These hot-spots can be used as local sources of parasitic wasps for future releases. Successive augmented releases of parasitic wasp over a few years can help build parasite populations to effective levels. Any chemical that kills CGW will also kill the parasitic wasps. The parasitic wasps are able to tolerate up to 5-hour's heat stress at 40°C if they have access to water. Light watering before releases during a hot day will enhance the survival of released parasitic wasps. As a first step toward mass rearing the parasitoids, a mother culture of the parasitoids has been established.

Two soil-applied systemic insecticides were found to be effective for controlling wasp larvae inside galls. One is now registered for CGW suppression in late spring and the other is available for CGW control in late spring under a permit. One of the two soil applied insecticides and a foliar-applied systemic insecticide demonstrated potential for larval control in the autumn. A kaolin-based product is highly repellent to the adult wasps and has shown excellent control of CGW in heavily infested citrus blocks.

To help growers time their controls of CGW, degree-day models have been developed for adult emergence and egg hatch. Degree-day is a measure of heat units. Insects require certain numbers of degree-days to complete development. An interactive, online tool based on the phenology models has been developed allowing growers to use local weather station data to predict when adult wasps are likely to emerge and when eggs laid by the wasps are likely to hatch. The online tool can be accessed at https://citrusgallwasp.shinyapps.io/predict/.

Integrated pest management (IPM) strategies based on monitoring, cultural control, biological control, and judicial use of chemicals are needed for sustainable management of CGW. In new incursion areas where galls are present in isolated trees, any galls found should be removed to delay the build-up of damaging CGW populations. Pruning (skirting/hedging) is also important to manage moderate to high infestations as it removes large numbers of individuals from local CGW populations. Further reductions of local CGW populations can be achieved with the use of registered chemicals. Where infestation is severe, multiple strategies including the use of repellents and systemic insecticides may be required. All chemicals are disruptive to beneficial arthropods. Frequent use of chemicals will suppress CGW parasitoid populations and may cause flare-ups of secondary pests. Setting aside an area of the orchard for minimal chemical use will help build up a local repository for the CGW parasitoids.

Keywords

Citrus gall wasp, chemical control, biological control, pruning, phenology models, IPM

Introduction

Citrus Gall Wasp (CGW) (Figure 1), *Bruchophagus fellis* (Hymenoptera: Eurytomidae) is an endemic pest of citrus in Australia (Noble 1936). Heavily infested trees are covered with galls of various sizes, some over 50 cm long (Figure 2). Severe infestation may cause yield loss and reduction of fruit size. Citrus production in the southern regions is currently valued at over $12m annually. Assuming a yield reduction of 5% as a result of CGW infestation, the annual loss would be over $600,000.

Figure 1. Female (left) and male (right) citrus gall wasp.

Figure 2. CGW Galls (left) and individual cells constructed by the larvae.

Adult wasps emerge from galls in the spring. They immediately mate and lay eggs. Female wasps lay eggs between the bark and wood of current season spring shoots, fruit stems, or leaf petioles. After hatching, the larvae burrow into the soft bark tissue and feed there in individually constructed cells (Figure 2) until pupation. The area of a shoot housing multiple feeding cells swells as the season progresses and eventually forms a characteristic gall. The lifecycle completes in a year (Figure 3).

CGW was traditionally a pest in southeast Queensland and central to northern NSW with a notable absence in the orange (*Citrus* x *sinensis*) production centres of Riverina, Sunraysia, and Riverland in southern Australia (Smith et al. 1997). In the late 1990s CGW was first detected in Sunraysia (Cannard 2007). In 2008, it was found in backyard citrus trees in Griffith in the Riverina (Hardy and Creek 2009). In 2012, it was found in commercial citrus orchards in Renmark and Loxton in the Riverland (K. Thiel, personal communication, September 2012). In 2013, it was found on the outskirts of Perth in Western Australia (A. Szito, personal communication, May 2013). By September 2015, the wasp had become widespread in the Sunraysia and the Riverland and on a number of farms in the Riverina.

Figure 3. Lifecycle of a citrus gall wasp.

In its natural habitats, CGW is attacked by two parasitoid species, *Megastigmus brevivalvus* and *M. trisulcus* (Hymenoptera: Torymidae) (Nobel 1935, Smith et al. 1997) (Figure 4). Where the parasitoid populations are high, over 90% of CGW larvae can be parasitised (Smith et al. 1997). Both parasitoid wasp species have established in the new incursion areas in the south after repeated releases, however, their populations are not yet high enough to effectively control CGW. In Queensland, where the parasitoids are well established, CGW populations still sometimes flare up and chemical interventions are needed to bring the CGW populations down to acceptable levels.

Figure 4. Parasitoid species of the gall wasp: *Megastigmus brevivalvus* (left) and *M. trisulcus* (right).

At the start of this project in September 2015, methidathion was the only registered insecticide for CGW control. Methidathion is an organophosphate insecticide. Due to its broad-spectrum activity and high mammalian toxicity, methidathion has been under constant review by APVMA. Queensland citrus growers have reported reduced efficacy of the insecticide in CGW control following a formulation change in the commercial products (M Wallis, personal communication, September 2017). New management options were urgently needed to stem the increasing tides of CGW infestations in the southern citrus regions.

In response to industry concerns, a pilot project (Horticulture Australia Ltd CT10021) was conducted in the Sunraysia region during 2010-2013 to better understand the biology of the wasp and identify potential management options for its management. The project (1) obtained data on CGW adult lifespan, oviposition pattern and egg development, (2) developed a preliminary forecast model to predict the emergence of the adult wasps and egg hatch, and (3) identified petroleum spray oil (PSO) and imidacloprid as potential (as yet unregistered) alternative chemical options (Mo *et al*. 2014). The project outputs have improved our understanding and management of CGW, however, more data and tools are needed to develop sustainable CGW management strategies.

An immediate need of the industry is to have effective management options. Pruning is an integral component in CGW management. In newly infested orchards, galls are usually found in isolated clusters of trees. Cutting galls before the wasps come out can eliminate or significantly reduce infestation sources and thus delay the spread of infestation to other parts of the orchards. In heavily infested orchards, annual pruning/hedging can significantly reduce local CGW populations. If pruning is done early, cut galls will dry out over time eventually killing the larvae/pupae inside. However, cut galls from late pruning may need to be mulched or burnt to prevent adult emergence. Finding out the cut-off dates for zero adult emergence will help growers time their punning operations and deciding on the need for mulching or burning.

Biological control plays an import role in CGW management. After repeated introductions, both *M. brevivalvus* and *M. trisulcus* have established in the southern citrus regions (Flett 2011). Further releases are needed to broaden their establishment. Until recently, the parasitoids were sourced exclusively from Queensland. To reduce release cost, it is desirable that local sites of relatively high parasitoid populations ('hot-spots') be located and reserved as local release sources in the future. For more reliable supply of the parasitoids, the feasibility of setting up artificial rearing facilities for the parasitoids needs to be investigated. In Queensland, CGW populations still sometimes flare up despite a long history of natural biological control by the two parasitoid species. This raises the question of whether natural parasitoid populations alone can offer sustainable control of CGW. To date, CGW is the only known host of both parasitoid species. The lack of alternative hosts tends to result in cyclic fluctuations in both the host and its parasitoid populations (Hassell 2000). This could explain the occasional flaring up of CGW populations in Queensland. In addition to the innate nature of CGW-parasitoid interactions, other factors may also have contributed to the flaring up of CGW populations in Queensland. In recent years, heatwaves have become more frequent and severe as a result of climate change. Heatwaves may destroy parasitoid populations allowing CGW populations to rapidly reach outbreak levels.

Project CT10021 identified PSO as an oviposition deterrent; however, commercial trials in the Riverland showed that PSO sprays often failed to bring down CGW populations (C. Swanbury, personal communication, August 2015). One suggested reason for the unsatisfactory control was that CGW females were forced to lay eggs in oil-treated shoots because there were no clean shoots nearby. The hypothesis needs to be tested before further development of the PSO option. In addition to PSO, other materials that change the physical/chemical properties of citrus foliage may also repel CGW oviposition. One such material is kaolin. Sprayed as a wettable powder, it forms a barrier film on the foliage which persists for months. This film changes surface texture of citrus shoots and may deter CGW females from laying eggs in them. One kaolin based product is currently registered for sunburn protection in citrus.

Chemical control of the citrus gall wasp targets either the adult wasps or larvae. For adult wasp control, many foliar insecticides should be effective as the wasp has not been under any intensive pressure to develop insecticide resistance. However, use of foliar insecticides for CGW control is not a preferred option for two reasons. Firstly, foliar insecticides are highly disruptive to citrus IPM. Secondly, citrus gall wasp females lay eggs soon after emergence (Mo *et al*. 2014) and by the time they are killed by foliar insecticides they may have already laid many eggs. CGW spends most of its life as larvae inside galls. Finding a systemic insecticide that kills the larvae should be a key to CGW management. Project CT10021 showed some potential of imidacloprid in CGW larval control. More data was needed to confirm the efficacy of imidacloprid. It is also worth investigating the efficacy of other systemic insecticides. They may offer similar or more effective control of CGW larvae.

Timing is important in CGW management. Papacek and Smith (1989) showed that methidathion application was best timed toward the end of the oviposition period and before the current year citrus shoots have hardened. In central Queensland this timing corresponds to early December. The same timing cannot be assumed for other locations due to temperature differences. A preliminary degree-day model was developed in CT10021 to predict the emergence of adult wasps (Mo and Stevens 2014). The model parameters were based on data collected on 'Navel' oranges in the Sunraysia during 2010-2012. For more accurate predictions across the southern citrus regions, the model needs to be validated and updated with data from different regions.

This project aims to develop coordinated national strategies to manage the increasing pressure from the gall wasp in the southern citrus regions. The priority research areas are (1) cultural control, (2) biological control, (3) IPM-compatible chemical options, and (4) a degree-day tool to guide control timing. Studies are also conducted to better understand the biology and behaviour of the wasp and its interactions with its parasitoids. Integrated strategies are developed based on available management options.

Methodology

Pruning

The effect of pruning was investigated with respect to (1) cut-off date of pruning to ensure zero emergence of adult CGW and (2) susceptibility of post-pruning shoots to CGW attack (Appendix-2).

Host selection

Relatively high CGW infestations have been reported for some citrus varieties such as lemon and grapefruit. In some orchards, heavily infested lemon trees and lightly infested orange trees were found side by side. Sucker shoots (basal shoots grown from the tree trunk below the graft union) are also seen as particularly susceptible to CGW. To verify the reported varietal differences in CGW attack and understand the underlying mechanisms, varietal differences in CGW oviposition were investigated using freshly cut shoots in the laboratory and potted trees (Appendix-3). The laboratory investigation consisted of a series of choice tests comparing shoots from Eureka lemon, Valencia orange, and Washington navel. The polyhouse trial compared Washington navel, Valencia orange, Eureka lemon, and Marsh grapefruit. In addition, the role of plant odour and odour from virgin CGW females on the orientation (direction of movement) of adult CGW were investigated in Y-tube tests (Appendix-3).

Dispersal

To estimate the dispersal range of adult CGW, a mark-recapture experiment was conducted during October-December 2017 in a block of Valencia orange trees in Leeton (Appendix-4). Adult CGW were marked with fluorescent powder, released from a central point, and recaptured by yellow sticky traps placed at various distances to the release tree in a grid pattern.

'Hot-spots' of CGW parasitoids

CGW Parasitism in the Riverina and Sunraysia were surveyed in spring 2016 to find orchards with relatively high parasitoid populations (hot-spots) (Appendix-5). CGW Parasitism in the Riverland was routinely checked as part of Fruit Doctors' monitoring services with supplementary survey data at previous release sites in 2016.

Effects of heat stress on survival of CGW parasitoids

Heat stress was simulated by 1, 2, and 5 hour's exposure to 40°C. Survival of the primary CGW parasitoid *M. brevivalvus* exposed to the heat stress was investigated in the laboratory with or without the provision of water (Appendix-6).

Rearing of CGW parasitoids

The prospect of artificial rearing of the CGW parasitoids was investigated in Loxton, SA, using caged rootstock trees (Appendix-7). Various stocking rates (total wasps introduced per cage) and ratios of parasitic wasps to CGW were investigated.

Pre-emergence control

Feasibility of controlling CGW close to adult wasp emergence was investigated in three field trials in the Riverina during September-October 2015 (Appendix-8). The purpose was to see if any chemicals applied to the galls at this late stage would reduce the emergence of adult wasps or kill them during their emergence process. Five insecticides with systemic or translaminar activities were investigated. Test insecticides were applied to tagged galls with a paint brush at 4, 20, and 33 days before gall wasp emergence. After completion of gall wasp emergence, the number of emerged wasps on each tagged gall was counted.

Adult wasp control

Potential insecticides for controlling adult CGW were investigated with respect to direct contact activity and indirect residual contact activity in the laboratory at the Yanco Agricultural Institute (Appendix-8). Direct contact activity was investigated by applying a 0.5 µL drop of an insecticide solution to the thorax of a test wasp with a micro pipette. Residual contact mortality was investigated by exposing wasps to leaves from sprayed potted trees. Test insecticides were chosen after consultations with the citrus industry and chemical companies.

Adult repellent

Investigations of adult repellents focused on Surround® (950 g/kg processed and refined kaolin), as the kaolin-based product is already registered in citrus for sunburn protection and growers had reported reduction of CGW following Surround® application. Three replicated large-plot field trials were conducted to determine the repellence of this product by comparing gall reductions in Surround®-treated plots and water-only plots (Appendix-8). One trial also collected data on red scale infestation to investigate the suspected side effect of Surround® sprays on red scale infestation.

Proof of efficacy of oil

Efficacy of Biopest® oil in deterring CGW females from laying eggs in citrus shoots was investigated in choice and no-choice experiments in the laboratory (Appendix-8). Three choice experiments and three no-choice experiments were conducted. Current season lemon shoots were dipped in water or 0.5-1% Biopest oil before being exposed to newly emerged CGW adults. After designated exposure periods, test shoots were debarked and checked under a stereo microscope to count the number of eggs.

Larval control

Confidor® Guard (350 g/L imidacloprid), Samurai® (500 g/kg clothianidin), and Movento® (240 g/L spirotetramat), were investigated for larval control in seven field trials, three for larval control in the spring (October-November) and four for larval control during summer to autumn (January-April) (Appendix-8). Efficacy of the insecticides in spring application trials was assessed by reduction of galls relative to untreated control plots. Efficacy in the summer-autumn application trials was assessed by suppression of adult gall wasp emergence.

Potted-tree trials

Separate investigations for potential chemicals for controlling adult wasp, wasp larvae and for repelling adult wasps were conducted in Loxton during 2015-2016 using potted rootstock trees (Appendix-9).

Impacts of Samurai® on non-target arthropods

The approval of a permit for the use of Samurai® for CGW control in citrus in 2016 has warranted an investigation of the potential impacts of the insecticide on beneficial arthropods. The project investigated the effects of Samurai® on (1) survival of the red scale predator *Chilocorus circumdatus*, (2) field parasitism of red scale, and (3) abundance of arthropods foraging in citrus canopies (Appendix-8). The effect of the insecticide on CGW parasitoids was not investigated as the insecticide is intended to kill CGW larvae and when a CGW larva is killed the parasitoid inside the CGW larva, if present, will also be killed. Effect of the insecticide on the survival of the red scale parasitoid *Aphytis* was investigated as part of the sampling data on red scale parasitism.

Phenology models

Emergence of adult wasps and egg hatch were monitored at four sites each in the Riverina and Sunraysia for three seasons during 2015-2017. Data of accumulated proportions of emerged adult wasps and hatched eggs and the corresponding data of degree-days (DD) were fitted to the Weibull distribution function. The fitted Weibull function was then used to estimate the time for median adult wasp emergence and egg hatch.

Optimal values for DD parameters (starting date for DD accumulation and the development threshold temperature) were found by cross-validation. Detailed model development and validation process is described in Appendix-10.

Statistical analyses

Oviposition data from choice experiments on PSO were recoded as presence-absence data of eggs and then analysed by chi-squared contingency table tests with Monte Carlo simulations (Hope 1969).

Differences in numbers of eggs laid in cut shoots from different citrus varieties were analysed by Kruskal-Wallis rank-sum test (Hollander and Wolfe 1973). Test units where no eggs were found in shoots from any citrus varieties being compared were excluded from the analysis because they provided no information on CGW's ovipositional choice.

Data from Y-tube olfactory tests were analysed by Fisher's exact binomial test (Kinley 1978).

All other data were analysed by general linear models (GLM) followed by analysis of variance (ANOVA) (Venables and Ripley 2002). For data from randomised blocks, effect of the blocking factor was removed before estimating the treatment effect. Proportional data (mortality, proportion of emerged wasps, proportions of red scale infested fruit) were 'arcsine' transformed before being analysed. 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 performed in R (R Core Team 2012).

Outputs

Cultural control

- One data set has been collected on the relationship between pruning dates and CGW emergence (Appendix-2).
- One data set has been collected on the relationship between pruning dates and production/susceptibility of spring shoots to CGW attack (Appendix-2).
- Two sets of data have been collected on the relative attractiveness of citrus shoots from different varieties to CGW attack (Appendix-3).
- One set of data has been collected on the movement range of CGW adults (Appendix-4).

Biological control

- Approximately 700 CGW parasitoids were released at 15 sites in Riverina in spring 2015 and approximately 30,000 CGW parasitoids were released at 20 sites in Riverland in spring 2014 and 2015. Survey in spring 2016 showed the parasitoids were present at all sites in the Riverina, Sunraysia, and Riverland and no further releases were made thereafter (Appendix-5).
- Hot-spots of parasitoid populations have been identified in the Riverina, Sunraysia and Riverland (Appendix-5).
- One set of laboratory data has been collected on the effect of heat stress on the survival of parasitoids (Appendix-6).
- A mother culture of parasitoids has been established in potted rootstock trees in the Riverland (Appendix-7).

Chemical control

- One set of field efficacy data has been collected on potential insecticides for pre-emergence control of CGW (Appendix-8).
- Three sets of laboratory efficacy data have been collected on potential insecticides for controlling CGW adults (Appendix-8).
- Three sets of field efficacy data have been collected on the effects of Surround® in repelling CGW adults (Appendix-8).
- Three sets of field efficacy data have been collected on the effects of the soil-applied systemic insecticides Samurai® and Confidor® Guard in controlling CGW larvae in late spring (Appendix-8).
- Four sets of field efficacy data have been collected on the effects of Samurai® and Confidor® Guard and the foliar-applied systemic insecticide Movento® in controlling CGW larvae in late summer and autumn (Appendix-8).
- Four sets of efficacy data from potted trees have been collected on potential insecticides for controlling CGW adults and larvae, and for repelling CGW adults (Appendix-9).
- One set of laboratory efficacy data has been collected on the effects of petroleum spray oil (PSO) in repelling CGW adults (Appendix-8).
- One set of data each have been collected on the impact of Samurai® on the survival of a commercially

available red scale predator in the laboratory, survival of red scale parasitoids in the field, and abundance of general predators and parasitoids in citrus canopy (Appendix-8).

Phenology models

- A total of 17 datasets (site by season data) each have been collected on adult wasp emergence and egg hatch in the Riverina and the Sunraysia during 2015-2017 (Appendix-10).
- Phenology models for adult emergence and egg hatch have been updated with the new data (Appendix-10).

Communications

- 17 Grower meetings/field days - 7 in the Riverina, 4 in the Sunraysia, 4 in the Riverland, and 1 each in WA and QLD (Appendix-11)
- 14 articles in industry magazines/newsletters (Appendix-11)
- 2 project workshops participated by the project team and industry representatives (Appendix-11)
- An online tool on gall wasp emergence and egg hatch (https://citrusgallwasp.shinyapps.io/predict/) (Appendix-10).

Outcomes

Cultural control

- ❖ Best timing for pruning has been estimated

  To ensure no adult wasps would emerge from pruned galls, pruning should be done at least 56 days before expected adult wasp emergence if pruned galls are left in the shade or at least 28 days before expected adult wasp emergence if pruned galls are left in the open. Otherwise pruned galls should be burned or mulched.

  Pruning encourages growth of young shoots, which are preferred oviposition sites of CGW. Treating pruned trees with a repellent or a systemic insecticide will reduce CGW establishment or survival in these shoots.

- ❖ Potential of the use of trap trees for CGW management has been evaluated

  Use of trap trees in CGW control does not appear promising for two reasons. First, choice testes in this project did not detect a consistent preference in CGW females for a particular citrus variety for oviposition or for odour from a particular citrus variety. The observed heavier infestations in grapefruit, lemon, and rootstock trees are likely due to reasons other than CGW's innate preference to lay eggs in these varieties. Rather, these varieties may produce more suitable shoots for CGW to lay eggs in or CGW eggs and larvae in these varieties survive better than they do in other citrus varieties. Results from a mark-recapture experiment in this project confirmed the observations that CGW females tend to stay close to trees of their emergence rather than flying away to search for new oviposition sites. As such, trap trees are unlikely to pull CGW adults away from surrounding trees even if they are more attractive.

Biological control

- ❖ CGW parasitoids have now established across the southern citrus production regions.

  Following repeated releases before and during this project, CGW parasitoids are now widely established in the Riverland, Sunraysia and the Riverina, although parasitism is below 5% at most sites.

- ❖ Hot-spots' of CGW parasitoids in the Riverina, Sunraysia, and Riverland have been identified.

  Hot spots of 20% or higher parasitism were found at one site in the Riverina and two sites in the Sunraysia. In addition, galls collected from two lemon trees at a site in the Riverina produced an average of 20 parasitic wasps per gall. The highest recorded parasitism rate in the Riverland was 12% and was found from one of the first sites used for parasitic wasp releases in the region.

  Protecting the hot-spots from excessive use of insecticides will help build up local parasitoid populations, which can be used as local sources for future parasitoid releases.

- ❖ CGW parasitoids are able to tolerate heat stress if they have access to water

  The primary parasitic wasp species of CGW, *M. brevivalvus*, tolerated up to 5 h of 40°C heat stress without suffering significantly higher mortality as compared to unstressed wasps when they were provided with access to water (Appendix-6). When they were deprived of water access, however, heat-stressed wasps suffered 24-61% higher mortality than unstressed wasps within the first day.

  Light watering during a hot day will improve the survival of parasitoids. Parasitoid populations are unlikely to collapse during heatwaves as citrus orchards are regularly irrigated.

- ❖ Future biological control of CGW has been enhanced with the establishment of a mother culture.

  As a first step toward mass rearing the parasitoids, a mother culture of the parasitoids has been established in the Riverland using rootstock trees.

Chemical control

- Four chemical products have shown potential for controlling CGW larvae.
  - Surround® demonstrated excellent efficacy in reducing CGW oviposition in three replicated, large-plot field trials. Two applications of Surround® applied at a total rate 7.5 kg/100 L to the foliage just before and during gall wasp emergence reduced galling by as much as 96%. Using Du-Wett® as the adjuvant, spray volume of Surround® can be reduced from 4000 L/ha to 2000 L/ha without compromising the repellence, effectively halving the cost.

    Surround® is not yet registered for CGW control.

  - Soil-applied systemic insecticide Samurai® provided consistent control of CGW larvae in late spring in three replicated field trials. A single application of the product at 8 g/tree in late spring reduced galling by 53-83%. In a potted-tree trial, a single application of Samurai® at 3 g/tree in December completely eliminated adult wasp emergence in the following spring.

    Samurai® has also shown potential for larval control in autumn. A single application of Samurai® at 8 g/tree in late March reduced emergence of adult wasps in the following spring by 55%.

    Samurai® does not appear to affect the abundance of parasitic wasps and predators foraging in citrus canopy, however, it may reduce the emergence of the red scale parasitoid *Comperiella bifasciata*.

    Samurai® has now been permitted for use for CGW control in late spring in citrus (APVMA PER81925 and PER82831).

  - Soil-applied systemic insecticide Confidor® Guard demonstrated moderate to high efficacy for larval control in late spring in two replicated field trials. A single application of the product at 9 ml/tree in late spring reduced galling by 71-90%.

    Confidor® Guard is now registered for CGW suppression in citrus in late spring (APVMA Product Label 55753).

  - Foliar-applied systemic insecticide Movento® has shown potential for larval control in autumn. Two applications of Movento® at 40 ml/100 L plus Hasten at 50 ml/100 L during March/April reduced emergence of adult wasps in the following spring by as much as 98%. A single spray of Movento® at a high water rate has also shown potential for CGW control.

    Movento® is not yet registered for CGW control.

- Control of CGW adults is possible but not a preferred option.

  Adult gall wasps are readily killed by foliar insecticides, some of which have shown good residual activity against the adult wasps. However, foliar insecticides are disruptive to populations of beneficial arthropods in citrus. Furthermore, CGW adults lay eggs immediately after emergence and by the time they are killed by the foliar insecticides they would have already laid many eggs.

Phenology models

- Phenology models have been developed that predict the time when adult CGW are likely to come out and when CGW eggs are likely to hatch using local temperature data. The information allows growers to schedule CGW management operations in the coming season.

- An easy-to-use online tool has been developed based on the phenology models. The online tool is easy to use. Growers simply select the closest weather stations and the predicted timing for adult CGW emergence and egg hatch will be shown. Current version of the online tool can be accessed at https://citrusgallwasp.shinyapps.io/predict/.

Communications

Growers and citrus industries have been kept up to date with project progress and project findings through a series of grower meetings and industry articles. The project team has also maintained regular contacts with the industries on issues of their concerns in relation to CGW management through face-to-face meetings, telephone conversations, and emails. Industry representatives participated in the review of project progress and discussion of project plans. As a result of the communications, growers and industries have improved understanding of CGW and are aware of available chemical, biological, and cultural options, and the online resources for CGW management.

Monitoring and evaluation

Has the project provided new information on the cultural control of CGW?

The project has provided new information on pruning timing and relative attractiveness of different citrus varieties.

Pruning is an important cultural option in CGW management. In newly infested orchards, galls are usually found in isolated clusters of trees. Cutting galls before the wasps come out can eliminate or significantly reduce infestation sources and thus delay the spread of infestation to other parts of the orchards. In heavily infested orchards, annual pruning/hedging can significantly reduce local CGW populations. To help growers time their pruning operations, the project has estimated the cut-off dates of pruning that will ensure zero-emergence of adult wasps from cut galls without the need for mulching/burning. The cut-off date for cut galls left in the open is 28 days before gall wasp emergence and that for cut galls left in the shade is 56 days before gall wasp emergence.

Pruning also has the undesirable side effect of promoting the growth of young shoots, which are preferred oviposition sites for CGW. The project found that shoots produced after pruning from July to October are all susceptible to gall wasp attack and that larger galls are more likely to form on shoots produced from early pruning (July/August) than on shoots produced after late pruning (October/November). However, late pruning may not be practical as the timing is close to citrus flowering and pruning this late may reduce flowering and fruiting.

It appears no single timing is optimal for pruning in all situations. In addition to cut-off dates for zero-emergence of CGW adults and susceptibility of post-pruning shoots to CGW attack, a number of other factors have to be considered when selecting a pruning date including tree phenology, harvest dates and resource availability. Many growers prune their trees in winter after harvest to maintain productivity and manage tree canopies. Winter pruning is a good practice for CGW management as it will also ensure no adult wasps would emerge from any cut galls regardless of whether or not the galls are left in the open or shade.

Trap crops are used in pest management in a number of crops. For the technique to work, the trap crop has to be much more attractive to the pest of concern than the target crop and that the pest will move away from the target crop to the trap crop. CGW infestation appears to be heavier in grapefruit, lemon and rootstock trees than in orange trees. Can the seemingly more attractive trees be used as trap trees in CGW management? To answer the question, the project investigated the relative attractiveness of different citrus varieties in attracting CGW oviposition. No significant ovipositional preferences were found. The project also investigated responses of CGW females to odours from different citrus varieties. Again no consistent odour preferences were detected. The results suggest that use of trap trees to manage CGW may not be a viable option. The observed heavier infestations in grapefruit, lemon, and rootstock trees are likely due to reasons other than CGW's innate preference to lay eggs in these varieties. Rather, these varieties may produce more suitable shoots for CGW to lay eggs in or CGW eggs and larvae in these varieties survive better than they do in other varieties. Results from a mark-recapture experiment in this project confirmed the observations that CGW females tend to stay close to trees of their emergence rather than flying away to search for new oviposition sites. As such, trap trees are unlikely to pull CGW adults away from surrounding trees even if they are more attractive.

Has the project improved the chance of biological control of CGW?

The project has improved the chance of biological control of CGW through (1) parasitoid releases, (2) identifications of local hot-spots of parasitoid populations, (3) estimation of the effect of heat stress on parasitoid survival, and (4) establishment of a mother culture of the parasitoids.

Parasitoid releases started soon after the detection of CGW in commercial citrus properties in the Coomealla district in the Sunraysia in early 2000s. By 2011, the parasitoids were found to have been established at the release sites in the Sunraysia (Flett 2011). First parasitoid releases in the Riverland were made in early 2010s. Further releases of parasitoids were made in the region in 2015 and 2016 as part of the project. In spring 2015, the parasitoids were first released in the Riverina. Surveys in the Riverland, Sunraysia and Riverina in 2016 detected the presence of the parasitoids at all sites, albeit at low levels. It is exciting to note that the surveys also identified hot-spots of over 20% parasitism in the Riverina and Sunraysia. With minimal use of insecticides, parasitoid populations at the hot-spots can expect to rise further in the next few years, potentially serving as local sources for future parasitoid releases.

Natural parasitoid populations take a long time to build up to a level sufficient for satisfactory control of CGW. The parasitoids rely exclusively on CGW for existence, have only one generation per year, and each parasitised CGW only produces one parasitoid offspring. Building up parasitoid populations in commercial citrus is also challenging. Faced with the rapid increases of CGW infestations, many growers will resort to using chemicals to bring CGW populations down. Any chemical that kills CGW larvae will also kill the parasitoid developing inside the larvae. Finally, the CGW-parasitoid system is unstable due to the one to one relationship. When a local CGW population crashes, the local parasitoid population will also crash. To overcome the constraints and provide a stable supply of parasitoids in the future, a mother culture of parasitoids has been established in the Riverland by the project collaborator Fruit Doctors. Early results are encouraging but more work is needed to increase the cost-effectiveness of the rearing system.

In Queensland, CGW populations still sometimes flare up despite a long history of natural biological control. The flare-ups may have been a reflection of cyclic fluctuations inherent to any host-parasitoid systems in which there are no alternative hosts (Hassell 2000). Another suspected reason was heatwaves. In recent years, heatwaves have become more frequent and severe as a result of climate change. Sustained high temperatures can destroy parasitoid populations allowing CGW populations to break free and rapidly reach outbreak levels. The project has shown that CGW parasitoids are able to tolerate up to 5-hour's 40°C heat stress if they have access to water. Citrus is an irrigated crop. As the parasitoids emerge over a period of time (Mo *et al*. 2014), water should be available to some parasitoids during a heatwave. It is therefore believed unlikely that heatwaves will cause a total crash of the parasitoid population.

Has the project identified effective insecticides for CGW control?

The project has identified several promising insecticides for CGW control, including the registered methidathion for controlling CGW adults, two insecticides for controlling CGW larvae in the spring and two effective insecticides for controlling CGW larvae in the autumn. No effective insecticides have been found for CGW control shortly before adult wasp emergence.

Control of CGW adults

Five new insecticides, chlorpyrifos, spinetoram, bifenthrin, thiamethoxam and natural pyrethrum, were screened in the laboratory for their effectiveness in controlling CGW adults along with the registered methidathion. All demonstrated good acute toxicity to the adult wasps. Spinetoram, bifenthrin and thiamethoxam have shown good residual activity against CGW adults. A screening trial using potted rootstock trees identified bifenthrin as the most promising insecticide for adult wasp control.

The results show CGW adults are readily killed by foliar insecticides, however, use of foliar insecticides for CGW control is not considered as a good practice for two reasons. First, foliar insecticides are highly disruptive to populations of beneficial arthropods in citrus orchards. Secondly, foliar insecticides may not deliver a desired level of control as female wasps mate and lay eggs immediately after emergence and are likely to have laid many of their eggs before being killed by the foliar insecticides.

Control of CGW larvae in spring

Two soil-applied systemic insecticides, Samurai® and Confidor® Guard, demonstrated consistent efficacy for larval control in spring in three field trials, reducing galls by over 50% in comparison to untreated controls. A potted-tree trial achieved over 99% reduction of CGW population by the two insecticides.

Permits have now been obtained for their use for CGW control/suppression in spring. Both insecticides are known to kill bees. It is important to avoid using them during citrus flowering. Data from a field trial showed that Samurai® at the label rate did not affect the abundance of parasitic wasps and predators foraging in citrus canopy, however, it reduced the emergence of the red scale parasitoid *Comperiella bifasciata*, from parasitised red scales.

Control of CGW larvae in autumn

Clothianidin demonstrated moderate efficacy for larval control in autumn in two field trials. Spirotetramat demonstrated moderate to high efficacy in the three field trials. March/April applications of spirotetramat reduced CGW emergence by as much as 98%.

Finding effective insecticides for CGW control in autumn is good news to citrus growers with infested Valencia trees. Chemical options for CGW control in spring cannot be used on Valencia trees as mature fruit are present on the trees at the time. Spirotetramat is a foliar-applied systemic insecticide and has a relatively short residual period in comparison to clothianidin and imidacloprid, making it a particularly attractive future option for CGW control.

What other effective chemical options has the project found for CGW control?

The project identified Surround® as a highly promising non-insecticidal chemical option for CGW control n three large-plot field trials. It reduced galling by as much as 96%. Surround® is registered for sunburn protection in citrus but not yet for CGW control. It is an excellent future option for reducing CGW populations in heavily infested blocks.

Three factors may hamper the development and adoption of Surround® for CGW control. First, current cost of Surround® sprays is high due to the high recommended water rate. The project has shown that the cost can be halved by using Du-Wett® as the adjuvant and less water without compromising the efficacy. There may be scopes for further reductions of water rate and hence cost. Secondly, frequent Surround® sprays may lead to an increase of red scale infestations, as indicated in one trial. This side-effect is not surprising as Surround® spray droplets consist of essentially fine clay particles and dust particles are known to be harmful to red scale parasitoids. Thirdly, some growers may be reluctant to use Surround® because of the perception that it does not directly kill the wasps and only push them away to other places. We suspect that although Surround® is non-insecticidal, it may exhaust the wasps and consequently increase their mortality by making them spend more time finding suitable host sites.

In addition to Surround®, laboratory choice and no-choice experiments in the project have confirmed the effectiveness of a petroleum spray oil (PSO) product in repelling CGW adults. Potassium Silicate® also demonstrated a moderate repellence effect against CGW adults in a potted-tree trial. Further investigations of PSO were not conducted as it was not considered a high priority by the project reference group.

Has the project improved the timing of CGW control?

Phenology models have been developed to predict when CGW adults emerge and when eggs laid by the wasps hatch. Cross-validation with independent data showed that predicted median adult emergence date (50% emergence) and median egg hatch date (50% egg hatch) were, on average, within three days of the observed median dates.

To help growers make the predictions, an easy-to-use prediction tool has been developed and is available online. Growers simply select a weather station from a dropdown list and the prediction results will be shown. Three panels of prediction results are presented. The ‘current status’ panel shows the current status of CGW development. The ‘Predicted dates’ panel shows the predicted dates of adult emergence and egg hatch in the current season. The ‘Previous records’ panel shows the recorded dates of adult emergence and egg hatch in previous years.

Adult wasps are the only visible stage in the CGW lifecycle and are only present for about four weeks. Control options targeting the adult wasps such as repellents have to be applied when the adult wasps are most active. The registered methidathion is most effective against newly hatched larvae. The prediction tool allows growers to know in advance of CGW adult emergence date and the following egg hatch date. This gives them time to plan for CGW management in the coming season and organise logistics.

What are the communication strategies of the project?

Growers and citrus industries have been kept up to date with project progress and project findings through a series of grower meetings and industry articles. The project team has also maintained regular contacts with the industries on issues of their concerns in relation to CGW management through face-to-face meetings, telephone conversations, and emails. Industry representatives participated in the review of project progress and discussion of project plans. As a result of the communications, growers and industries have improved understanding of CGW and are aware of available chemical, biological, and cultural options, and online resources for CGW management.

Two demonstration sites were setup in March 2017 in the Sunraysia, one at the 2016 trial site for Surround® efficacy on a farm in Buronga NSW and another at the trial site for pruning on the same farm. The plan was to have field days at the trial sites as part of the Citrus Technical Forum & Field Day 2017 in Mildura but the forum was fully booked and the plan did not eventuate. Findings of the trials have been reported to the industries during the NSW DPI Roadshows in Perth, Riverland, Sunraysia, and Riverina during September/October 2017 and industry articles (see Outputs).

Recommendations

CGW is now widespread in the southern citrus production regions and the infestation is expected to rise in the short-term. There is no need to panic and rush to spray. A holistic and integrated approach based on monitoring, cultural control, biological control, and chemical control is needed to effectively manage CGW.

Monitoring of CGW is best done from autumn onwards when galls are well formed. Galls tend to be more abundant in the lower canopy close to the skirt line. A good monitoring technique is to check underneath the canopy and look for shoots growing at a right angle from the branch. Severity of infestation within a citrus block can be estimated by checking the number of galls of different sizes on randomly selected branch units. A branch unit consists of all shoots in an approximately 30 cm long terminal branch containing 2-3 year's growth. Infestation is rated very low, low, moderate, high, and severe if the number of galls per branch unit is <0.2, 0.2-2, 2-5, 5-8, and >5, respectively. Very low to low infestations are also characterised by predominately small galls (<3cm), whereas high to severe infestations by the common occurrence of medium sized galls (3-5cm).

In citrus blocks where CGW is restricted to isolated trees (very low infestation), the best option is to remove any galls found. Galls cut off within eight weeks of adult wasp emergence should be mulched or burned. Finding and removing all galls in a low-infestation orchard is time-consuming and costly but the pay-off is great. By removing the early invasion galls, the starting CGW population is greatly reduced and it will take longer for the local CGW population to build up to damaging levels. Another option is to release parasitoids. Early establishment of the parasitoids in new CGW incursion areas can help slow down the progression of CGW infestation.

In severely infested blocks, trees can be heavily pruned by hedging and skirting. This can be done in winter following harvest and will remove a large proportion of the local CGW population. Heavy pruning will result in vigorous regrowth which will be highly attractive to the remaining CGW adults when they emerge. The regrowth shoots can be protected by using a registered repellent or systemic insecticide.

Annual pruning will also help reduce CGW populations in moderate to high infestations. Further reductions can be achieved by using a registered systemic insecticide and parasitoid releases. Chemical control may not be needed if the infestation level has been stable over the years or has decreased from last season. For an immediate effect with parasitoid releases, a large number of parasitoids need to be released. Small releases will not provide immediate control but will help build up local parasitoid populations and CGW control in the future. CGW parasitoids are not yet commercially available. To release parasitoids, galls need to be sourced from places known to have high parasitoid populations and placed in the target area. If a release is to be done on a hot day, light watering before releases will improve survival of released wasps.

CGW parasitoids have now established in the southern citrus production regions but their population levels are low in most places. Parasitoid populations will gradually build up over time, reducing the dependence on chemical control. Natural parasitoid populations fluctuate with CGW populations as the parasitoids rely exclusively on CGW for reproduction. Supplementary releases are needed when the parasitoid population has crashed to a low level.

Currently there are three registered chemical options for CGW control: methidathion, Samurai® and Confidor® Guard. Methidathion is a foliar-applied broad-spectrum insecticide and can kill the adult wasps but it is best used to target newly hatched larvae. In the southern citrus production regions, most CGW eggs have hatched by the end of December. So the best timing for methidathion is late December or early January. Use of the chemical for adult wasp control is discouraged as it is highly disruptive to beneficial insects and may not achieve a desired level of control. Female CGW mate and lay eggs immediately after emergence and by the time they are killed by the chemical they have already laid many eggs. Samurai® and Confidor® Guard are soil-applied systemic insecticides. They can be used to control CGW larvae in spring. It is important to wait until after citrus flowering before applying the insecticides to avoid killing bees. Both insecticides are neo-nicotinoids, which have been recently banned in Europe. Growers planning to export to Europe should avoid using these insecticides.

Repeated use of insecticides is not necessary and will promote development of insecticide resistance and may cause outbreaks of secondary pests. When planning for insecticide applications, it is a good practice to set aside a section of the orchard for minimal insecticide use. This will help build up parasitoid populations.

Each CGW female produces over 100 eggs. Even with a 50% natural mortality in all CGW stages (adults, eggs, and larvae), a control level of over 90% is needed to cause a reduction in a CGW population. Such a high level of control is difficult to achieve with a single control technique. Multiple control techniques including pruning, parasitoid releases and chemical applications are needed to reduce CGW infestations.

Adult CGW is the only visible stage in the CGW lifecycle and is only present for about four weeks. Growers planning for CGW control can consult the online CGW timing tool (https://citrsugallwasp.shiny.io/predict/) to find out in advance when the adult wasps are likely to emerge and when the eggs will hatch. The online tool is easy to use. Growers only need to select a weather station closest to their orchard and the prediction results will be shown.

Future R&D is needed to

1. Estimate the impacts of CGW infestation on yield.

   Formation and development of CGW galls consume energy and nutrients. Heavy infestations can potentially reduce yield and fruit size. The yield impacts of CGW infestation have not yet been studied. Quantification of the yield impacts is needed to estimate the economic threshold for CGW control and reduce unnecessary sprays.

2. Investigate cost-effective ways to rear CGW parasitoids.

   Establishing local parasitoid repositories is a key to sustainable CGW management. This project has demonstrated the feasibility of rearing CGW parasitoids using potted trees. Further investigations are needed to optimise the rearing system to increase production and reduce cost.

3. Collect further efficacy/residue data to support the registration of promising chemicals.

   In addition to Samurai® and Confidor® Guard, kaolin and spirotetramat have shown promise for CGW control in the project. The two chemicals are not currently registered for CGW control in citrus. Further efficacy data and residue may be needed to support their registrations.

4. Investigate effective control options for nursery trees.

   Commercial citrus nurseries have expressed concerns over the lack of chemical registration for citrus gall wasp control in nursery trees. Without adequate control, infested nurseries become a source of new infestations. New control options are needed to prevent the spread of CGW throughout the supply chain.

5. Collect CGW phenology data in WA and QLD to expand the application range of the phenology models.

   The online tool for the prediction of CGW adult emergence and egg hatch was based on phenology models developed from data collected in the Riverina and Sunraysia. Phenology data from WA and QLD are needed to validate/update the phenology models before the online tool is extended to cover the two regions.

6. Find safe and effective ways to manage CGW in peri-urban environment.

   CGW is now in the backyards of Melbourne, Adelaide and Perth. In Perth, CGW is not yet found in commercial citrus orchards. Safe and effective control options are needed to manage CGW in backyard trees to slow/stop the spread of the wasp into commercial orchards.

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Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report

Acknowledgements

This project has been funded by Hort Innovation using the citrus research and development levy and funds from the Australian Government. NSW Department of Primary Industries (NSW DPI) provided in-kind support to the project. Fruit Doctors, and WA Department of Agriculture and Food (DAFWA) are collaborators of the project. Project team: NSW DPI – Jianhua Mo, Steven Falivene, Andrew Creek, and Scott Munro; Fruit Doctors – Craig Swanbury, Naomi Galbraith and Narelle Borgmeyer; DAFWA – Sonya Broughton, Bronwyn Walsh, and Helen Newman. Andrew Harty (Costa Group), David Daniels (Citrus Australia), James Altman (Biological Servcies), Dan Papacek (Bugs for Bugs) Justin Lane (Mildura Fruit Company), Mal Wallis (Citri Care, QLD), Scott Dix (Costa Group), and Ben Bring (Costa Group) participated in discussion of project activities and offered advices. Kevin Cock (Buronga NSW), Graeme Beevers (Dareton NSW), Colin Nankivell (Nangiloc VIC), Peter Hill (Loxton SA), Barry Boeck-Hopley (Leeton NSW), Sam Musolino (Griffith NSW), Bart Brighenti (Griffith NSW), Vince Vitucci (Griffith NSW), Laurence Tovo (Griffith NSW), Tony Ciccia (Griffith NSW) provided trial/monitoring sites. Mal Wallis (Citri Care, QLD), Stephen Hegedus, Peter Symens, Jack Dunne, Hannah Draper, John Draper and Grant Napier helped with data collection/processing. Graeme Hardwick (AgNova) and Doug Paton (Sumitomo) supplied test chemicals. Amanda Warren-Smith (NSW DPI) provided comments to an earlier version of the report.

Appendices

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Appendix-2	Pruning timing
Appendix-3	Host selection
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Appendix-1 Branch method

Assessing Your Orchard for CGW—Branch Unit Method

Monitoring Citrus Gall Wasp (CGW)

The severity of citrus gall wasp infestation in your orchard can be assessed over the winter months using the following branch assessment method (adapted from the Queensland monitoring system created by Dan Papacek / *Bugs for Bugs).*

How to monitor

This system uses a 'branch unit' as the basis for monitoring assessments. A 'branch unit' consists of all shoots on an approximate 30cm length of 2-3 year old wood, see *Figure 1*. Note; where gall numbers are high, branches can be cut from the tree to allow easier assessment.

One branch is assessed per tree and the total number of branches sampled will vary depending on block size. *Table 1* provides an indicative guide, the more branches sampled, the more reliable the assessment.

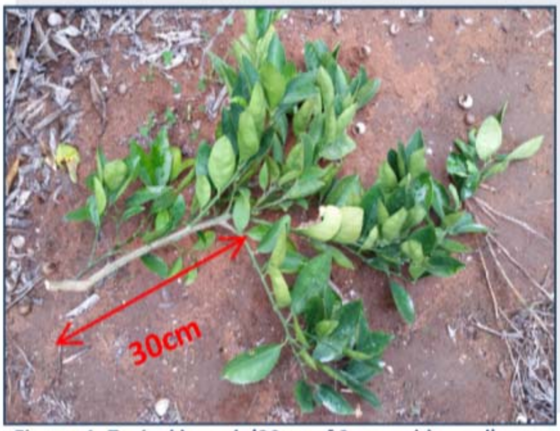


Figure. 1. Typical branch (30cm of 2 year old wood).

Branches should be taken from different aspects of the tree, typically between hip and head height. Count the number of current season galls and record the number under the appropriate size category (see *table 2*).

The ***percentage presence*** is calculated by dividing the number of branches with galls by the total number of branches assessed. The ***number of galls per branch*** is calculated by dividing the total gall number by the total number of branches assessed.

% presence = branches with galls / total branches assessed
= 4 / 10
= 40%

Gall no. per branch = total gall / total branches assessed
= 24 / 10
= 2.4

Table 1. Indicative guide for the number of branches to sample per block .

Block Size	No. Branches
<2 ha	30
2-3 ha	36
3-4 ha	45
4-8 ha	60
8-16 ha	75

Table 2. Example recording sheet.

Branch	Presence / Absence	Gall Length					
		1-3cm	3-5cm	5-10cm	10-15cm	>15cm	Total
1	1	7	3	1			11
2	1	3					3
3	0						
4	0						
5	0						
6	1	5					5
7	0						
8	1	4	1				5
9	0						

Total number of galls

September 2015

Assessing Your Orchard for CGW—Branch Unit Method

What do the numbers mean?

The average number of galls per branch, gall size and the percentage of branches with gall presence help frame the severity of CGW. The higher the level of branch infestation, the larger the galls, and the greater the level of galling per branch the higher the infestation, *Table 3*.

Table 3. Fruit Doctors' rating system.

Overall Rating	Extent of Infestation	Gall Severity	Galls per Branch
Very Low	< 10%	Galls are generally small < 3cm	< 0.2
Low	10-50%	Increasing number of small galls but generally no or very few medium to large galls above 5cm	< 2
Moderate	>50%	Increasing number of small galls, medium to large galls above 5cm present on <30% or branches	> 2
High	>50%	High number of small galls + medium to large galls present on more than 30% of branches	> 5
Severe	90-100%	High number of small galls + medium to large galls present on more than 75% of branches	> 8

The overall rating of a block is allocated based on the block meeting at least two of the three criteria listed for that particular category.

In Queensland, the action threshold for chemical intervention is reached when more than 1 in 3 branch units are found to contain medium sized galls (≥5cm) - this equates to the 'high' rating category in *table 3*. High numbers of small galls (> 90% of branch units) may be considered acceptable in QLD where parasites are known to be present in good number.

In the Riverland, the most significant economic damage appears to occur once infestations reach the high to severe rating category. As parasite abundance is extremely low in our district intervention to manage CGW should occur earlier than QLD with initiatives potentially even taken prior to blocks reaching a moderate rating.

Appendix-2 Pruning timing

Introduction

Pruning is an integral component in CGW management. In newly infested orchards, galls are usually found in isolated clusters of trees. Removing galls before the wasps come out can eliminate or significantly reduce infestation sources and thus delay the spread of infestation to other parts of the orchards. In heavily infested orchards, annual pruning/hedging can significantly reduce local CGW populations. If pruning is done early, cut galls will dry out over time eventually killing the larvae/pupae inside. However, cut galls from late pruning may need to be mulched or burnt to prevent adult emergence. Pruning is also known to stimulate the growth of young shoots, which are the preferred oviposition sites for CGW. This study investigated the effects of pruning timing on (1) adult CGW emergence and (2) the susceptibility of post-pruning shoots to CGW attack.

Materials and Methods

Timing of pruning and CGW survival

The trial was conducted in a block of 'Salustiana' trees on a citrus farm in Leeton, NSW. Twenty medium sized, current season galls were randomly cut off weekly from late August to mid-October 2016. Half of the galls were placed in a basket on the orchard floor in an unshaded area just outside the citrus block and half in a basket under the tree canopy where it was always shaded. In late December 2016 when wasp emergence had completed, the number of emergence holes on each gall was counted. Daily temperature and humidity in shaded and unshaded areas in the orchard during the study period was monitored with Tinytag data loggers (Hastings Data Loggers, Port Macquarie, NSW).

Susceptibility of post-pruning shoots to CGW attack

The investigation was conducted on a citrus farm in Buronga, NSW. Three trees in a block of Valencia orange trees were side hedged on both sides with a chainsaw to a depth of approximately 30cm on five dates between 25 July and 7 October 2016. Two trees in a block of navel orange trees on the farm were similarly hedged on 3 dates between 26 August and 7 October 2016. On 18 May 2017, 20 current season shoots each from a hedged Valencia tree, a hedged navel tree, two un-hedged Valencia trees on either side of the hedged Valencia tree, and two un-hedged navel trees on either side of the hedged navel tree were checked for galls. All galls on selected shoots were measured for length.

Results and Discussion

The last pruning date resulting in zero-emergence of CGW adults from cut galls left in the open was 28-day before gall wasp emergence and that from cut galls left in the shade was 56 days before gall wasp emergence (Table A2-1). It is of interest to note that pruning dates as late as 19 days before adult wasp emergence also largely eliminated emergence of adult wasps from cut galls. Average temperature range during the trial period at the trial site was 10-21°C, which was slightly lower than the mean long-term minimal and monthly temperature range during October-November for the region (12-27°C). As such, the estimated cut-off dates are likely earlier than needed.

In the post-pruning susceptibility trial, spring shoots were produced in abundance following hedging on trees hedged on all dates (Figure A2-1). All post-pruning shoots (100%) were attacked as seen by the presence of galls regardless of pruning date and variety. In Valencia trees, total gall length in shoots from trees hedged in September or August was 39-67% longer than that in shoots from un-hedged trees, whereas that in shoots from trees hedged in September or October were slightly shorter than that in shoots from un-hedged trees (Figure A2-2). In navel trees, total gall length in shoots from trees hedged in August was 56% longer than that in shoots from un-hedged trees. Total gall length in shoots from trees hedged in September or October was again slightly shorter than that in shoots from un-hedged trees (Figure A2-2). The results suggest that pruning in August or earlier may result in larger galls than unpruned trees. The reason was likely because shoots produced from early pruning were longer than those produced from late pruning at the time of CGW attack and longer shoots provided CGW with more oviposition sites than shorter shoots.

Table A2-1. Percentage of cut galls from which no CGW adults emerged for galls pruned on different dates and placed in different positions.

Position	Pruning date [1]					
	19	28	35	41	49	56
Open	2.47 (81)[2]	0.00 (39)	0.00 (39)	0.00 (56)	0.00 (60)	0.00 (43)
Shade	2.17 (46)	3.77 (56)	5.55 (36)	0.00 (48)	2.32 (86)	0.00 (35)

[1] Days before the first CGW adults emerged from uncut galls at the site; [2] Numbers in brackets indicate sample size (N).

In summary, the cut-off date for pruning without the need to burning or mulch cut galls is 56 days or earlier before gall wasp emergence. Such timing would ensure no adult gall wasps would emerge from pruned galls regardless where they are placed (open or shade). Shoots produced after pruning between July and October are all susceptible to gall wasp attack. Larger galls are more likely to develop in shoots produced from earlier pruning than from later pruning. However, late pruning may affect citrus flowering and thus may be impractical.

Figure A2-1. Spring shoots produced after hedging.

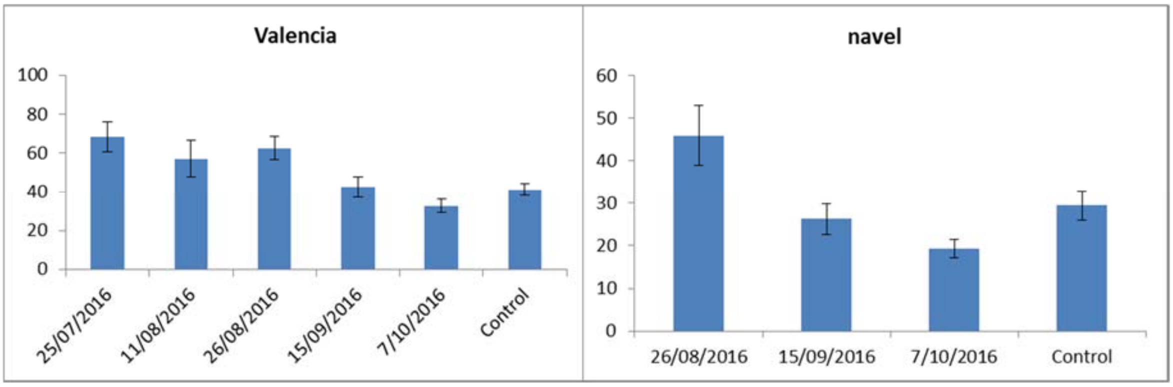


Figure A2-2. Relationship between gall size (length, mm) and pruning date. Wire bars show standard errors.

Appendix-3 Host selection

Introduction

Relatively high CGW infestations have been reported for some citrus varieties such as lemon and grapefruit. In some orchards, heavily infested lemon trees and lightly infested orange trees were found side by side. Sucker shoots (basal shoots grown from the tree trunk below the graft union) are also seen as particularly susceptible to CGW. To verify the reported varietal differences in CGW attack and understand the underlying mechanisms, this study investigated varietal differences in CGW oviposition and the role of plant odour and odour from virgin CGW females on CGW movement.

Materials and Methods

Choice tests with cut shoots

Varietal differences on CGW oviposition in cut shoots was investigated in the laboratory using the same test arena as that used in the investigation of oil repellence (Appendix-8). Freshly cut, current season shoots of different citrus varieties were exposed side by side to CGW males and females in the same test unit for 24 hours at 25°C, after which the shoots were debarked and the number of eggs laid in each shoot was counted under a stereo microscope. Shoot length varied from 10 to 19 cm, however, length of shoots in the same group was similar. The number of CGW adult females introduced per test unit varied from 2 to 119 depending on availability of the wasps at the time of the tests. Ten choice tests were conducted in 2017 comparing 'Eureka' lemon, Valencia orange, and Washington navel. Two to three varieties were compared in each test with a minimum of four replicates.

Choice tests with potted trees

Varietal differences on CGW oviposition in potted trees was investigated in a polyhouse at the Yanco Agricultural Institute during October-November 2017. Potted Washington navel, Valencia orange, Eureka lemon, and Marsh grapefruit trees were placed in five groups (replications) along the perimeter of a 2.5 m-radius circle (Figure A3-1). The sequence of varieties within a group was randomised. CGW adult wasps were released at the centre of the circle by placing cut galls collected previously from lemon trees in a commercial citrus farm in Griffith, NSW. After no more live wasps were spotted either on the galls or potted trees, 10 current season shoots were removed from each tree and the number of eggs in each shoots was counted under a stereo microscope.

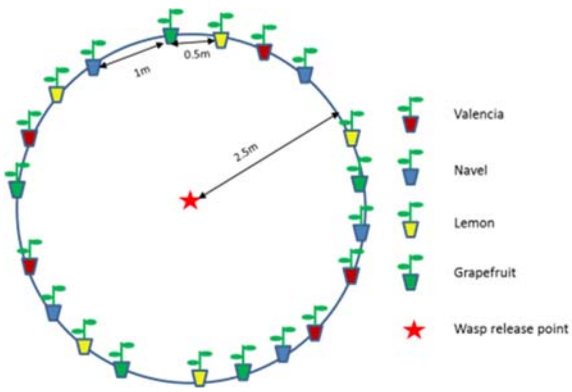


Figure A3-1. Layout of polyhouse choice test in 2017.

The numbers of eggs found in potted trees of different citrus varieties were analysed by general linear models (GLM) for randomised blocks (replication as the blocking factor) followed by analysis of variance (ANOVA) (Venables and Ripley 2002). Where a significant treatment effect was detected by ANOVA, treatment means were separated by Fisher's LSD test (Steel and Dickey 1997).

Y-tube tests

The role of plant odour and odour from virgin CGW females on the orientation (direction of movement) of adult gall wasps were studied in Y-tube tests (Figure A3-2). The Y-tube test unit (olfactometer) has an air purification and delivery unit to clean the air and maintain constant airflow to the two arms of the Y-tubes. Odours from two different sources pass through separate arms of the Y-tube and go out through the stem end. Female wasps were introduced individually at the stem end of the Y-tube. The number of females choosing each arm was recorded.

A series of Y-tube tests were conducted comparing responses of CGW females to odours from leaves of different citrus varieties, leaves from an eucalypt plant (*Eucalyptus cladocalyx*), and paper. Y-tube tests were also conducted investigating the odour response of CGW males to virgin CGW females. All tests were conducted in dark with a centrally placed red light to remove the effects of sight on CGW response. Test leaves and paper were cut to small discs (0. 5 cm diameter) and then placed in gelatin capsules before being placed in the odour source chamber in the Y-tube. Virgin females were also placed in gelatin capsules.

Female gall wasps were used to test odour response to plant materials or paper. Male gall wasps were used to test odour response to virgin female wasps.

Data were analysed by Fisher's exact binomial test (Kinley 1978).

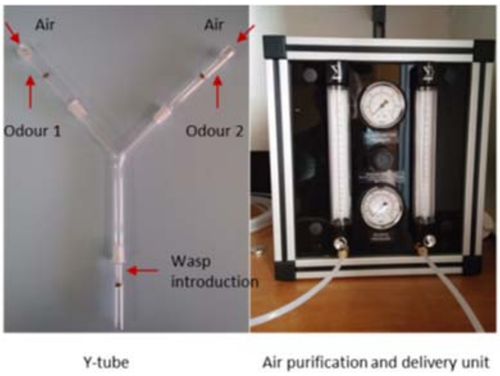


Figure A3-2. Y-tube olfactometer.

Results and Discussion

Five tests compared lemon shoots to Valencia shoots and five comparing lemon, Valencia and navel shoots. In all but one test, cut shoots from lemon, Valencia, and navel trees attracted similar numbers of eggs (Table A3-1). In the only test where a significant difference was detected, Valencia shoots attracted more CGW eggs on more occasions than lemon shoots.

In the polyhouse trial with potted trees, grapefruit trees attracted, on average, over twice as many eggs than trees of other citrus varieties. Lemon trees also attracted over twice as many eggs than navel and Valencia trees. However, the differences were not significant ($F = 0.21, DF = 3, 12, P = 0.2090$) (Figure A3-3). This was due to large within-variety variations in number of eggs.

Female CGW showed a significant preference for odour from Valencia orange leaves to that from eucalypt (*Eucalyptus cladocalyx*) leaves ($P < 0.05$) (Table A3-2). However, they did not discriminate odours from leaves of different citrus varieties or odours from citrus leaves and paper ($P > 0.05$) (Table A3-2). Male CGW showed a significant preference for odour from virgin CGW females to that from paper ($P < 0.05$) (Table A3-2), suggesting presence of female sex pheromone.

It is often observed that grapefruit, lemon, and rootstock trees are more heavily attacked by CGW than orange trees. Our results suggest that, if the varietal differences in CGW infestation are true, the underlying cause is unlikely because female CGW prefers to lay eggs in citrus trees of certain varieties or they are attracted to the odour from these citrus varieties. Two alternative reasons may have been responsible: (1) grapefruit, lemon, and rootstock trees produce more suitable shoots for CGW oviposition during the time when adult CGW are around than orange trees; (2) CGW eggs and larvae survive better in grapefruit, lemon, and rootstock trees than in orange trees.

Table A3-1. Relative attractiveness of cut shoots from lemon, Valencia, and navel trees to CGW oviposition.

Test	Frequency of choice [1]			n [3]	chi-squared	df	P
	Lemon	Navel	Valencia				
1	**1**	NA [2]	**3**	**4**	**5.5**	**1**	**0.0220**
2	2	NA	1	3	0.56	1	0.4561
3	0	NA	2	4	3.5	1	0.0614
4	0	NA	2	4	3.33	1	0.0679
5	0	NA	1	3	1.67	1	0.1967
6	2	2	2	7	0.20	2	0.9058
7	1	1	0	3	1.33	2	0.5134
8	3	2	0	6	4.19	2	0.1229
9	2	2	1	5	2.10	2	0.3504
10	0	4	1	8	5.07	2	0.0792

[1] Frequency of choice: number of replicates in a test where shoots of from a particular host had more CGW eggs than shoots from the other hosts. [2] NA: not included in the test. [3] n: number of replicates in a test.

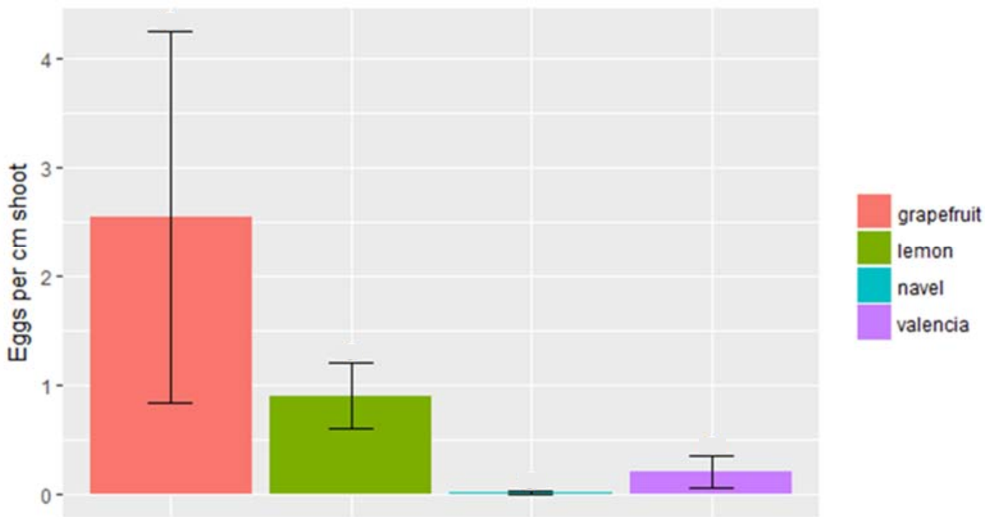


Figure A3-3. Number of eggs (± SE) in potted grapefruit, lemon, navel and Valencia trees. There were no significant differences in the numbers of eggs between any of the citrus cultivars (P > *0.05)*.

Table A3-2. CGW selections in Y-tube tests.

Comparison	№ of wasps tested	№ of wasps selecting A	P
Valencia (A) vs eucalypt (B)	18	14	0.0309
lemon (A) vs Valencia	22	13	0.5235
Lemon (A) vs late lane	43	19	0.5424
Lemon (A) vs paper (B)	32	16	1.0000
Late lane (A) vs paper (B)	17	7	0.8036
Virgin female (A) vs paper	23	18	0.0106

Appendix-4 Dispersal

Introduction

It is commonly observed that adult CGW stay around trees of their emergence. There have also been reports of new CGW infestations turning up in places at considerable distances from existing infestation sites. This study is the first attempt to estimate the dispersal distance of CGW. A commonly used method to study dispersal is mark-recapture (Haffaker and Rabb 1984), in which insects are marked either externally or internally, released at central point, and recaptured by traps placed at varying distances to the release point. The numbers of marked insects recaptured at different distances to the release point provide information on the dispersal distances of the target insects.

Materials and Methods

A mark-recapture experiment was conducted during October-December 2017 in a block of Valencia orange trees in Leeton to estimate the dispersal range of CGW adults. Trees in the experiment block were spaced at 5 m between rows and 3 m within a row. Current season galls were placed in a white tray and covered with fluorescent powder before being placed under a centrally located tree in the block. Emerged wasps from the galls were automatically marked by the fluorescent powder during their emergence process. Four yellow sticky traps were placed on each tree along four concentric rings around the release tree to capture marked wasps (Figure A4-1). There were 12, 24, 46, and 96 trees in the inner most, second, third and the outermost ring. Distance of the trap trees to the release tree varied from 5 to 77 m. The sticky traps were retrieved after wasp emergence had finished. Wasps caught on the sticky traps were examined individually under a UV light to determine if it was marked or unmarked.

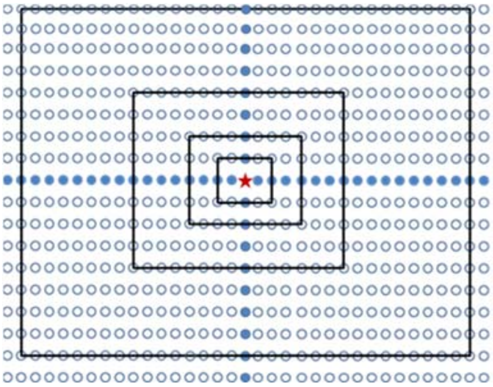


Figure A4-1. Layout of the mark-recapture experiment showing the wasp release point (red star) and four rings around the release tree where every tree on the ring had four yellow sticky traps to capture marked wasps. The right image shows galls covered with green fluorescent powder.

Results and Discussion

Twenty-one marked CGW adults (Figure A4-2) were caught by the yellow sticky traps, of which 20 were caught on the release tree (95%) and 1 on a tree 5 m away (5%). All but one of the captured CGW adult were males. Traps placed further away (8-77 m) did not catch any marked wasps. Interestingly, traces of fluorescent powder were also detected in 10 other traps where no marked CGW adults were present. Three of the traps were positioned over 45 m away from the release tree. While the likelihood of the traces being left by visiting marked wasps cannot be ruled out, it is suspected those traces of fluorescent powder were due to accidental human contamination during trap inspection.

The results support our observations that citrus gall wasps do not fly far and are happy to stay in the same tree of their emergence. However, CGW adults can be carried over long distances by wind or movement of infested citrus materials.

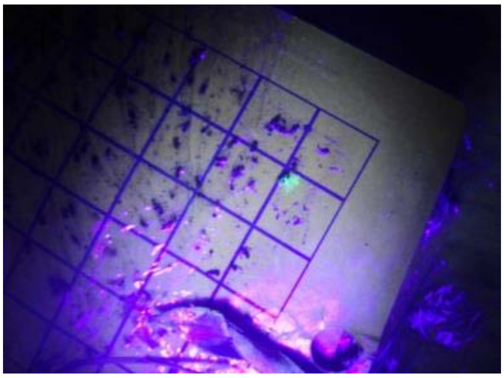


Figure A4-2. A marked CGW adult (green) caught by a yellow sticky trap. The marked wasp appears brightly green under a UV light, in contrast to the dark un-marked wasps.

Appendix-5 Hot-spots of parasitoids

Introduction

After repeated introductions, the two parasitoid species of CGW, *Megastigmus brevivalvus* and *M. trisulcus* have established in the southern citrus regions. Further releases are needed to broaden their establishment. Until recently, the parasitoids were sourced exclusively from Queensland. To reduce release cost, it is desirable that local sites of relatively high parasitoid populations ('hot-spots') be located and reserved as local release sources in the future.

Materials and Methods

CGW parasitoids were released at 15 sites in the Riverina (8 in Griffith and 7 in Leeton) in spring 2015. Parasitoids were sourced from a block of Valencia trees in the Sunraysia and a block of lemon trees in Griffith. The total number of parasitoids released was about 700. In spring 2014 and 2015, about 30,000 parasitoids sourced from Queensland were released at 20 sites across the Riverland (Loxton, New Residence, Pyap, Lyrup, Yamba, Berri, Renmark, Murtho, Paringa, Lindsay Point, Taylorville, and Waikerie). Parasitoids were not released in the Sunraysia as CGW parasitoids are known to have established in the region (Mo *et al*. 2014). Two parasitoid releases were made in spring 2015 in Perth WA.

Population levels of CGW parasitoids were surveyed in the Riverina and Sunraysia in spring 2016 to see if there were any hot-spots of the parasitoids in the two regions. Ten citrus farms with moderate to high CGW infestations were targeted in the survey in each region. A random sample of 50 galls was collected fortnightly on three occasions from each farm starting at the first sight of adult gall wasp emergence. Cut galls were placed in transparent 1.9 L clear plastic bottles with mesh-covered lids for ventilation. The containers were left in a 20°C constant temperature room until after all wasps (CGW or parasitic wasps) had emerged. Emerged wasps were transferred to glass vials containing 75% alcohol and counted by species under a stereo microscope. Parasitism was estimated as the proportion of emerged parasitic wasps in all wasps emerged (CGW or parasitic wasps).

Parasitism level in the Riverland was routinely checked as part of Fruit Doctors' monitoring services. Additional parasitism data were collected from four sites in the region in spring 2016 using a similar method as described above.

Results and Discussion

Results from the 2016 survey showed widespread distribution of CGW parastioids in the Riverina, Sunraysia and Riverland, with parasitoids found at all survey sites. However, parasitism rate was below 5% at most sites. It is encouraging to note the presence of parasitoids at the Riverina and Sunraysia sites where there were no known records of previous parasitoid releases. It is possible the non-release sites may have had unrecorded private releases but the more likely explanation is that the parasitoids had spread from release sites to non-release sites by spring 2015. The results suggest that CGW parasitoids have now widely established in the southern citrus production regions and that no further releases were needed to broaden their establishment.

Hot-spots of 20% or higher parasitism were found at one site in the Riverina and two sites in the Sunraysia (Table A5-1). In addition, galls collected from two lemon trees at a site in the Riverina produced an average of 20 parasitic wasps per gall. The highest recorded parasitism rate in the Riverland was 12% (Table A5-2). This site was one of the first sites used for parasitic wasp releases in the region. It is suggested the hot-spots be protected as local sources of parasitods for future releases with minimal use of insecticides.

Megastigmus brevivalvus was the dominant parasitoid species, accounting for over 80% of all parasitic wasps recovered in the Riverina and the Sunraysia (Figure A5-1). *M. trisulcus* was also consistently recovered but in lower numbers (<15%).

Establishment of released parasitoids in Perth WA were not checked due to the extreme low CGW populations.

Table A5-1. Number of and percent parasitism at the surveyed sites in the Riverina and Sunraysia in spring 2016. Sites in bold were previous release sites of parasitoids.

Riverina			Sunraysia		
Site	№/gall	Para %	Site	№/gall	Para %
MD	0.3	2.3	NK	6.4	13.6
TV	3.0	13.1	**BV**	0.4	1
BG	2.3	5.2	**CK1**	12.4	25.8
VT	0.6	1.5	BV	0.03	0.1
ST	3.9	20.3	**CK2**	13.6	25.8
ML	0.4	3.1	NK	0.8	1.7
BH	0.4	1.7	UN	0.7	NA
DG	20.0	NA*	JN	4.6	NA
GT	0.1	NA	GG	9.9	NA
MR	4.6	NA			

* Parasitism was not estimated due to missing data on early emerged gall wasps.

Table A5-2. Number of and percent parasitism at the surveyed sites in the Riverland in spring 2016

Parasitism rate – monitoring 2016

Site	**Parasitism**	
	Para №	**%**
Bioservices	1024	12.2
LRC	216	6.0
Barry	18	0.4
Loyd	110	0.6

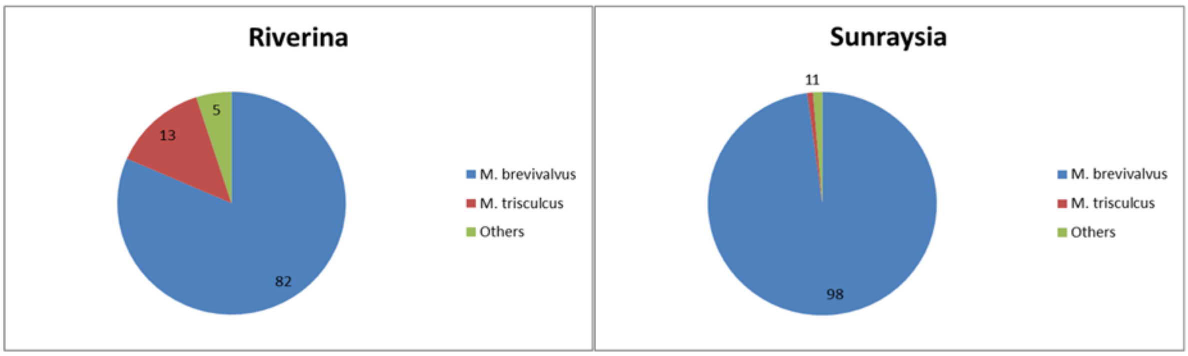


Figure A5-1. Composition of parasitic wasp species in the Riverina and the Sunraysia.

Appendix-6 Effect of heat stress on survival of parasitoids

Introduction

In Queensland, CGW populations still sometimes flare up despite a long history of natural biological control by CGW parasitoids. In recent years, heatwaves have become more frequent and severe in Australia. Heatwaves may destroy parasitoid populations allowing CGW populations to rapidly reach outbreak levels. This study investigated the effect of heat stress on the survival of the primary CGW parasitoid *Megastigmus brevivalvus.*

Materials and Methods

Heat stress was simulated by 1, 2, and 5 hour's exposure to 40°C. Newly emerged *M. brevivalvus* (<48 hour-old) were placed in groups of 10 in 70 ml clear plastic containers. The wasp containers were placed either in a 40°C incubator or in a 25°C incubator (control). After the target exposure periods, wasp containers in the 40°C incubator were moved to the 25°C incubator. Mortality of the wasps was checked 1, 2, and 5-hour later on the first day and then daily until all had died. Four experiments were conducted, two with the test wasps having access to water and two without water access.

Results and Discussion

The primary parasitic wasp species of CGW, *M. brevivalvus*, tolerated up to 5 h of 40°C heat stress without suffering significantly higher mortality as compared to unstressed wasps when they were provided with access to water (Figure A6-1, Exp-1 and Exp-4). When they were deprived of water access, however, heat-stressed wasps suffered 24-61% higher mortality than unstressed wasps within the first day (Figure A6-1, Exp-2 and Exp-3). In particular, over 90% of test wasps subjected to 5-h heat stress died within 24 hours post stress. The results suggest that light watering will improve the survival of released wasps if the releases are to be conducted during a hot day.

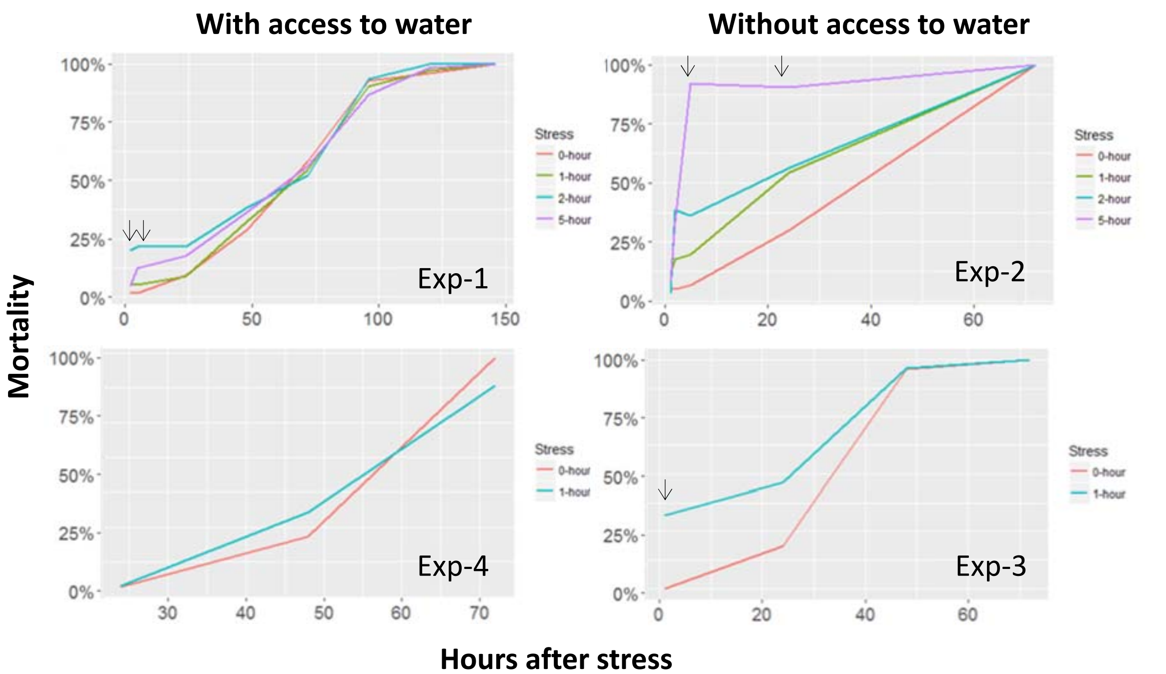


Figure A6-1. Post-stress mortality of test wasps subjected to 0, 1, 2, and 5 hour's heat stress at 40°C. Arrows indicates points of time when mortality rates were significantly different ($P < 0.05$) between stress durations. Test wasps in Exp-1 and Exp-4 had access to water, whereas thouse in Exp-2 and Exp-4 did not have access to water.

Appendix-7 Rearing of parasitoids

Introduction

Following the completion of insecticide screening and efficacy evaluations our focus moved towards rearing and evaluating the Australian native parasitoid *Megastigmus brevivalvus*. The principal motivation was to provide industry with an ecologically based pest management alternative and to reduce insecticide reliance.

A classical biological control approach was thought to have a low chance of succeeding due to a number of factors. Firstly *B. fellis* (CGW) and *M. brevivalvus* have only a single generation each year. Hence a small population of M. brevivalvus would take many years to establish and be out competed by an already established *B. fellis* population. Secondly in the absence of a selective chemical choice growers would then be forced to treat orchards with a systemic insecticide to maintain tree viability before a tipping point was reached. Mass rearing on rootstock trees seemed to provide an opportunity to overcome these constraints by introducing high numbers of *M*. *brevivalvus* to the field in a single season, hastening the rate of establishment and potentially averting or at least deferring the need for chemical intervention.

Materials and Methods

The potential of rearing CGW parasitic wasps was investigated in Loxton, SA. Rootstock trees were enclosed in tent cages (Figure A7-1) at a density of 4 trees per cage. CGW adult females were introduced to the cages over a 5 day period. Parasitic wasps were introduced 7-10 days after final CGW introduction. Four stocking rates (total wasps introduced per cage) and four ratios of parasitic wasps to CGW were investigated: 30 wasps per cage at the ratio of 1:10, 75 wasps per cage at the ratio of 1:4, 100 wasps per cage at the ratio of 1:3, and 150 wasps per cage at the ratio of 1:2. In the following spring/early summer, the number of parasitic wasps reared per cage was counted. In 2016 the first steps were taken to build an *M*. *brevivalvus* mother culture. *M. brevivalvus* were sourced from three locations, two local sites in Loxton S.A. and one from NSW.

Figure A7-1. Tent cages used in rearing *E. brevivalvus*.

Results and Discussion

In the first year approximately 200 two year old citrange trees were inoculated with 17,000 *B. fellis* adult wasps and an estimated 8,000 *M*. *brevivalvus* wasps. An emergence assessment in spring 2017 confirmed establishment with an estimated 60,000 *M. brevivalvus* wasps reared in the first season.

B. fellis and *M. brevivalvus* wasps were reintroduced onto a fresh batch of approximately 200 three year old citrange rootstock trees in spring 2017. Based on trends observed in the first season it is anticipated that these trees will be carrying a high parasitoid load building the culture population to approximately 350,000 wasps.

The intention for spring 2018 will be to expand the *M. brevivalvus* mother culture to approximately 500 trees subject to tree availability and greenhouse space. Multiplication for field release and evaluation will then be possible from 2019 onwards.

Apart from establishing a *M revivalvus* mother culture, an experiment has been implemented to help identify the likely tipping point where the *M. brevivalvus* wasps could be confidently relied upon to provide control of *B. fellis* in the field.

The controlled experiment examined four replicated population scenarios which are summarised in Table A7-1. Results of this experiment will be assessed in spring and early summer of 2018 and be used to guide *M. brevivalvus* field release rates in 2019. In addition it's anticipated that these results will provide a helpful support for shaping management decisions in the field post release and during *M. brevivalvus* establishment.

Table A7-1. Ratio trial for brevivalvus - 2017

Stocking Rate Trial	Sex	21% (4.7:1)	57% (1.75:1)	91% (1.1:1)	150% (0.7:1)
Total CGW introduction (per cage)	Female	1050	420	315	210
	Male	70	70	70	70
Total Brevivalvus introduction (per cage)	Female	119	140	175	210
	Male	119	140	175	210
Daily CGW introduction (per cage) **	Female	150	60	45	30
	Male	10	10	10	10
Daily Brevivalvus introduction (per cage) **	Female	17	20	25	30
	Male	17	20	25	30

*** Introductions spread evenly over 7 days*

Stocking Rate Trial	21% (4.7:1)	57% (1.75:1)	91% (1.1:1)	150% (0.7:1)
Trees / cage	10	4	4	4
Number Cages / Reps	2	2	2	2
Total Trees required	20	8	8	8

Appendix-8 Chemical options

Summary

Chemical options have been investigated for pre-emergence control, adult wasp control, repelling adult wasps, and larval control. Application of foliar insecticides to the galls close to adult wasp emergence did not affect emergence of adult wasps. Adult wasps can be easily controlled by foliar insecticides, some of which have shown good residual activity against the adult wasps. However, foliar insecticides are disruptive to populations of beneficial arthropods in citrus orchards and may not always deliver a desired level of control as female wasps are likely to have laid many eggs before they are exposed to the foliar insecticides. A better control option targeting the adult wasps is the use of repellents. A kaolin based product demonstrated strong repellence to the adult wasps, reducing next-season galls by over 90%. This product has the potential for CGW control in heavily infested citrus blocks but is not registered for this use. Frequent use of this product is discouraged as it may cause local flaring up of red scale populations. Two soil-applied systemic insecticides demonstrated efficacy for larval control in late spring and both now have permits for CGW control/suppression during this period. One of the two systemic insecticides and a foliar-applied systemic insecticide demonstrated efficacy for CGW control during late-summer and autumn, which is encouraging as late-spring applications of systemic insecticides is not feasible for Valencia trees due to the presence of mature fruit.

Introduction

Methidathion was the only registered insecticide for CGW control at the time when this project commenced. It is a broad-spectrum organophosphate insecticide with high mammalian toxicity and harmful to beneficial arthropods. The Australian Pesticides and Veterinary Medicines Authority (APVMA) is currently reviewing this insecticide for possible withdrawal. Project CT10021 identified Biopest® (paraffinic oil) as a potential new chemical option for CGW management (Mo *et al*. 2014) but it is not currently registered for gall wasp control in citrus. It repels CGW adults and thus reduces the chances of current season spring shoots being attacked. In small-plot trials, two sprays of 0.5% Biopest® during late October - early November reduced next season galls by >50%. However, subsequent large-plot trials of paraffinic oils produced inconsistent results (C. Swanburry, personal communication, August 2015). Other possible reasons include insufficient spray coverage and rapid decline of oil repellence with time. There is a need to unequivocally determine the efficacy of oil sprays and, if proven, to ‘fine-tune’ oil sprays with respect to product selection, timing, rate, frequency, and spray volume.

In addition to paraffinic oils, other non-insecticidal chemicals that mask or change host odour/texture may also repel the adult wasps. Surround® WP (950 g/kg processed and refined kaolin) is processed and refined kaolin in the form of a wettable powder. It is registered in Australia citrus for sunburn protection (but not for gall wasp control) and in Canada for protection against a number of insect pests in apple, grape, and vegetables (Anon. 2018). After spray, Surround® WP forms a thin barrier film on the host foliage. The film is highly reflective protecting the foliage from sunburn and heat stress, while at the same time allowing covered plant tissue to breathe. The protective property of Surround® WP against insects is attributed to the barrier film confusing the insects and making the coated host tissues less likely to be accepted for oviposition and feeding. The barrier film may also cause insects to deem the fruit or leaves unsuitable. If insects recognize the crop and land on it, clay particles from the coating may stick to the insects causing them to become agitated and stimulated to move to other more attractive plants.

CGW control by insecticides targets either the adult wasps or larvae. Many foliar insecticides would be effective against the adult wasps as the pest has not been under intensive pressure to develop resistance to any insecticides. The key to adult wasp control is to find insecticides with long residual toxicity so that adult wasps emerged on different dates would be killed before they lay eggs. For larval control, systemic insecticides or insecticides with good translaminar activity are needed. One group of widely used systemic insecticides are neoticotinoids. Despite their negative impacts on bees (Wood and Goulson 2017), neonicotinoids are still widely used in the control of sap-sucking insects worldwide. Project CT10021 trialled one neonicotinoid (imidacloprid) for controlling CGW larvae (Mo *et al*. 2014). The results are not conclusive but suggest the insecticide may be effective. Another neonicotinoid insecticide, clothianidin, was recently registered in Australia for controlling mealybugs, aphids, fruit feeding moths, and fruit flies in tree crops and vines. Spirotetramat is from a different class of systemic insecticides. Unlike neonicotinoids which translocate mainly via plant xylem (Sur and Stork 2003), spirotetramat translocates via both plant xylem and phloem and is particularly effective controlling the juvenile stages of pests (Nauen 2008). This dual translocation mode may enhance the uptake of spirotetramat in in the feeding cells of CGW larvae, which lie between xylem and phloem in the cambium layer (Noble 1936).

A series of field trials, laboratory experiments, and potted tree trials were conducted to investigate alternative chemical options for controlling CGW adults and larvae.

Materials and Methods

Pre-emergence control

Feasibility of controlling CGW close to adult wasp emergence was investigated in three field trials in the Riverina during September-October 2015. The purpose was to see if any chemicals applied to the galls at this late stage would reduce the emergence of adult wasps or kill them during their emergence process. Five insecticides with systemic or translaminar activities were investigated at their label rates: Movento® (240 g/L spirotetramat) at 40 ml/100 L, Supracide® (400 g/L methidathion) at 125 ml/100 L, Actara® (250 g/kg thiamethoxam) at 30 g/100 L, Exirel® (100 g/L cyantraniliprole) at 75 ml/100 L, and Karate® (250 g/L lambda-cyhalorthrin) at 5 ml/100 L. All three trials were designed as completely randomised blocks with 10 replicates. A replicate was a CGW infested tree. On each tree, six similarly sized galls were selected and randomly assigned to each of the five chemical treatments and a control. To determine the number of emerged wasps, the selected galls were individually caged in modified lunch boxes (500 ml) (Figure A8-1) before any wasps had emerged. The base of the lunch box was cut out and replaced with a piece of insect-proof mesh for ventilation. Treatments were applied to the tagged galls with a paint brush at 4, 20, and 33 days before gall wasp emergence in Trial 1, Trial 2, and Trial 3 respectively. After completion of gall wasp emergence, the number of emerged wasps inside each lunch box and the number of emergence holes on each tagged gall were counted. Tagged galls were then debarked to count the numbers of un-emerged gall wasps (adult, pupae or larvae).

Figure A8-1. Lunch box enclosure used in the investigation of chemicals for pre-emergence control of gall wasp.

Adult wasp control

Five unregistered insecticides were investigated for their contact activity against CGW adults in the laboratory at the Yanco Agricultural Institute (YAI) (Table A8-1). The purpose was to find which chemicals had the potential for adult wasp control. Efficacies of test insecticides were compared with the registered methidathion and a water only control. Two experiments tested for direct contact mortality and one for indirect residual contact mortality.

Table A8-1. Insecticides tested for the control of adult CGW at YAI

Product	Active	Rate
Supracide® 400EC	methidathion (400 g/L)	125 ml/100 L
Pyrethrum®	pyrethrins (13 g/L)	30 ml/5 L
Talstar® 250EC	bifenthrin (250 g/L)	20 ml/100 L
Fortune® 500	chlorpyrifos (500 g/L)	50 ml/100 L
Actara®	thiamethoxam (250 g/kg)	30 g/100 L
Success® Neo	spinetoram (120 g/L)	20 ml/100 L

Direct contact mortality was investigated by applying a 0.5 µL drop of an insecticide solution to the thorax of a test wasp with a micro pipette. Test wasps were anesthetised with CO_2 before insecticide applications. After insecticide applications, test wasps were transferred to rearing units in a 20°C constant temperature room, where their survival was monitored daily until all had died. A rearing unit consists of two 70- ml clear plastic vials (43 mm diam. 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 (Figure A8-2). The experiments were designed as complete randomised blocks with five replicates. In each replicate, seven groups of 10 newly emerged adult wasps (< 24-h old, 5 females and 5 males) were tested for each insecticide and control. Test wasps were sourced from current season galls collected before any wasps had emerged. To remove possible effects of source galls on wasp mortality, test wasps used in the same replicate were sourced from the same galls.

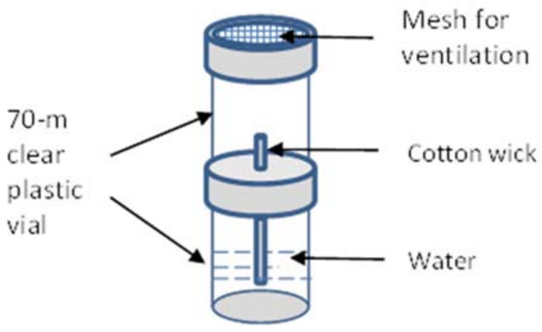


Figure A8-2. Gall wasp rearing unit.

Residual contact mortality was investigated by exposing wasps to leaves from sprayed potted trees. Potted lemon trees (Lisbon on Citrange, 3-year old) were sprayed to runoff with a handheld sprayer. Sprayed trees were left in the shade in a polyhouse. On 0, 3, and 7-days post spray, leaves were removed from sprayed trees and placed in the upper vials of the rearing units. Depending on the sizes of the leaves, 1-2 leaves were placed in each rearing unit to ensure complete coverage of the interior walls of the upper vial. Twenty newly hatched (<24-h old) CGW adult wasps (10 males and 10 females) were introduced to the upper vial of each rearing unit. The rearing units were then placed in a 20°C constant temperature room, where mortality of the test wasps was monitored daily until all had died. Leaves collected on different dates were tested in separate experiments, each designed as completely randomised blocks with five replicates. Test wasps used in the same replicate were sourced from the same galls.

Screening of potential chemicals for adult wasp control was also investigated in a potted-tree trial (Appendix-9).

Adult repellent

1. Field trials of Surround®

The effect of Surround® in repelling CGW adults was investigated in three large-plot field trials (Table A8-2). Two sprays of Surround® 10-15 days apart were applied at a total rate of 7.5 kg/ha. Two trials investigated Surround® at the recommended water rate of 4000 L/ha. One trial compared Surround® applied at 2000 L/ha to 2000 L/ha water rates to see if similar control of CGW can be achieved at a lower water rate. All trials were designed as completely randomised blocks with five replicates. A plot was a rectangular section of citrus trees five rows wide and of 10 trees long. Trials SRD15SUN and SRD15RLD were each conducted in a single block of citrus trees with the same citrus variety and rootstock. Trial SRD16MUL was conducted on three citrus farms, with one replicate in a block of grapefruit trees on a citrus farm in Griffith, NSW, two replicates in a block of Valencia orange trees on a separate citrus farm in Griffith, NSW, and two replicates in a block of Valencia orange trees on a citrus farm in Buronga, NSW.

Two sets of gall data were collected in each trial, one on previous season galls and another on current season galls formed after Surround® applications. Data on previous season galls were collected by the branch method (Appendix-1). Data on current season galls in trial SRD15RLD were collected using the same method and from the same branch units as that for previous season galls. Data on current season galls in the other two trials were collected using a 50 x 50 x 50 cm wireframe. The wireframe was pushed into the tree canopy and all galls within the frame were removed and measured for individual lengths and total weight.

To investigate suspected side effect of Surround® sprays on red scale infestation, trial SRD16MUL also collected data on red scale infestation. Twenty random fruit from each of the 4 central trees in each plot were inspected to count the numbers of fruit infested by 0, 1-10, and >10 red scales.

2. Repellence by oil – proof of efficacy

Efficacy of Biopest® oil in deterring CGW females from laying eggs in citrus shoots was investigated in choice and no-choice experiments in the laboratory. Three choice experiments and three no-choice experiments were conducted. Current season lemon shoots were dipped in water or 0.5-1% Biopest® oil before being exposed to newly emerged CGW adults. The test arena consisted of a wide-mouthed, 1.9 L clear plastic bottle sitting on a 1.3 L bucket filled with water (Figure A8-3). Test shoots were placed vertically in the test arena through a small hole cut out at the centre of the plastic bottle with the lower ends in the water. In choice experiments, shoots from different oil treatments were placed in the same test unit. In no-choice experiments, shoots from different oil treatments were placed in separate test units. The exposure period varied from 24-144 h. After the designated exposure period, test shoots were debarked and checked under a stereo microscope to count the number of eggs.

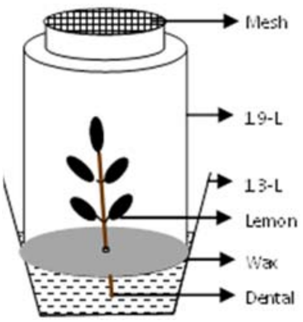


Figure A8-3. Test unit for oil

Potential repellents were also screened in a potted tree trial (Appendix-9).

Larval control

Confidor® Guard (350 g/L imidacloprid), Samurai® (500 g/kg clothianidin), and Movento® (240 g/L spirotetramat), were investigated for larval control in seven field trials, three for larval control in the spring (October-November) and four for larval control during summer to autumn (January-April) (Table A8-2). Confidor® Guard was investigated at 9 ml/tree, Samurai® at 4-12 g/tree, and Movento® at 40 ml/100 L. Confidor® Guard and Samurai® were applied to the soil along the drip lines with pre- and post-application watering as per instructions from the chemical companies. Movento® was applied to the foliage together with Hasten® (adjuvant). All trials were designed as completely randomised blocks with five replicates. A plot was three consecutive trees in the same row. Neighbouring plots in the same row of trees were separated by a 2-tree buffer.

Table A8-2. Summary of field trials

Trial ID	Target	Season	Location	Variety	Treatment [1]
SYS15RLD	Larva – spring	2015-16	Loxton SA	'Chislet' Navel	Confidor Guard 9 ml/tree Samurai 4, 8, 12g /tree Movento 40 ml/100 L control
SYS15SUN	Larva - spring	2015-16	Buronga NSW	Lemon	Confidor Guard 9 ml/tree Samurai 4, 8, 12 g/tree Movento 40 ml/100 L control
SYS17QLD	Larva - spring	2017-18	Munduberra QLD	'Washington' Navel	Confidor Guard 9 ml/tree Samurai 8 g/tree control
SYS16RVR	Larva - autumn	2016-17	Griffith NSW	Valencia	Confidor Guard 9 ml/tree Samurai 12 g/tree Movento 40 ml/100 L control
SYS16RLD	Larva - autumn	2016-17	Loxton SA	'Chislet' Navel	Movento 40 ml/100 L – Jan 25 Movento 40 ml/100 L – Feb29 Movento 40 ml/100 L – Apr 5 control
SYS17RVR	Larva- autumn	2017-18	Griffith NSW	Grapefruit	Movento 40 ml/100 L – Feb 16 Movento 40 ml/100 L – Mar 17 Movento 40 ml/100 L – Apr 13 control
SYS17SUN	Larva- autumn	2017-18	Buronga NSW	Valencia	Confidor Guard 9 ml/tree Samurai 8 g/tree Samurai 12 g/tree control
SRD15SUN	Adult repellent	2015-16	Buronga NSW	Valencia	Surround with Agral 4000 L/ha control
SRD15RLD	Adult repellent	2015-16	Buronga NSW	Valencia	Surround with Agral 4000 L/ha control
SRD16MUL	Adult repellent	2016-17	Buronga NSW [2] Griffith NSW	Valencia Valencia, Grapefruit	Surround with Agral 4000 L/ha Surround withDu-Wett 2000 L/ha control

[1]Movento was applied with Hasten at 50 ml/ha. [2] Two farms in Griffith and one farm in Buronga NSW.

Two sets of data were collected for spring application trials: size and quantity of previous season galls and size and weight of current season galls. Data on previous season galls were collected by the branch method

(Appendix-1). Data on current season galls were collected in May of the following year when current season galls had well-formed using a wireframe. Suppression of gall wasp populations was measured by reduction of galls in treated plots as compared to that in untreated control plots.

Assessment data for summer-autumn application trials were collected during December-January in the following season after gall wasp emergence had completed. A minimum of 10 random galls were removed from each plot and brought to the laboratory, where lengths and numbers of emergence holes of individual galls were recorded. Total weight of all galls in each plot was also recorded in three of the four trials. Suppression of wasp emergence was measured by reduced number of emergence holes per centimetre of gall and/or per gram of gall weight in treated plots as compared with that in untreated control plots.

Potential chemicals for larval control were also investigated in a potted-tree trial (Appendix-9).

Impacts of Samurai on non-target arthropods

Possible impacts of Samurai® on natural enemies of red scale were investigated during November 2016 and March 2017 at the Yanco Agricultural Institute. The investigation targeted the commercially available *Chilocorus circumdatus* ladybird beetle and field parasitism of red scales. Valencia orange trees that were heavily infested with red scale were used for the investigation. Samurai® was applied to three trees at the rate of 8 g/tree on 23 November 2016. The other trees were left untreated. Treated and untreated trees were separated by at least 7 metres. Mature, red scale infested leaves were collected from treated and untreated trees at 10-day before and 7, 15, 42, 56, 70, and 84-day after Samurai application. The leaves were placed individually in glass petri dishes (70 mm diameter) lined with moist filter paper. Five *C. circumdatus* ladybird beetles from Bugs for Bugs (Toowoomba, QLD Australia) were introduced to each petri dish. Survival of the ladybird beetles was checked daily until all had died. Five petri dishes each were used for treated and untreated leaves collected on each of the seven dates.

The effect of Samurai® on field parasitism of red scale was investigated by collecting 20 red scale infested fruit each from treated and untreated trees. The fruit were placed in individual containers with mesh-covered ventilation holes in the lids and left in a 20°C constant temperature room for four weeks. The containers and fruit were then rinsed with 70% ethanol into petri dishes and any parasitoids and predators present were identified and counted. One hundred mature scales from each fruit were checked under a stereo microscope for parasitism.

General impacts of Samurai® on non-target arthropods were invested in a large-plot field trial at the Yanco Agricultural Institute during the same period. Eight small blocks (4-5 rows x 8-10 trees) of Valencia orange trees originally setup for investigation of planting density were used for the trial. The trees were small and the average canopy size was less than half of that of commercial citrus trees. For this reason, we have chosen the half label rate of 4 g/tree for Samurai® in the investigation. Four blocks were randomly selected for Samurai® treatment and the other four as controls. Samurai was applied through driplines on 17/11/2016. D-vac samples of arthropods were collected weekly to fortnightly on eight occasions after Samurai application from both sides of tree canopies of the central trees in each plot. Common arthropods were identified to species. Others were identified to families or higher orders.

C. circumdatus is a red scale predator. The effect of the insecticide on the survival of *C. circumdatus* was investigated in the laboratory by feeding the ladybird beetle adults with red scale collected from treated and untreated trees. The effect of the insecticide on field parasitism of red scale was investigated by random samples of red scale infested fruit from treated and untreated trees. General impacts of the insecticide on the abundance of arthropods foraging in citrus canopies were invested in a replicated, large-plot field trial at the Yanco Agricultural Institute.

Statistical analysis

Oviposition data from choice experiments on PSO were recoded as presence-absence data of eggs and then analysed by chi-squared contingency table tests with Monte Carlo simulations (Hope 1969). All other data were analysed by general linear models (GLM) followed by analysis of variance (ANOVA) (Venables and Ripley

2002). For data from randomised blocks, effect of the blocking factor was removed before estimating the treatment effect. Proportional data (mortality, proportion of emerged wasps, proportions of red scale infested fruit) were 'arcsine' transformed before being analysed. 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 performed in R (R Core Team 2012).

Results

Pre-emergence control

None of the test chemicals significantly reduced gall wasp emergence when applied directly to the galls at 4, 20, and 33-day before gall wasp emergence (Figure A8-4).

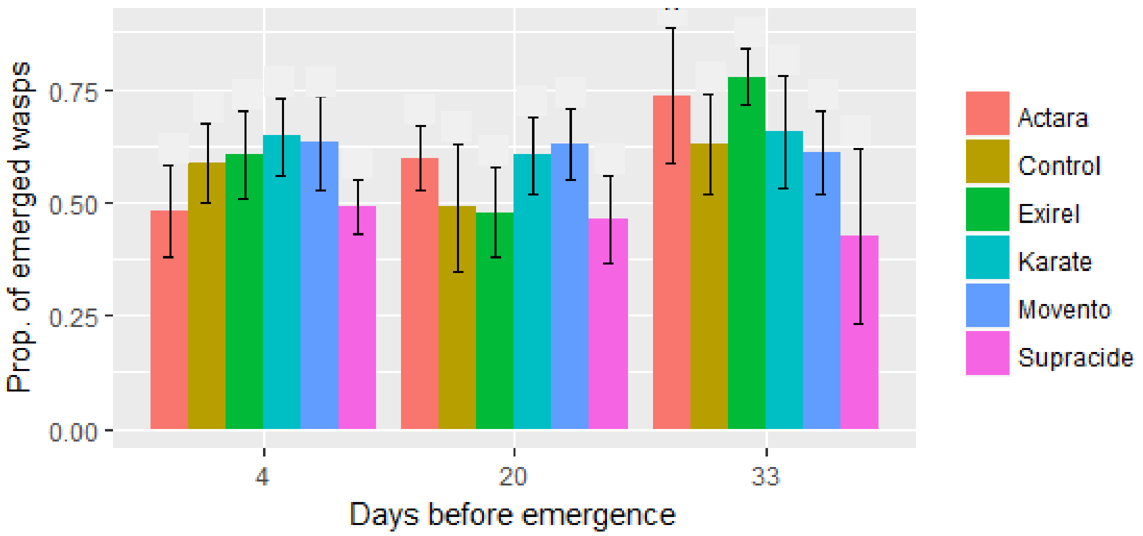


Figure A8-4. Mean proportions (± SE) of emerged wasps from galls treated with the test chemicals on three farms in the pre-emergence chemical control trial. There were no significant differences between treatments within any groups (*P > 0.05*).

Adult wasp control

1. Laboratory experiments

When applied directly on wasp bodies, Fortune®, Success® Neo, Talstar®, Actara® and natural pyrethrum all caused significantly higher mortality in test wasps than water-only control in one experiment (Figure A8-5). In another experiment, only Fortune®, Success® Neo, Supracide®, and natural pyrethrum showed significant direct contact mortality to the test wasps (Figure A8-5).

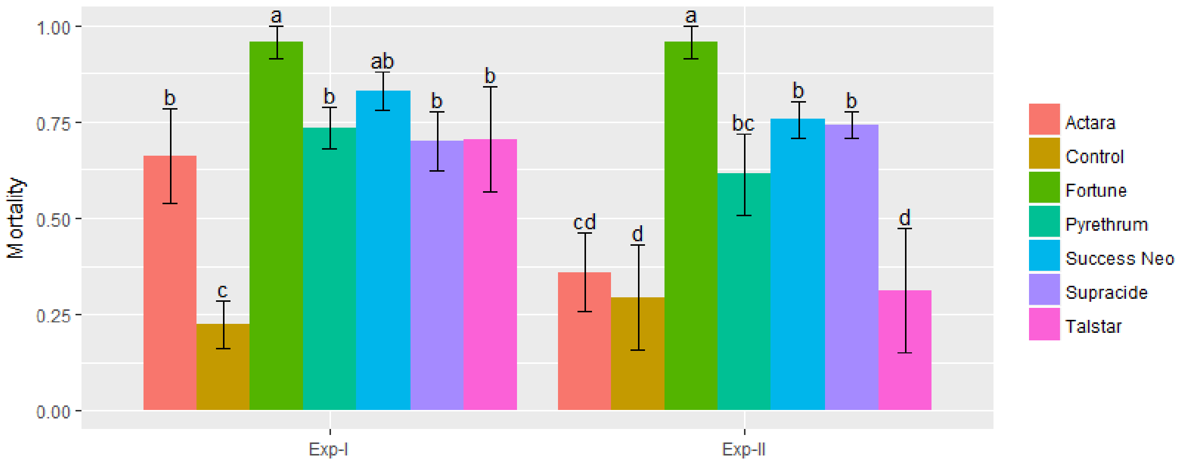


Figure A8-5. Mean first-day mortality (± SE) of test wasps after a small droplet of the test insecticide was placed on them. Bars in the same group labelled with different letters are significantly different at *α = 0.05*.

When applied to citrus leaves, all tested insecticides caused over 90% mortality in adult wasps on the day of spray (Figure A8-6). Leaves treated in with Fortune, Success® Neo, Talstar®, and Actara® remained toxic to adult wasps for up to 7 days, killing > 80% of the test wasps (Figure A8-6). The residual activity persisted to 14 days for Success® Neo, Talstar® and Actara®, which was longer than that for Supracide® (7 days).

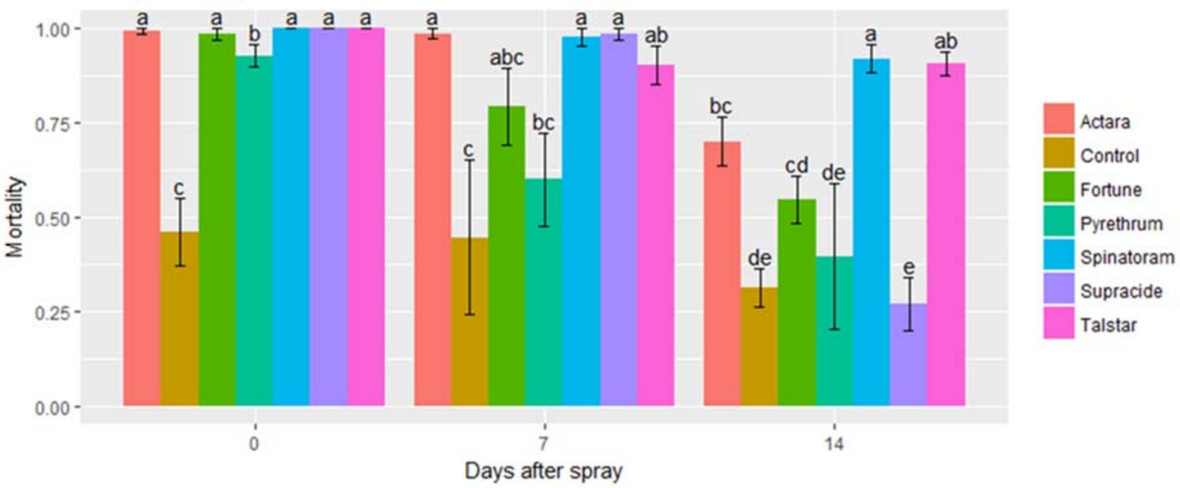


Figure A8-6. Mean first-day mortality (± SE) of adult citrus gall wasps after being confined with citrus leaves picked from trees sprayed with a test insecticide 0, 7, and 14 days ago. Bars in the same group labelled with different letters are significantly different at *α = 0.05*.

2. Potted-tree trial

Single and double sprays (7-days apart) of Talstar® reduced gall wasp emergence holes in the following spring by at least 99% relative to the control (Appendix-9). The next best treatments were single and double sprays of Suprathion® and single and double sprays of Actara® plus oil, reducing emergence by 89-93% and 78-89% respectively. Single and double sprays of Success® Neo provided the least control compared to the other treatments but still reduced emergence by 69-71%.

Adult repellent

1. Field trials of Surround

Trial SRD15SUN

Before the trial, treated and untreated plots had similar numbers of medium sized galls (30-100mm), however, treated plots had significantly fewer small galls (<30mm) than untreated plots and the reverse was true for large galls (>100mm) (Figure A8-7). In the season following Surround® applications, numbers of small, medium, and large galls in treated plots were reduced by 87, 94 and 99% respectively relative to control plots (Figure A8-7). Total gall weight (g) was reduced by 96% in treated plots relative to control plots (Figure A8-7).

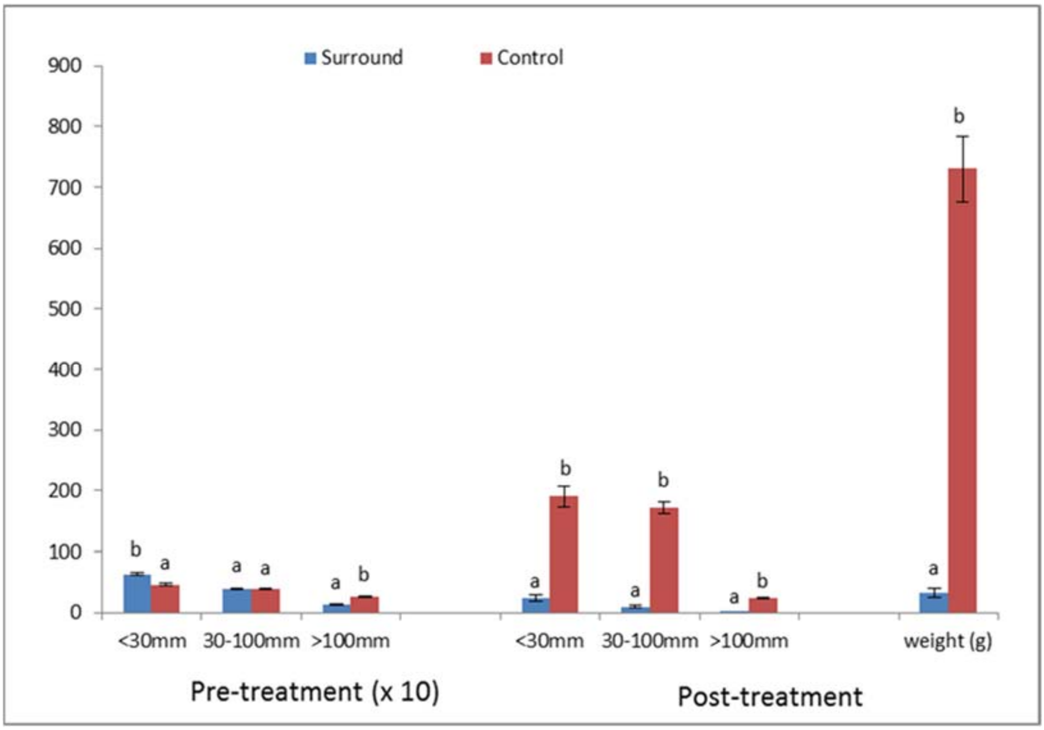


Figure A8-7. Mean numbers of galls (± SE) by size category in treated (Surround®) and untreated (control) plots in the field trial of Surround in Buronga, NSW in 2015/16 (trial SRD15SUN). Surround was applied twice 11 days apart at a total rate of 7.5 kg/ha and the water rate of 4000 L/ha. Pre-treatment data were collected by branch method and the numbers of galls have been multiplied by 10 (e.g pre-treatment <30mm actually about 7 not 70 for Surround). Surround was applied twice at the total rate of Post-treatment data were collected by wireframes. Bars in the same group labelled with different letters are significantly different at $\alpha = 0.05$.

Trial SRD15RLD

Numbers of previous season galls were similar between treated and untreated plots in all size categories (Figure A8-8). In the season following Surround® application, there were 94% less galls in treated plots relative to untreated plots. In comparison to the previous season, total number of galls in Surround treated plots had decreased by 77%, whereas that in control plots had increased by nearly 3-fold.

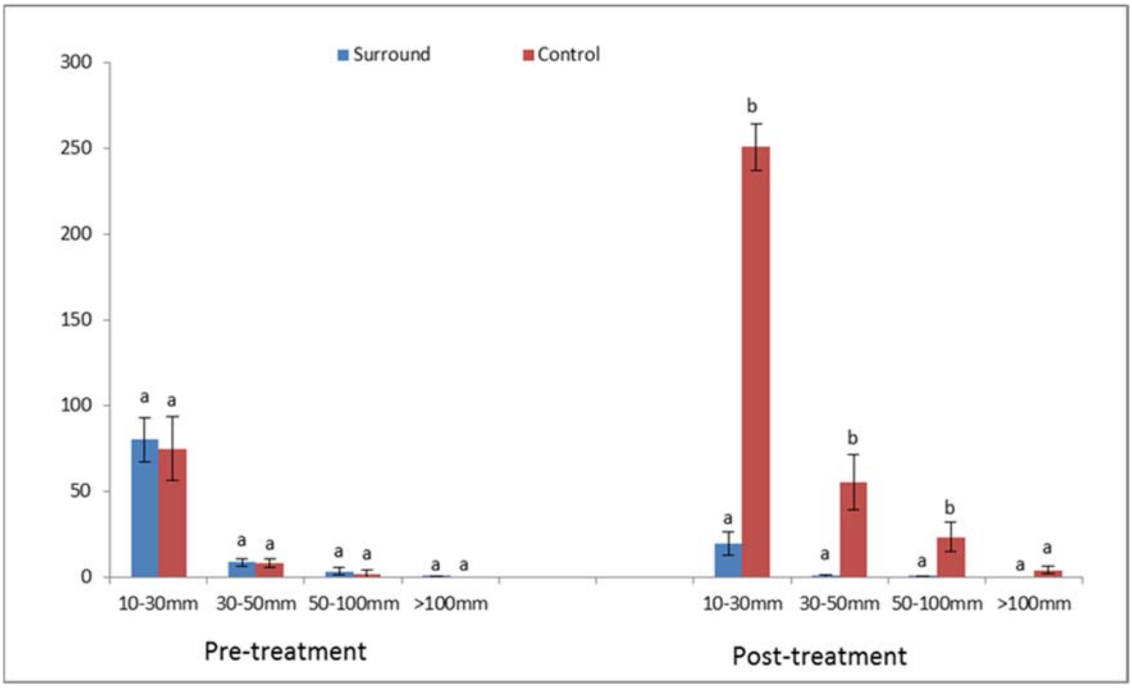


Figure A8-8. Mean numbers of galls (± SE) by size category in treated (Surround®) and untreated (control) plots in the field trial of Surround in Loxton, SA in 2015/16 (trial SRD15RLD).). Surround was applied twice 15 days apart at a total rate of 7.5 kg/ha and the water rate of 4000 L/ha. All data were collected by the branch method. Bars in the same group labelled with different letters are significantly different at *α = 0.05*.

<u>Trial SRD16MUL</u>

Before the trial, all treatments had similar numbers of medium and large galls, however, plots treated with Surround plus Agral® at 4000L water/ha (Agral4000) had significant more small galls than plots in the other two treatments (Figure A8-9). In the season following Surround® applications, numbers of small, medium, and large galls in Agral4000 plots were reduced by 86, 92 and 84% respectively relative to control plots, and those in plots treated with Surround® plus Du-Wett® at 4000 L water/ha (Du-Wett2000) by 78, 75, and 89% respectively (Figure A8-9). The reductions were similar between the two Surround® treatments. Total weight (g) of galls in Agral4000 plots was reduced 90% relative to control plots and that in Duwett2000 plots by 78% (Figure A8-9). The difference was significant at *α = 0.1* but not at *α = 0.05* (*F = 1.14; DF = 2, 8; P = 0.0736*).

Both Surround® treatments significantly increased red scale infestation relative to control plots, with the Agral4000 treatment having twice as more fruit infested by over 10 red scales (Figure A8-10).

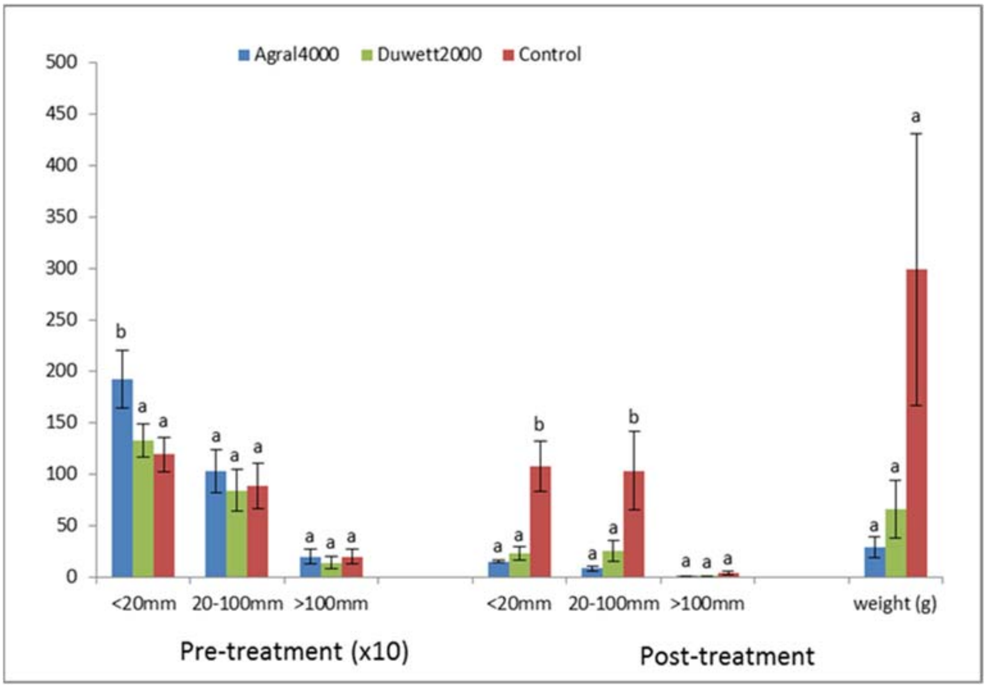


Figure A8-9. Mean numbers of galls (± SE) by size category, and post-treatment gall weight in plots treated with Surround® plus Agral® at 4000L water/ha (Agral4000), plots treated with Surround® plus Du-Wett® at 2000 L water/ha, and control plots in the field trial of Surround in Griffith and Buronga, NSW in 2016/17 (Trial SRD16MUL). Surround was applied twice 10-11 days apart at a total rate of 7.5 kg/ha and the water rate of 4000 L/ha. Pre-treatment data were collected by the branch method and the numbers of galls have been multiplied by 10. Post-treatment data were collected by wireframes. Bars in the same group labelled with different letters are significantly different at *α = 0.05*.

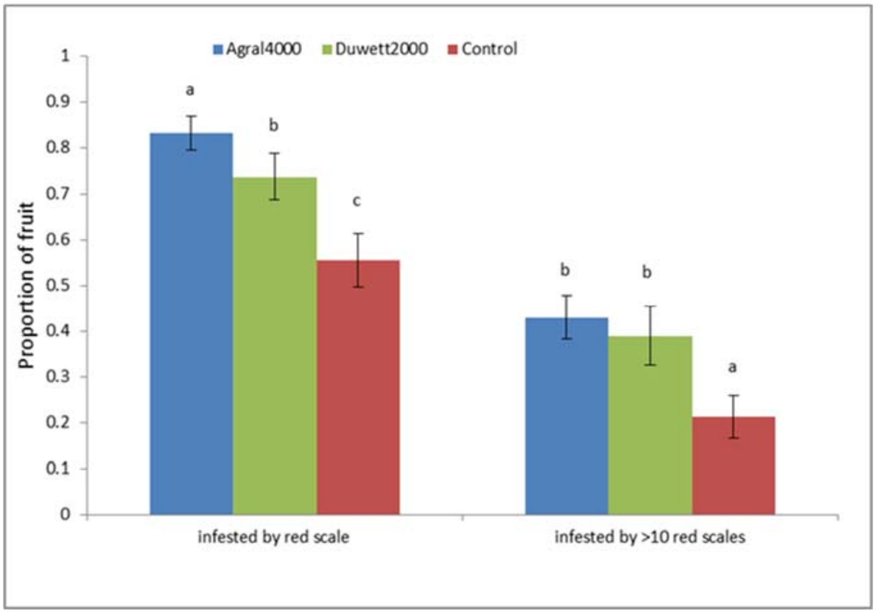


Figure A8-10. Mean proportions (± SE) of red scale infested fruit and fruit infested by over 10 red scales in plots treated with Surround plus Agral at 4000L water/ha (Agral4000), plots treated with Surround® plus Du-Wett® at 2000 L water/ha, and control plots in in the field trial of Surround in 2016/17 (trial SRD16MUL). Bars in the same group labelled with different letters are significantly different at *α = 0.05*.

2. Biopest oil – Laboratory experiments

No eggs were found in any of the test shoots in choice experiment I. In choice experiment II, an average of 4.2 and 3.2 eggs were found in shoots treated with water and 0.5% oil, respectively, and no eggs in shoots treated with 1% oil (Table A8-3). The differences were not significant ($\lambda^2 = 4.2, P = 0.2409$). The oil treatments in this experiment may have deterred CGW oviposition but the sample size was not large enough to detect the effect statistically. In choice experiment III and the three no-choice experiments, CGW eggs were exclusively confined to water treated shoots. The differences were highly significant ($P < 0.001$) in all three no-choice experiments and marginally significant in choice experiment III ($P < 0.1$).

Table A8-3. Effects of Biopest® oil on gall wasp oviposition in current-year lemon shoots

experiment	oil rate	№ of eggs (mean ± se)	№ of shoots	№ of female wasps	exposure period(h)
choice I	0.5%	0	5	11-21	48
	1%	0	5		
	water only	0	5		
choice II	0.5%	3.2 ± 2.0	5	61-108	24
	1%	0	5		
	water only	4.2 ± 4.0	5		
choice III	0.5%	0	5	>50	24
	1%	0	5		
	water only	17.8 ± 13.2	5		
no choice I	0.5%	0	5	4-28	144
	1%	0	5	7-16	
	water only	11.8 ± 6.0	5	3-33	
no choice II	0.5%	0	5	61-131	24
	1%	0	5	84-129	
	water only	68.4 ± 50.7	5	42-235	
no choice III	0.5%	0	6	>50	24
	1%	0	5	>50	
	water only	57.4 ± 34.8	5	>50	

3. Potted-tree trial

Two sprays of Surround and a single spray of silica both demonstrated a repellence effect against CGW (Appendix-9). The double Surround® treatment produced the strongest repellence effect reducing next-season gall wasp population by 81% relative to the control but still had a population increase of 61% in comparison to the number of wasp introduced. The silica treatment reduced next-season gall wasp population by 50% relative to the control however the size of the next-season gall wasp population was 329% of that initially introduced. The single Wettable Sulphur® treatment had no measurable repellence effect against CGW adults.

Larval control

1. Field trials of systemic insecticides – spring applications

<u>Trial SYS15SUN</u>

Gall wasp pressure was similar across the treatments at the start of the trial, as indicated by similar numbers of small, medium, large and very large previous season galls (Figure A8-11). In the season following the treatments, number of very large (longer than 100 mm) galls was significantly reduced in Confidor® Guard and the three rates of Samurai® as compared to that in the control (Figure A8-11). Number of large (50-100 mm) galls was significantly reduced in Confidor® Guard, Movento®, and Samurai® 12 g/tree. Number of medium (10-50 mm) galls was significantly reduced in Confidor® Guard and Samurai® 12 g/tree. Number of small galls (<10 mm) was significantly reduced in all treatments except in Samurai® 4 g/tree. Total weight of current season galls in Confidor® Guard and Samurai 12 g/tree were reduced by 71% and that in Samurai® 4 g/tree and Samurai® 8 g/tree by 42 and 53% respectively (Figure A8-11). The weight reductions were all significant ($P < 0.05$). Movento did not significantly reduce the total weight of current season galls.

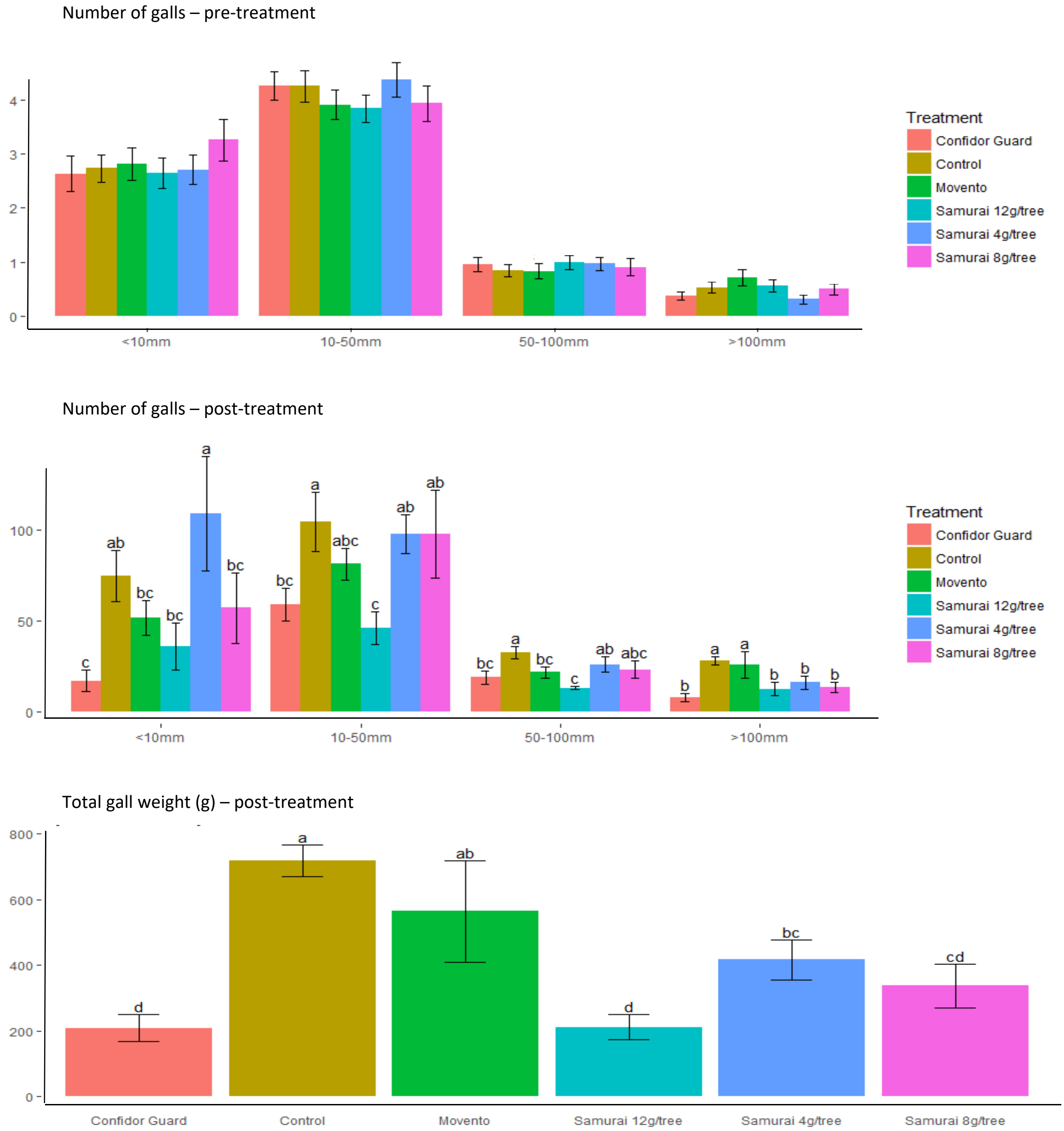


Figure A8-11. Pre- and post-treatment numbers of galls (mean ± SE) by size category and post-treatment gall weight (mean ± SE) in the spring application trial of systemic insecticides in Buronga, NSW in 2015/16 (trial SYS15SUN). Confidor Guard was applied at 9 ml/tree. Movento was applied at 40 ml/100L plus Hasten at 50 ml/100L. Pre-treatment data were collected by the branch method. Post-treatment data were collected by wireframes. Bars in the same group labelled with different letters are significantly different at $\alpha = 0.05$. Pre-treatment numbers of galls did not differ significantly between treatments in any size categories ($P > 0.05$).

Trial SYS15RLD

Gall wasp pressure was similar across the treatments at the start of the trial, as indicated by similar numbers of small, medium, large and very large previous season galls (Figure A8-12). In the season following the treatments, number of very large (longer than 100 mm) galls was significantly reduced in Confidor® Guard, Samurai® 12 g/tree and Samurai® 8 g/tree as compared to that in the control (Figure A8-12). Number of large (50-100 mm) galls was significantly reduced in Confidor® Guard and Samurai® 12 g/tree. Number of medium (10-50 mm) galls and number of small galls (<10 mm) were significantly reduced in Confidor® Guard, Samurai® 12 g/tree and Samurai® 8 g/tree. Total weight of current season galls was significantly reduced in Confidor® Guard, Samurai® 8 g/tree, and Samurai® 12 g/tree, with a reduction rate of 90, 83, and 66% respectively.

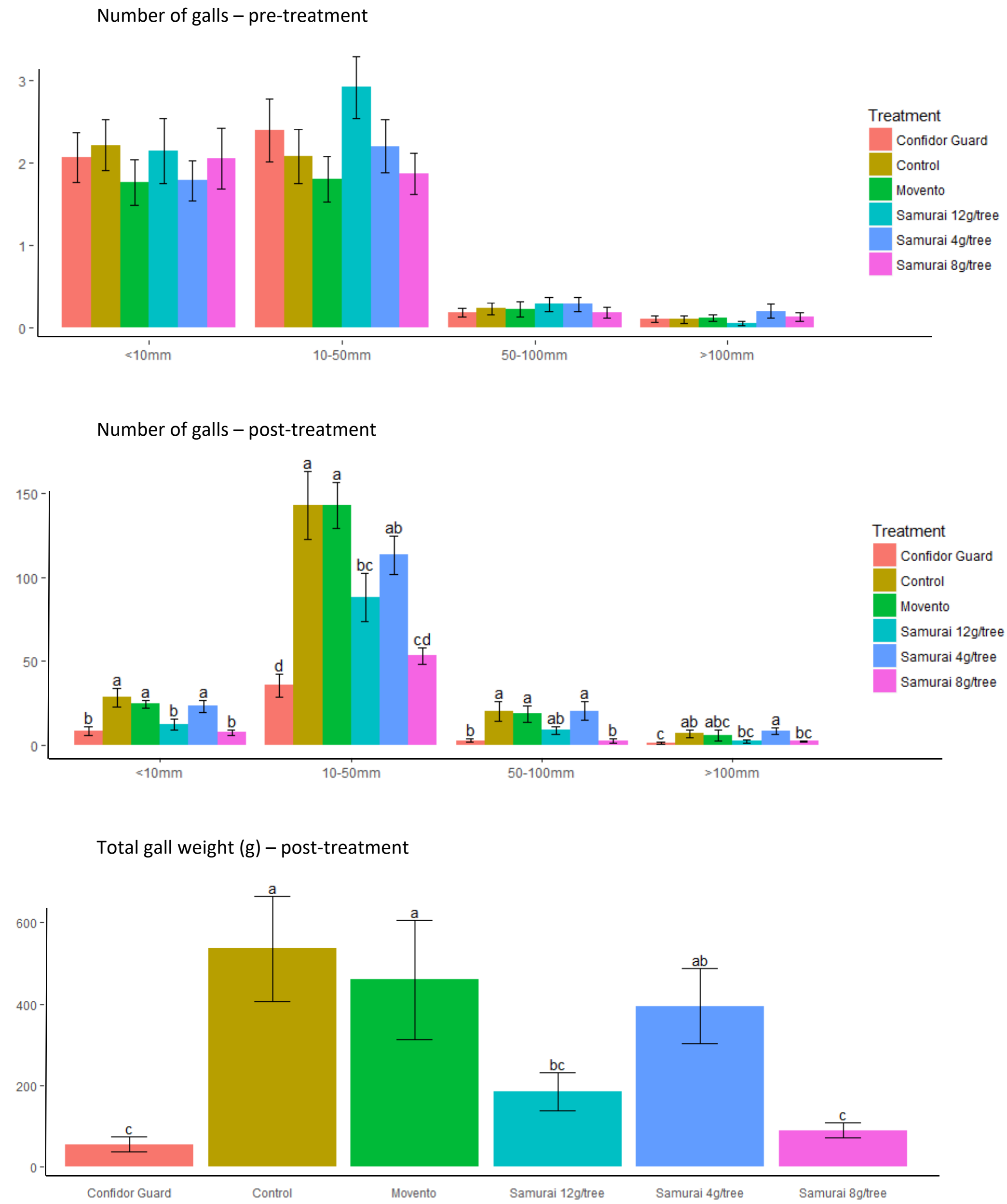


Figure A8-12. Pre- and post-treatment numbers of galls by size category and post-treatment gall weight (mean ± SE) in the spring application trial of systemic insecticides in Loxton, SA in 2015/16 (trial SYS15RLD). Confidor Guard was applied at 9 ml/tree. Movento was applied at 40 ml/100L plus Hasten at 50 ml/100L. Pre-treatment data were collected by the branch method. Post-treatment data were collected by wireframes. Bars in the same group labelled with different letters are significantly different at *α = 0.05*. Pre-treatment numbers of galls did not differ significantly between treatments in any size categories (*P > 0.05*).

Trial SYS17QLD

Gall wasp pressure was similar across the treatments at the start of the trial, as indicated by similar numbers of small, medium, and large previous season galls (Figure A8-13). In the season following the treatments, total weight of current season galls was reduced by 70% in plots treated with Samurai® 8 g/tree as compared to that in control plots and the difference was significant. Confidor® Guard also reduced total gall weight but the reduction was not significant relative to the control.

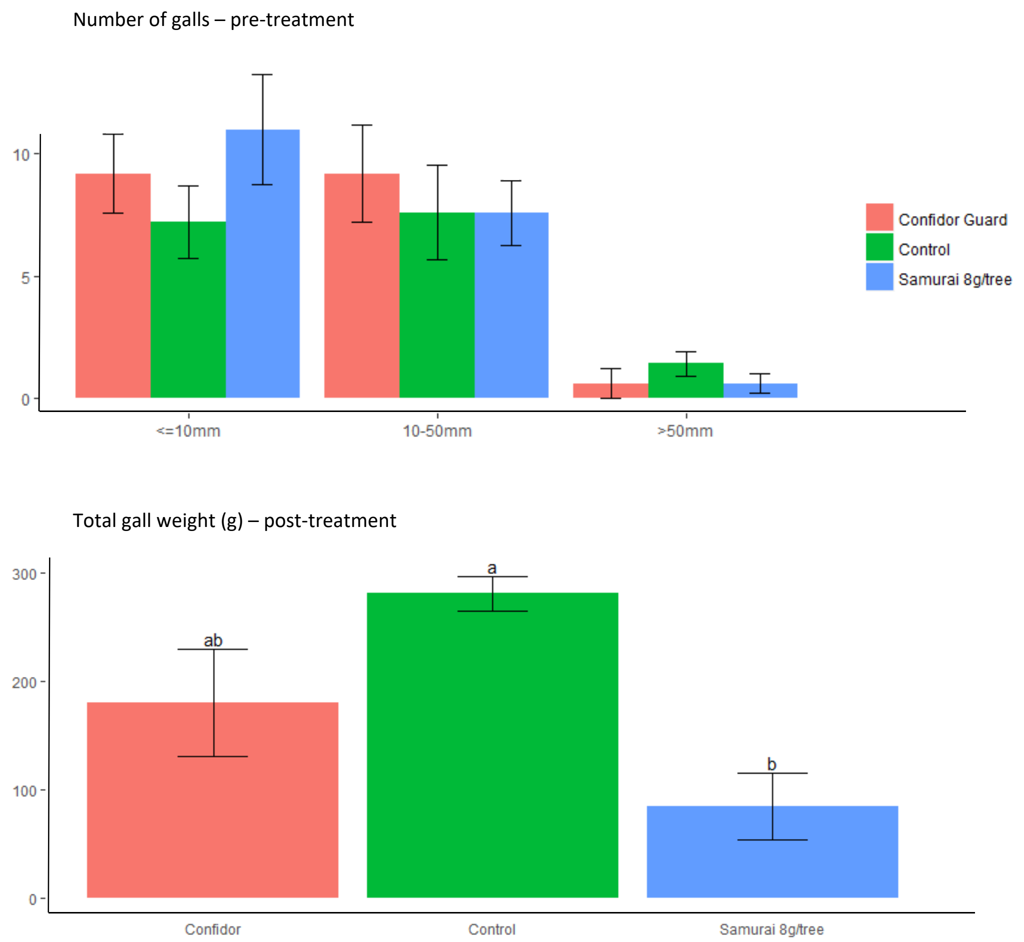


Figure A8-13. Numbers of galls (mean ± SE) by size category at pre-treatment and total gall weight (mean ± SE) at post-treatment in the spring application trial of systemic insecticides in Mundubbera, QLD in 2017/18 (trial SYS17QLD). Confidor Guard was applied at 9 ml/tree. Pre-treatment data were collected by the branch method. Post-treatment data were collected by wireframes. Bars in the same group labelled with different letters are significantly different at $\alpha = 0.05$. Pre-treatment numbers of galls did not differ significantly between treatments in any size categories ($P > 0.05$).

2. Field trials of systemic insecticides – summer-autumn applications

Trial SYS16RVR

Autumn applications of Movento® at 40 ml/100 L plus Hasten at 50 ml/tree and Samurai® 12 g/tree applied reduced gall wasp emergence by 69 and 62% respectively relative to the control (Figure A8-14). By contrast, Confidor® Guard at 9 ml/tree did not suppress gall wasp emergence.

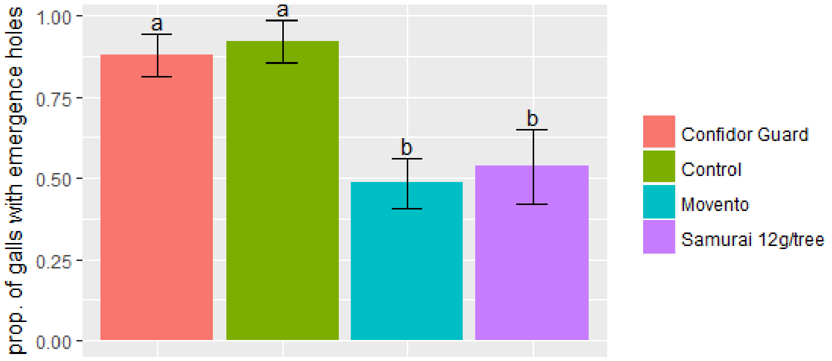


Figure A8-14. Mean proportions (± SE) of galls with emergence holes in the summer-autumn application trial of systemic insecticides in Griffith in 2016/17 (trial SYS16RVR). Confidor Guard was applied at 9 ml/tree. Movento was applied at 40 ml/100L plus Hasten at 50 ml/100L. Bars labelled with different letters are significantly different at *α = 0.05*.

Trial SYS16RLD

Movento® at 40 ml/100 L plus Hasten® at 50 ml/tree applied on January 25, February 23, and April 5 all significantly suppressed gall wasp emergence, reducing the number of emergence holes per 100g of galls by 71, 95, and 98% respectively relative to the control (Figure A8-15). Significantly better suppression was achieved with the February 23 and April 5 timings than with the January 25 timing.

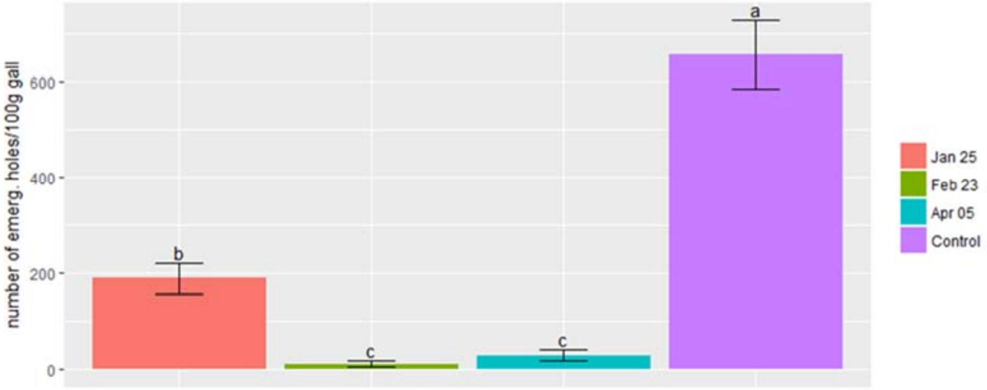


Figure A8-15. Mean numbers of CGW emergence holes per 100 g of galls (± SE) in trees sprayed with Movento® at 40 ml/100 L plus Hasten® at 50 ml on three different dates in the summer-autumn application trial of Movento in Loxton, SA in 2016/17 (trial SYS16RLD). Bars labelled with different letters are significantly different at *α = 0.05*.

Trial SYS17RVR

Movento® at 40 ml/100 L plus Hasten at 50 ml/tree applied on March 17 and April 16 significantly suppressed gall wasp emergence, reducing the number of emergence holes per centimetre gall length by 62 and 80% respectively relative to the control (Figure A8-16). No significant suppression was seen in the February 16 timing.

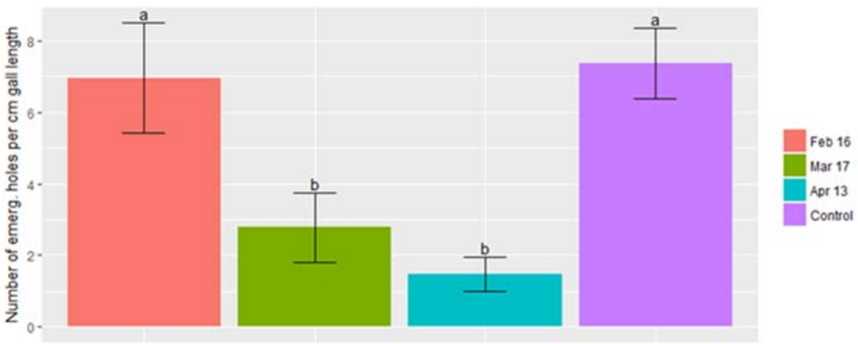


Figure A8-16. Mean numbers of CGW emergence holes per cm gall length (± SE) in trees sprayed with Movento® at 40 ml/100 L plus Hasten® at 50 ml/tree on three different dates in the summer-autumn application trial of Movento in Griffith, NSW in 2017/18 (trial SYS17RVR). Bars labelled with different letters are significantly different at *α = 0.05*.

<u>Trial SYS17SUN</u>

Autumn applications of Samurai® at 8 g/tree and 12 g/tree significantly suppressed gall wasp emergence, both reducing the number of emergence holes per centimetre gall length by 55% relative to the control (Figure A8-17). No significant suppression was observed in the Confidor® Guard 9 ml/tree treatment.

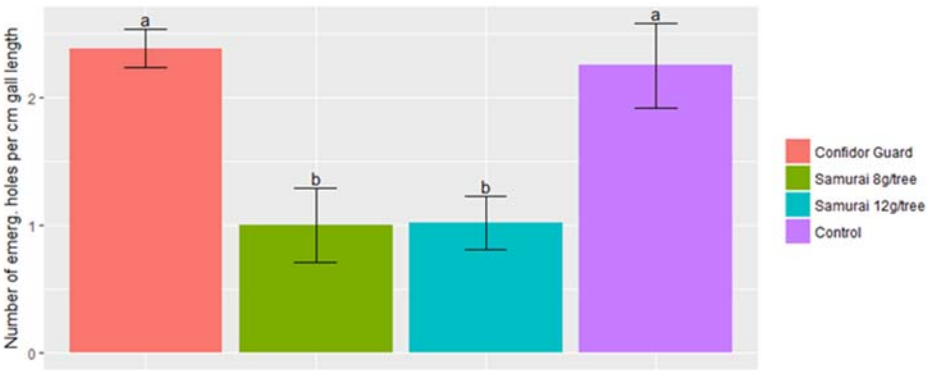


Figure A8-17. Mean numbers of CGW emergence holes per cm gall length (± SE) in trees sprayed with Confidor® Guard at 9 ml/tree, Samurai® at 8 g/tree and Samurai® at 12 g/tree in the summer-autumn application trial of systemic insecticides in Buronga, NSW in 2017/18 (trial SYS17SUN). Bars labelled with different letters are significantly different at *α = 0.05*.

3. Potted tree trials

Single applications of Samurai® at 3 g/tree and Suprathion® at 125 ml/100 plus Hasten® at 200 ml/100 in December provided 100% control of gall wasp larvae in potted trees as indicated by nil emergence of adult gall wasps in the following spring (Appendix-9). A single application of Confidor® Guard at 1 ml/tree in December reduced the number of emergence holes by 99% relative to the control. A single application of Movento® on March 5, double applications of Movento® in December, and double applications of the Bayer experimental chemical DC-154 in December provided moderate levels of control of gall wasp, reducing next-season wasp emergence by 76-85% relative to the control. The other treatments also reduced gall wasp emergence but levels of control were much lower (48-68%).

4. Impacts of Samurai on non-target arthropods

No significant negative impact of Samurai® was observed on the survival of the red scale predator *Chilocorus circumdatus* in the laboratory after 15 days of treatment application (Figure A8-18). There was, however, a slight but significant reduction of the lifespan (< 20%) at 7-day post-treatment compared with the control. Field samples of red scale infested fruit from Samurai® treated trees and control trees showed similar numbers of the red scale parasitoid *Aphytis* spp. and the red scale predator *Rhyzobius lophanthae* (Figure A8-19). However, significantly more *Comperiella bifasciata* were recovered from untreated control trees than from Samurai treated trees. *C. bifasciata* is a common predator of the red scale in the southern citrus regions.

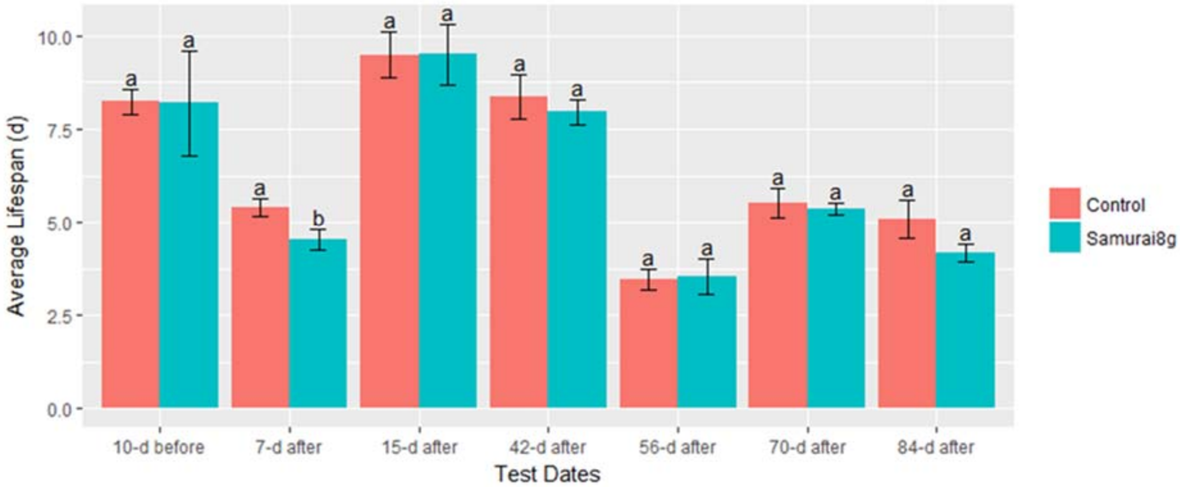


Figure A8-18. Mean Lifespan of *Chilocorus* ladybird beetles fed on red scales from Saumrai® treated trees and control trees. Bars labelled with different letters are significantly different at $\alpha = 0.05$.

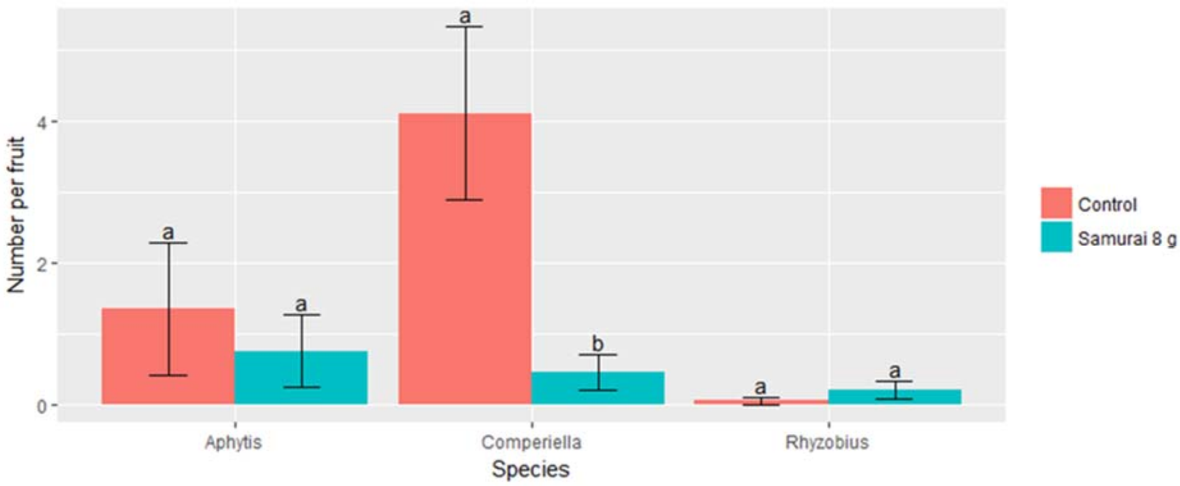


Figure A8-19. Numbers of red scale parasitoids *Aphytis* spp., *Comperiella bifasciata*, and red scale predator *Rhyzobius lophanthae* from trees treated with Samurai® 8 g/tree and control trees. Bars in the same group labelled with different letters are significantly different at $\alpha = 0.05$.

Results from a large-plot field trial showed that Samurai® applied at equivalent label rate did not significantly affect the abundance of general parasitic wasps, predatory beetles, other predatory insects and mites, and spiders foraging in citrus canopies at 8-100 day post application (Figure A8-20). The only exception was the abundance of ants, which was significantly lower in Samurai® treated trees than in control trees.

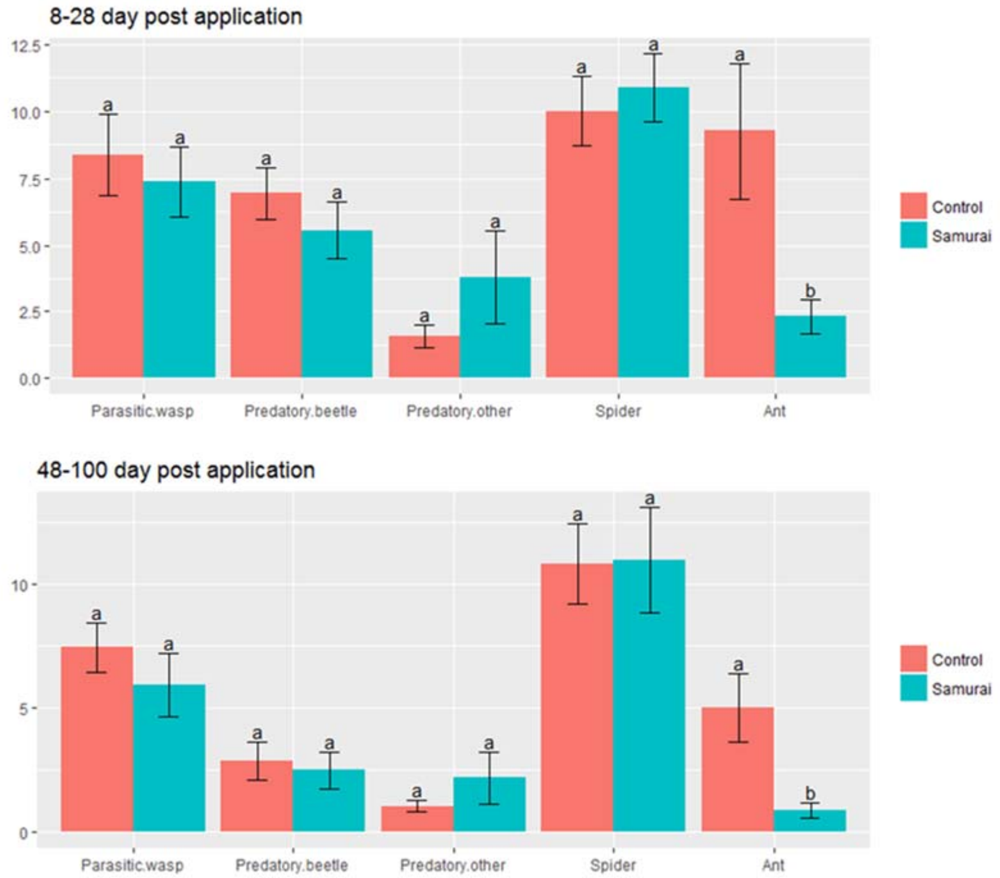


Figure A8-20. Mean abundance (± SE) of selected groups of non-target arthropods in canopies of Samurai® treated trees and control trees 8-28 day post application and 48-100 day post application as estimated by D-Vac samples. Bars in the same group labelled with different letters are significantly different at *α = 0.05*.

Discussion

A series of laboratory, polyhouse, and field trials/experiments were conducted to investigate new chemical options for CGW control. New chemical options were investigated for (1) pre-emergence control, (2) adult wasp control, (3) repelling adult wasps, and (4) larval control. Several promising new chemical options have been identified as a result of these investigations. Efficacy data collected in the project have resulted in the registrations of two new chemicals for citrus gall wasp control in Australia. Efficacy data for two other promising chemicals have been sent to the chemical companies for consideration of future registration.

Pre-emergence control targets mature CGW larvae, pre-pupae and pupae. Five insecticides with systemic or translaminar activities were directly applied to the galls 4, 20 and 33 days before adult wasp emergence. None reduced the emergence rate. The results were not surprising as two of the targeted stages, pre-pupae and pupae, were non-feeding stages and therefore would not be affected by the insecticides. Some mature larvae may have been present in the trial for the 33-day pre-emergence timing but they would be the minority compared to pre-pupae and pupae. The results also suggest that insecticides applied to the galls close to gall wasp emergence were either present at a non-toxic level on the gall surface or not picked up by the adult wasps during the emergence process.

Adult wasp is the only exposed stage in the CGW lifecycle. Five foliar insecticides were investigated at label rates for their activity against the adult wasps in the laboratory: Fortune® (500 g/L chlorpyrifos), Success® Neo (120 g/L spinetoram), Talstar® (250 g/L bifenthrin), Actara® (250 g/kg thiamethoxam) and natural pyrethrum. All demonstrated good contact toxicity against the adult wasps. Success® Neo, Talstar® and Actara® sprayed leaves remained toxic to the adult wasps for 14 days, longer than that for the registered Supracide (7 days).

In a potted-tree trial, Talstar® reduced next-season gall wasp population by 99% and Supracide® and Actara® by 78-89%. The results suggest adult citrus gall wasps can be readily controlled by many foliar insecticides. However, relying on foliar insecticides for CGW control has two major drawbacks. First, most of the insecticides are broad-spectrum. Frequent use of these insecticides risks decimating populations of beneficial arthropods in citrus orchards and consequently causing outbreaks of secondary pests. Secondly, as CGW females lay eggs immediately after emergence, some females may have already laid many eggs before they were exposed to and killed by the foliar insecticides.

An alternative to direct control of the adult wasps is the use of non-insecticidal chemicals that repel the adult wasps and thus reduce eggs laid in citrus shoots. Repellents are more IPM-compatible than foliar, broad-spectrum insecticides and strong, persistent repellence can lead to a significantly reduction in local gall wasp populations. Four potential repellents were investigated in the project: oil, silicate, sulphur, and kaolin. A petroleum spray oil (PSO) product, Biopest®, showed some potential in repelling the adult wasps at the application rate of 0.5% in small-plot trials in a previous investigation (Mo *et al*. 2014). In response to conflicting reports on the effects of CGW control with spray oils in commercial-scale trials, choice and no-choice experiments were conducted to establish/reject proof of efficacy for Biopest®. The results confirmed the repellence effect of Biopest® at 0.5%, however, complete repellence (elimination of oviposition in treated citrus shoots) was only achieved at the 1% rate. Considering that the same rate was also used for red scale control in citrus with PSOs, we believe PSOs remain as viable repellents for CGW control. Use of high-quality PSO and good coverage are essential for best effects (Beattie 2002). Potassium Silicate® also demonstrated a moderate repellence effect against adult CGW in a potted trial in this project. Field efficacy data are needed to validate the repellence potential of this product.

Kaolin is naturally occurring clay resulting from weathering of aluminous minerals. Kaolin-based Surround® is registered for sunburn protection in horticultural crops including citrus in Australia. In the US, Surround® is also labelled as supressing insects. Three mechanisms have been suggested for the insect suppressing property of the product: (1) it repels insects by creating an unsuitable surface for feeding or egg-laying, (2) it disrupts insect's host finding capability by masking plant tissue colour, and (3) it irritates insects when kaolin particles become attached to their bodies triggering an excessive grooming response (Showler 2002, Glenn and Puterka 2005, Silva and Ramalho 2013). Three large-plot field trials were conducted to collect efficacy data of Surround® for CGW control. Surround® demonstrated strong repellence against the adult wasps in all three trials. Two applications of Surround® applied at a total rate 7.5 kg/100 L and a water rate of 4000L/ha in late October reduced next-season galls by over 90% relative to untreated control. In one trial the reduction of galls was also seen across seasons, indicating a population decrease of CGW at the trial site. Three factors may limit the use of Surround® for CGW control. First, current cost of Surround® sprays is high due to the high recommended water rate. We have shown that the cost can be halved by using Du-Wett® as the adjuvant and less water without compromising the efficacy. There may be scope for further reduction of water rate and hence cost. Secondly, frequent Surround® sprays may lead to an increase of red scale infestations, as indicated in one trial in the project. This side-effect is not surprising as Surround® spray droplets consist of essentially fine clay particles and dust particles are known to be harmful to red scale parasitoids (Bugs for Bugs 2018). Thirdly, some growers may be reluctant to use Surround® because of the perception that it does not directly kill the wasps and only push them away to other places. We suspect that although Surround® is non-insecticidal, it may exhaust the wasps and consequently increase their mortality by making them spending more time finding suitable host sites.

Larva is the longest of all CGW lifecycle stages lasting over 10 months. CGW larvae feed and develop inside galls, which protect them from predators, airborne pathogens and most contact chemicals. Chemical control of the larvae is possible only with systemic insecticides or insecticides with strong translaminar activity. In this project, we investigated three systemic insecticides for the control of gall wasp larvae: Confidor® Guard, Samurai®, and Movento®. Seven replicated field trials were conducted, three investigating spring applications and four summer-autumn applications of the insecticides. Confidor® Guard at 9 ml/tree and Samurai® at 8 g/tree demonstrated significant efficacy for larval control in the spring, reducing next-season galls by 53-90%. Results from potted-tree trials were even more promising. Both insecticides and Suprathion® reduced next-season CGW population by 99% and over. The efficacy data have resulted in the registrations of the two chemicals for CGW control/suppression in citrus. For larval control during late-summer and autumn, Samurai® at 8 g/tree and Movento® at 40 ml/tree plus Hasten® at 50 ml/tree were promising. Samurai® applied in

March/April showed a similar level control of CGW larvae as when it was applied in late spring. March/April applications of Movento® reduced gall wasp emergence in the following spring by up to 98%. Demonstration of the autumn efficacy of Samurai® and Movento® is encouraging as it opens the potential for growers to control CGW in Valencia trees, for which spring applications of systemic insecticides are not feasible due the presence of mature fruit and consequent residue concerns.

The approval of a permit for the use of Samurai® for CGW control in citrus has warranted an investigation of the insecticide on beneficial arthropods. Our investigations in the laboratory and field did not detect significant negative impact of Samurai® on the survival of the red scale predator *Chilocorus circumdatus* or overall abundance of parasitic wasps and predatory beetles in citrus canopy. However, Samurai® has shown evidence of reducing the number *Comperiella bifasciata* emerging from red scales. *C. bifasciata* is a common predator of the red scale in the southern citrus regions. More studies are needed to confirm the negative impact.

In summary, chemical options have been investigated for pre-emergence control, adult wasp control, repelling adult wasps, and larval control. Gall wasp control close to adult wasp emergence does not appear to be a valid option. Adult wasps can be easily controlled by foliar insecticides, some of which have shown good residual activity against the adult wasps. However, foliar insecticides are disruptive to populations of beneficial arthropods in citrus orchards and may not always deliver a desired level of control as female wasps are likely to have laid many eggs before they are exposed to the foliar insecticides. A better control option targeting the adult wasps is the use of repellents. A kaolin based product demonstrated strong repellence to the adult wasps, reducing next-season galls by over 90%. This product has the potential for CGW control in heavily infested citrus blocks. Two soil-applied systemic insecticides demonstrated efficacy for larval control in late spring and both now have permits for CGW control/suppression during this period. One of the two systemic insecticides and a folia-applied systemic insecticide demonstrated efficacy for CGW control during late-summer and autumn, which is encouraging as late-spring applications of systemic insecticides is not feasible for Valencia trees due presence of mature fruit at the time.

Disclaimer

Surround® WP (950 g/kg processed and refined kaolin) and Movento® (240 g/L spirotetramat) are not registered for CGW control. Samurai® and Confidor Guard® are only permitted for use for CGW control/suppression in spring and early summer. Pesticide Act 1999 states chemicals must only be used for the purpose described on the product label.

Appendix-9 Potted-tree trials

Introduction

Citrus Gall Wasp *Bruchophagus fellis* (CGW) has emerged as a major pest for citrus producers in the southern growing regions of Australia, reducing tree productivity and profitability within heavily infected orchards. At the commencement of this screening trial the only insecticide registered for the management of CGW in Australia was methidathion, marketed as Suprathion® by Adama. Concerns by industry existed around placing too great a reliance on Suprathion® as the sole insecticide management for a number of reasons including; uncertainty of supply into Australia, the changing or removal of MRLs by key trading partners, pending review by the APVMA as well as anecdotal evidence of Suprathion offering only medium efficacy in the suppression of CGW. The primary objective for this potted tree screening trial was to identify which registered (or pending/potential) chemistry's may provide an alternative chemical choice for managing CGW populations. This trial has been funded as part of the citrus gall wasp national project (Horticulture Australia Ltd. Project CT15006).

Materials and methods

The potted tree trial was comprised of a number of bioassays which were designed to investigate current and potential Citrus Gall Wasp (CGW) management strategies. This study was conducted in a local greenhouse facility and took place over a period of approximately 4 weeks. Trees were housed in a shade cloth enclosure (Figure A9-1). Further insulation was installed overhead to provide more favourable conditions for wasp activity.

Carrizo citrange rootstock trees were used. Trees were allowed to grow for a period of 3 weeks to establish fresh growth. Trees were then graded according to total shoot length and selection was made to even out total shoot length between cages. The irrigation method used was a single dripper per bag and fertiliser was applied as required.

Figure A9-1. Cages with rootstock trees used in the potted tree trial.

Insects

CGW is a small, black wasp which attacks the spring flush of citrus trees (Figure A9-2). According to Mo *et al* (2014), each female wasp has the potential to lay up to 100 eggs. Under laboratory conditions (16.7-22.8°C) the average length of life for male and female wasps was 7.10 and 5.96 days respectively (Noble 1936).

Figure A9-2. Left: Female wasp; Centre: Citrus gall; Right: Wasps emerging from gall.

Wasp Collection & Introduction

As wasps were to be introduced to caged trees on a daily basis over a two-week period, a consistent supply of fresh wasps was needed. This was achieved by collecting galls from local orchards and placing them in insect rearing cages (32.5cm^3). Fresh galls were collected to ensure a consistent supply of fresh wasps, with three collections made during the course of the wasp exposure period. To ensure consistent fitness of wasps, cages were vacuumed after collection each day ensuring that all wasps were less than 24 hours old at the time of introduction.

Cages initially received 20 female wasps for the first four introductions and subsequently received 25 newly emerged wasps/day/cage for the remainder of the trial. Some males were also introduced to guard against the collection of virgin females. Wasps were aspirated from the collection cages early to mid-morning and placed in milkshake cups (Figure A9-3). Once aspiration was complete, wasps were then introduced to caged trees. During the afternoon observation, wasps that were still in the milkshake cups were considered dead and replaced.

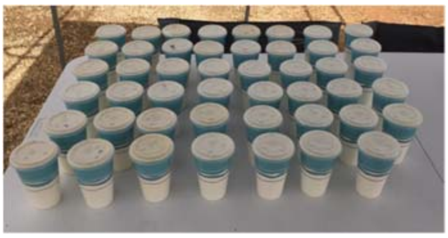


Figure A9-3. Milkshake cups with wasps to be released in cages.

A moistened piece of cotton wool was attached to the inside of the top of the cage and wetted twice a day during hot weather. This was done in order to maximise wasp longevity by providing a water source and increasing humidity.

Wasp Observation

Informal afternoon observations were undertaken during wasp exposure period. Observations were made on: wasp activity on flush, leaf and spike, wasps laying eggs and the number of dead wasps.

Bioassays

The trial was divided in four broad categories: adult toxicants; adult toxicants + surround; adult repellents and larvicides (Table A9-1).

Table A9-1. Products investigated.

Adult toxicant	Adult toxicant + Surround	Adult - Repellent	Larvicide
Actara® (250 g/kg thiamethoxam)	Surround® + Success® Neo	Potassium Silicate (SiO_2 32%)	Actara®
Suprathion® (400 g/L methidathion)	Surround® + Talstar®	Surround®	Exire® (100 g/L cyantraniliprole)
Success® Neo (120 g/L spinetoram)		Wettable Sulphur (800 g/kg sulphur)	Confidor® Guard
Talstar® (250 g/L bifenthrin)			DC-154 (test chemical from Bayer)
			Suprathion®
			Movento®
			Samurai®

The bioassay was conducted in a completely randomised design. Each replicate consisted of a tree and each cage contained four trees. Trees were tagged and kept in fully enclosed insectary cages (75 x 75 x 115cm dome) to enable wasp pressure to be regulated. The treatments had either four, eight or twelve replicates. Trees were sprayed to point of run off in accordance with treatment type using a hand sprayer, with the exceptions of Confidor and Samurai which were applied as a soil drench. Treated trees were placed to dry in the shade. All products were measured accurately using pipettes, graduated cylinders and scales.

All treatments were exposed to wasps for a total of 14 days, after this period trees were cleaned and placed in a wasp free environment.

<u>Efficacy of Adult Toxicants</u>

The aim of this bioassay was:

- To determine if the insecticides are toxic to the adults, thereby preventing egg laying activity, and
- To observe if the efficacy is improved with a second application after 7 days.

  This bioassay consisted of a total of nine treatments (Table A9-2). As the trial took place over a period of 14 days, repeat spray applications were required for the 7 day insecticides.

Table A9-2. Adult toxicant treatments.

Treatments	Rate	Additives	Spray Timing
Actara® (7 day)	30 g/100 L	500 ml/100 L Trump oil	Start of the trial & day 8
Actara® (14 day)	30 g/100 L	500 ml/100 L Trump oil	Start of the trial
Suprathion® (7 day)	125 ml/100 L	500 ml/100 L Trump oil	Start of the trial & day 8
Suprathion® (14 day)	125 ml/100 L	500 ml/100 L Trump oil	Start of the trial
Success® Neo (7 day)	20 ml/100 L	10 ml/100 L wetter	Start of the trial & day 8
Success® Neo (14 day)	20 ml/100 L	10 ml/100 L wetter	Start of the trial
Talstar® (7 day)	20 ml/100 L	10 ml/100 L wetter	Start of the trial & day 8
Talstar® (14 day)	20 ml/100 L	10 ml/100 L wetter	Start of the trial
Control			

<u>Efficacy of Combining Adult Toxicants with Surround</u>

The aim of this bioassay was

- To investigate whether the level of control is increased by mixing an adult toxicant with Surround compared to either treatment sprayed in isolation.

A total of five treatments were investigated and are presented as in the table below.

Table A9-3. Adult toxicants & repellent treatments.

Treatment	Rate	Additive	Spray timing
Surround® + Success Neo	2.5 Kg/100 L Surround	10 ml/100 L wetter	Double spray at the start of the trial, insecticide to be added to second application once initial spray dry.
	2.5 Kg/100 L Surround + 20 ml/100 L Success Neo	10 ml/100 L wetter	
Surround® + Talstar	2.5 Kg/100 L Surround	10 ml/100 L wetter	
	2.5 Kg/100 L Surround + 20 ml/100 L Success Neo	10 ml/100 L wetter	
Success® Neo 14 day	20 ml/100 L	10 ml/100 L wetter	Start of the trial
Talstar® 14 day	20 ml/100 L	10 ml/100 L wetter	Start of the trial
Surround®	2.5 Kg/100 L	10 ml/100 L wetter + 2.5 g/100 L Guar Gum	Double spray at the start of the trial, second application applied once initial spray dry.
Control			

Efficacy of Repellents
The aim of this bioassay was

- To evaluate the repellent effect of silica, Surround® and sulphur on egg laying activity.

A total of four treatments were investigated and are presented as in the table below.

Table A9-4. Adult repellent treatments.

Treatments	Rate	Additives	Spray Timing
Silica	2.5 Kg/100 L	10 ml/100 L wetter + 2.5 g/100 L Guar Gum	Start of the trial & day 8
Surround®	2.5 Kg/100 L	10 ml/100 L wetter + 2.5 g/100 L Guar Gum	Double spray at the start of the trial, second application applied once initial spray dry.
Wettable Sulphur	500 g/100 L	10 ml/100 L wetter	Start of the trial & day 8
Control	-	-	-

Larvicidal Efficacy
The aim of this bioassay was:

- To evaluate the effectiveness of a selection of systemic / partially systemic insecticides targeting larvae.

A total of twelve treatments were investigated and are presented as in the table below.

Table A9-5. Treatments, rates, additives and timing in the potted tree trial of chemicals for larval control

Treatments	Rate	Additives	Timing
Actara®	30 g/100 L	Hasten® 200 ml/100 L	Single treatment, 15th December *
Confidor®	1 ml/tree	-	Single treatment, 1st December.
DC-154 Bayer	100 ml/100 L	-	Single treatment, 1st March.*
DC-154 Bayer Double Spray	100 ml/100 L	-	Double treatment, 1st and 15th December. *
Exirel®	75 ml/100 L	Hasten® 200 ml/100 L	Single treatment, 15th December *
Suprathion® / methidathion	125 ml/100 L	Hasten® 200 ml/100 L	Single treatment, 15th December.
Movento® Single Spray	40 ml/100 L	Hasten® 50 ml/100 L	Single treatment, 15th December.*
Movento® Single Spray	40 ml/100 L	Hasten® 50 ml/100 L	Single treatment, 1st March.*
Movento® Double Spray	40 ml/100 L	Hasten® 50 ml.100 L	Double treatment, 1st and 15th December.*
Samurai®	3 g/tree	-	Single treatment, 1st December.
Samurai®	3 g/tree	-	Single treatment, 15th December.
›ntrol			

* Product applied twice on the same day to improve uptake due to lower leaf area / potential uptake problems.

Method for Cleaning Trees
At the end of the two-week wasp exposure period, the trees and the cages were cleaned using a vacuum cleaner to remove all wasps.

Results

Rating and Measurement of Galls
In May 2016, after galls had reached their maximum size, gall area was assessed using a grid sheet ($25mm^2$) measuring only solid gall area. Galls were assessed as separate units where there was a reasonably clear break (Figure A9-4, D). Spikes and side shoots were measured separately to the main stem / shoot.

Figure A9-4. (A) Partial gall; (B) Complete gall; (C) Example Gall Segregation; (D) Clear break in situ; (E) Clear break with bark removed.

<u>Adult Toxicants</u>

All insecticides used in this bioassay had a toxic effect against adult CGW. Talstar® had the highest efficacy against CGW, with only a single wasp emerging in the 7-day treatment equating to a 99.9% reduction in population (Figure A9-5). The 14-day Talstar treatment demonstrated the second best efficacy reducing the population by 92%.

The 7-day methidathion, 7-day Actara® and 14-day methidathion were similar to the Talstar® treatments. However, there was not a high level of efficacy in terms of population reduction; 7-day methidathion gave a 38% population reduction, 7-day Actara® 10% and 14-day methidathion 7%. The 7-day and 14-day Success® Neo treatments and 14-day Actara® treatments had low efficacy, with population increases of 145%, 157% and 82% respectively. The control had a population increase of 756%.

In all treatments there was not a statistical difference between the 7-day and 14-day treatments. However, there was a positive trend in increased population reduction.

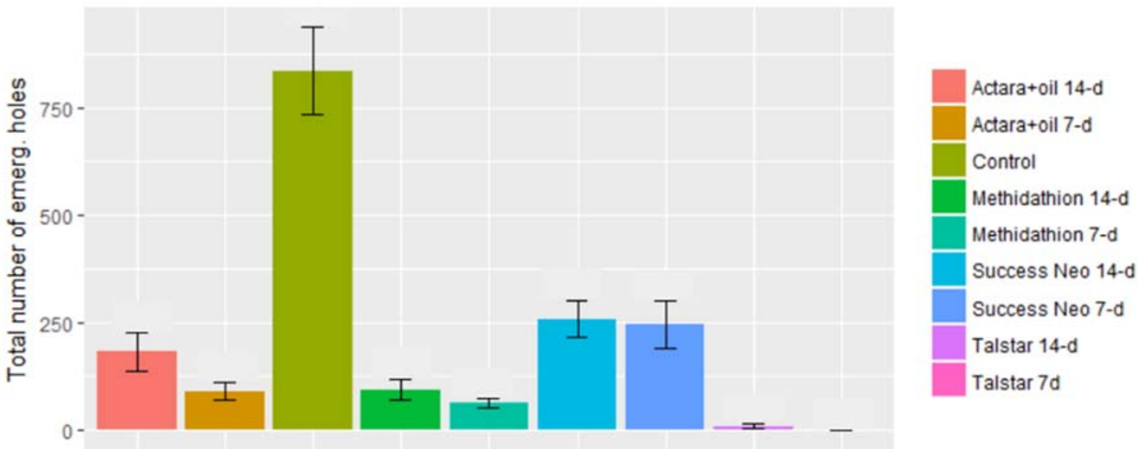


Figure A9-5. Numbers of emergence holes per tree (mean ± SE) in different treatments in the potted-tree trial of adult toxicants.

<u>Adult Toxicants in Combination with Surround®</u>

The insecticide + Surround treatments in this bioassay all had a toxic effect against adult CGW. The 14-day Talstar® + Surround® treatment had the highest efficacy reducing populations by 43% (Figure A9-6). The individual 14-day Talstar® treatment resulted in a 93% reduction in population, indicating that the inclusion of Surround® has a negative impact on the efficacy of Talstar®. Talstar + Surround® and Talstar® treatments provided similar levels of control.

The 14-day Success® Neo + Surround® treatment had a population increase of less than 1%. The individual 14-day Success® Neo treatment resulted in a 157% population increase, while the 14-day Surround® treatment yielded a 61% population increase. This indicates a positive synergistic effect of combining Surround with Success® Neo. Control levels were similar between Success® Neo + Surround®, Success® Neo and Surround® treatments.

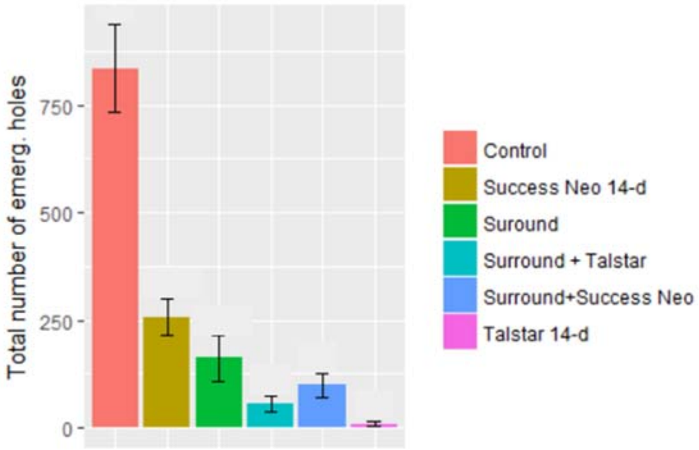


Figure A9-6. Numbers of emergence holes per tree (mean ± SE) in different treatments in the potted-tree trial of adult toxicants and Surround.

<u>Adult Repellents</u>
The 14-day Surround and 7-day Silica treatments both demonstrated a repellence effect against CGW (Figure A9-7). 14-day Surround produced the strongest repellence effect but still had a population increase of 61%. Silica had a population increase of 329%.

It should be noted that efficacy appeared to be lost due to fresh growth developing during the wasp introduction period (enabled wasps to rest and lay eggs onto uncovered shoot material). This was a greater issue for the 14-day Surround treatment which was sprayed only once at the commencement of wasp introductions.

The 7-day Wettable Sulphur treatment had no measurable repellency effect against CGW adults.

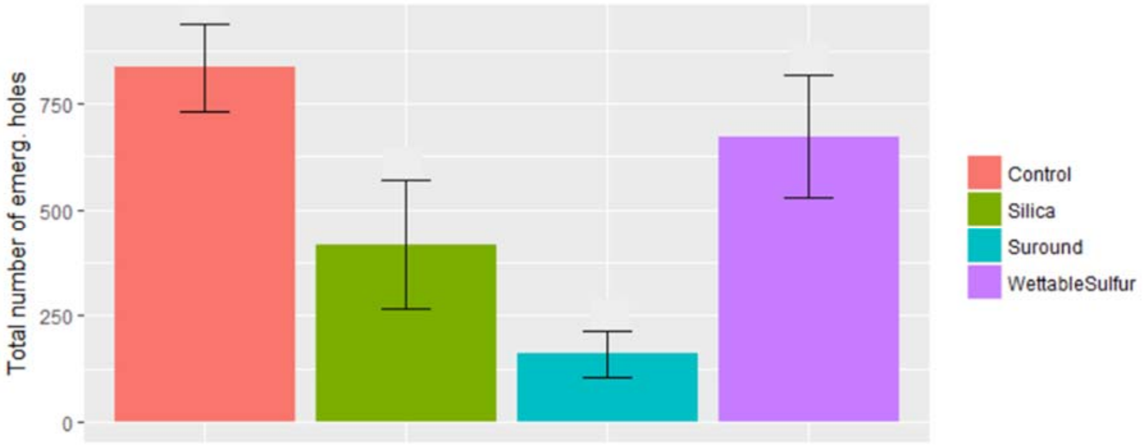


Figure A9-7. Numbers of emergence holes per tree (mean ± SE) in different treatments in the potted-tree trial of adult repellents.

Larvicides
All larvicide treatments used in this bioassay showed a positive reduction in the level of gall wasp emergence relative to the control (Figure A9-8).

Treatments of Samurai® and methidathion both provided complete control with a population reduction of 100%. Confidor also showed very high efficacy with a population reduction of 99%.

None of the remaining treatments demonstrated high efficacy. The next best performing treatments were March-Movento 19% population increase, December-Double Movento 79% and December-Double DC-154 Bayer 101%.
Actara, Exirel, December-Single Movento and March DC-154 Bayer had low efficacy at 164%, 259%, 332% and 319% population growth respectively.

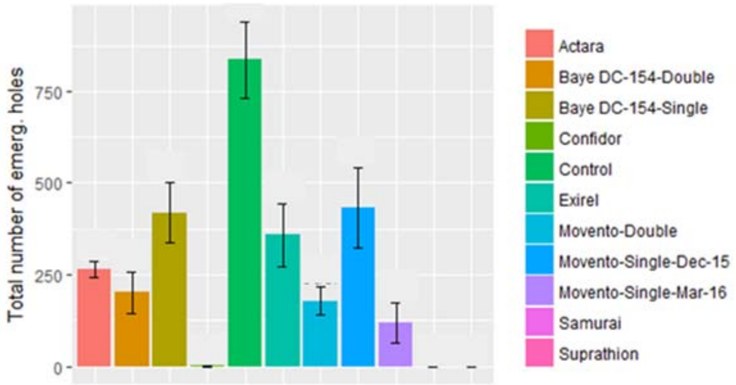


Figure A9-8. Numbers of emergence holes per tree (mean ± SE) in different treatments in the potted-tree trial of larvicides.

Summary and conclusions

The population growth potential for CGW on an annual basis is high, in this trial under greenhouse conditions the number of wasps for the control treatments multiplied from 1,170 wasps to 10,020 within one annual cycle, a population growth of 756%. Field observations have recorded annual population growths consistently around 400-600% under normal conditions.

Due to this exponential growth factor and relatively low establishment of biocontrol agents in many groves, industry requires insecticide management options which can deliver a high level of efficacy. Ideally population reductions in excess of 90% are desirable. Lower efficacy products closer to 50% would require a serial program of repeated treatment for three consecutive years before a one year break could be anticipated.

Talstar® was the only adult toxicant bioassay demonstrating high efficacy against CGW. Talstar® was included in this trial as a benchmark chemistry given its high and persistent toxicity to a broad range of insects rather than offering a desirable or realistic insecticide option to industry.

Methidathion demonstrated moderate efficacy but only at the 7-day interval, indicating that if applied regularly during wasp emergence it may provide some meaningful population suppression. Realistically Methidathion does not offer a stand-alone chemical option during the wasp emergence period due to the short protection period (7-days) and modest efficacy offered. In addition Methidathion is losing acceptance from major trading partners making it a less desirable chemical choice for growers targeting lucrative export markets. All other insecticides evaluated are unlikely to provide meaningful suppression of CGW when applied during the wasp emergence period.

Surround® has proven to be an effective repellent in previous greenhouse trials and field trials providing a high efficacy option to industry. The inclusion of Surround® in this trial was aimed at investigating efficacy at reduced rates and to provide a comparative benchmark for Silica and Wettable Sulphur bioassays. The Silica bioassay showed some repellence effect, however efficacy was poor relative to most insecticide bioassays.

Samurai®, Confidor® and methidathion larvicide treatments were the highest performing bioassays with a 99-100% population reduction. Samurai® (subject to registration) and Confidor® are anticipated to play a central role in the management of CGW, particularly in smaller to mid-size tree situations.

Experience has demonstrated that heavier textured soil types, application method and larger tree size can compromise the efficacy of Confidor under field conditions. At this point in time there has been no attempt to evaluate whether or not soil texture, organic matter levels or tree size will impact Samurai in a similar manner. The management of MRL's for the neo nicotinoids may also become an increasing challenge for industry with increased scrutiny occurring internationally.

Methidathion remains the only registered insecticide option for CGW in citrus. This trial validated methidathion's efficacy (in the context of complete plant coverage) and potential as a management tool when targeting larval stages. Anecdotal observations of reduced methidathion efficacy in field can now be more confidently linked to compromised spray coverage rather than a problem with chemical toxicity. As previously reported, the adoption of methidathion as a management tool will be restricted by the prohibition of key trading partners.

The March-Movento® Bioassay in this trial demonstrated efficacy but at a level lower than anticipated given positive results observed in-field during recent seasons. The lower than expected efficacy may be linked to the high severity of galling on the young trees and impaired tree function. Field trials of autumn applied Movento® are ongoing and are expected to confirm the relative merits of Movento as a management strategy.

No other insecticides demonstrated meaningful suppression of CGW when applied as a larvicide.

Additional comments

Trees were graded and allocated to cages in an attempt to equalise available shoot length per treatment however it was not possible to have each cage exactly the same. Replicates of varying shoot length were a factor affecting the standard deviations.

Observations were conducted during the wasp exposure period for the purpose of verifying that sufficient wasp pressure had been attained and wasps were behaving naturally. Aggregation was noted in some cages on single replicates but this was not investigated in detail during this trial.

Product movement within plants was variable between systemic treatments. As eggs hatch after a period of 2-4 weeks (Noble 1936), the objective was to have the chemical in the trees so that when the eggs hatched the larvae were immediately exposed to it. The rate at which the chemical moves into the plant and reaches the full dose level as well as its persistence influenced the timing of application.

Disclaimer

Surround® WP (950 g/kg processed and refined kaolin) and Movento® (240 g/L spirotetramat) are not registered for CGW control. Samurai® and Confidor Guard® are only permitted for use for CGW control/suppression in spring and early summer. Pesticide Act 1999 states chemicals must only be used for the purpose described on the product label.

Appendix-10 Phenology models

Summary

Adult CGW emergence and egg hatch were monitored at 4 sites each in the Riverina and the Sunraysia for three seasons during 2015-207. Together with data collected in a previous project, a degree-day model for adult emergence and a degree-day model for egg hatch were developed to predict the timings when adult CGW emerge from galls and when egg hatch. According to the adult emergence model, 5, 50, and 95% adult emergence occurs when 560, 723 and 835 degree-days above 8°C have been accumulated since August 1. According to the egg hatch model, 5, 50, and 95% adult emergence occurs when 1039, 1327, and 1521 degree-days above 2°C have been accumulated since October 1. The predicted median adult emergence date (50% emergence) and median egg hatch date (50% egg hatch) were, on average, within three days of the observed median dates. Based on the models, an interactive, online tool has been developed that allows growers to use local weather station data to predict when adult citrus gall wasps are likely to emerge from the galls and when the eggs laid by the current season wasps are likely to hatch.

Introduction

Timing is important in CGW management. Adult wasps are the only exposed stage in the CGW lifecycle, which lasts only about four weeks each year. The larval period is long (ca. 10 months) but larvae are inside the galls and shielded from natural elements and many insecticides. Two groups of insecticides can reach the larvae, systemic insecticides or non-systemic insecticides with good translaminar activity. Until recently, methidathion was the only registered insecticide for CGW control. It is a contact insecticide with translaminar activity. Methidathion is toxic to the adult wasps but its recommended use is to kill newly hatched larvae. To target newly hatched larvae, methidathion is best applied after most adult wasps have laid their eggs and before current-year citrus shoots have hardened (Papacek and Smith 1989).

Noble (1933, 1936) provided the first detailed account of CGW phenology, including durations of the egg, larval, and pupal period, and timing of adult emergence. A preliminary degree-day model was developed in CT10021 to predict the emergence of adult wasps (Mo and Stevens 2014). The model parameters were based on data collected on 'Navel' oranges in the Sunraysia during 2010-2012. For more accurate predictions across the southern citrus regions, we updated the model with new data from different locations and citrus varieties. We also developed separate degree-day (DD) models for egg hatch. Degree-day is a measure of heat units. Insects require certain numbers of degree-days to complete development.

Materials and Methods

Data collection sites

Emergence of adult wasps and egg hatch were collected from four sites each in the Riverina and Sunraysia for three seasons during 2015-2017 (Table A10-1). All monitoring sites were on commercial citrus farms with each site in a separate block of citrus trees. Citrus varieties at the Riverina sites were Salustiana orange, navel orange (Thompson), and lemon (mixed cultivars). Citrus varieties at Sunraysia sites were valencia orange, navel orange (Washington), grapefruit (Rio Red), and lemon (Euraka). Tree age at monitoring sites varied from 10 to 55 years. A minimum of two sites were used to monitor emergence of adult wasps and progression of egg hatch in each season (Table A10-1). A total of 17 datasets (site by season data) each were collected on adult wasp emergence and egg hatch.

In addition, five datasets of CGW adult wasp emergence and three datasets of CGW egg hatch collected from navel orange (Autumn gold) trees in the Coomealla Irrigation District in the Sunraysia during 2010-2013 (Mo *et al* 2014) were used in this study to develop the degree-day models for adult wasp emergence and egg hatch. These datasets were collected using similar methods as described in this report.

Monitoring of adult wasp emergence

Emergence of adult wasps was monitored with sticky cup traps (Figure A10-1). The trap was made from a disposable clear plastic cup (480 ml capacity, 85 mm diameter at rim). The interior cup surface was coated with a thin layer of Tangle-Trap® (The Tanglefoot Company, Grand Rapids, MI, USA) to capture emerging wasps. It was placed around a CGW gall through a 1 cm hole in the centre of the cup base and an L-shaped slit linking the cup opening to this hole. Twenty traps were used at each site in each season. At sites of mixed citrus varieties, 10 traps each were used for each variety.

Monitoring started before any adult CGW adults had emerged at the monitoring sites and finished after three consecutive monitoring dates of zero catch. Monitoring at site-BB1 in 2015 was conducted weekly in the first three weeks and twice weekly thereafter. At all other sites in all other years, monitoring was conducted twice weekly throughout the monitoring periods. On each monitoring date, the traps were individually checked to see if they had caught any wasps. If a trap had caught one or more wasp, the trap was replaced. Removed traps were individually wrapped in Glad Wrap®, taken back to the laboratory, and checked under a stereo microscope to count the number of wasps caught. Where gall wasps and parasitic wasps were found in the same trap, their numbers were counted separately.

Table A10-1. Description of the monitoring sites

Region	Site (Season)	Shire	GPS	Variety (Rootstock)	Age (yr)	Infestation
Riverina	BB1 (15-16)	Leeton	-34.573650 146.432638	Salustiana (Trifoliata)	10-15	Moderate
	BB2 (16-17)	Leeton	-34.581849 146.425930	Salustiana (Trifoliata)	10-15	Moderate
	BG (15-18)	Griffith	-34.299592 146.050540	Navel (TPSN)[3] (Lemon)	65	Moderate
	MS (15-18)	Griffith	-34.245728 146.042695	Lemon (Mixed)	15	High
Sunraysia	CK (15-18)	Buronga	-34.161243 142.185737	Valencia (Citrange)	33	High
	NK1 (15-18)	Morquong	-34.139865 142.181071	Navel (WSTN)[4] (Sweet orange)	50-55	High
	NK2 (15,18)[1]	Morquong	-34.139865 142.181071	Grapefruit (Mixed)[5] (Citrange)	10-15	High
	BV2 (16-17) [2]	Coomealla	-34.071577 142.135843	Lemon (Eureka) (Benton)	50-55	High

[1]Shoot data were not collected in the 2016-17 season; [2] Shoot data only; [3] Mixed varieties of Thompson Navel and Valencia trees. Traps were only put on Navel trees; [4] Washington Navel; [5] Primarily of the 'Rio Red' variety.

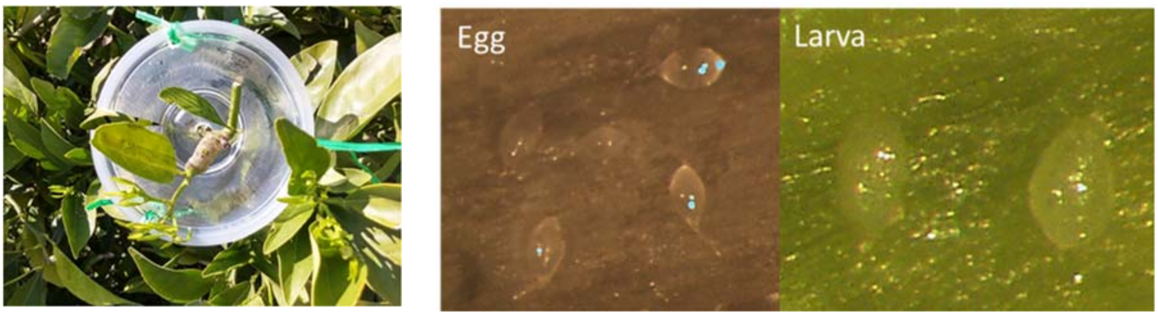


Figure A10-1. Left: A CGW gall inside a yellow sticky cup trap; Right: CGW eggs and larvae under the bark.

Monitoring of egg hatch

Progression of egg hatch was monitored by periodically dissecting shoots and counting the numbers of total eggs and hatched eggs (larvae) (Figure A10-1). Data collection started before any eggs had hatched and finished after all eggs had hatched. Actual starting and finishing dates varied with season. On each monitoring date, a minimum of 20 current season citrus shoots were cut off and placed immediately in automotive radiator coolant containing ethylene glycol (65 g/L) to stop egg development. In the laboratory, 20 shoots were de-barked and examined under a stereomicroscope at 18-25x magnification to count the number of eggs and larvae. Shrivelled eggs were not included in the counts.

Temperature data

Daily maximum and minimum temperatures at two weather stations in the Riverina (Griffith Airport and Yanco Agricultural Institute) and one weather station in the Sunraysia (Mildura Airport) during 2015-2018 were downloaded from the Australian Bureau of Meteorology website (http://www.bom.gov.au). Daily temperature data at these weather stations were used for degree-day (DD) estimations at the monitoring sites, with the closest of the three weather stations chosen for each monitoring site (Griffith Airport for site BG and and MS, Yanco Agricultural Institute for site BB1 and BB2, and Mildura Airport for site CK, NK1, NK2, and BV2. Site descriptions are in Table A10-1). Distance from a monitoring site to its closest weather station was < 50km.

Data analysis

With the inclusion of five datasets of adult wasp emergence and three datasets of egg hatch from project CT10021 (Mo et al 2014), 22 datasets were available for the analysis of adult wasp emergence and 20 for the analysis of egg hatch. All 22 adult wasp datasets were used in the analysis of adult wasp emergence. However, two datasets of egg hatch were excluded in the analysis as they contained data points outside the normal data range, leaving 18 datasets for the analysis of egg hatch.

The accumulated proportion of emerged wasps by a given monitoring date was estimated by the proportion of all wasps caught by any traps by that date over all wasps caught by any traps during the entire monitoring period. Accumulated proportion of hatched eggs by a given monitoring date was estimated by the proportion of larvae over the sum of eggs and larvae in the 20 dissected shoots. Accumulated DD by a given monitoring date were estimated from daily maximum and minimum temperature data using the single-sine method with horizontal cut-off (Roltsch et al., 1999).

To predict the timing when a given proportion of adult wasps have emerged or a given proportion of eggs have hatched, the accumulated proportions (P) and DD were fitted the following Weibull distribution function (Weibull 1961):

$$P(DD) = 1 - \exp(-(\frac{DD}{\lambda})^k) \quad (1)$$

where λ and k are parameters to be estimated.

Parameters λ and k were estimated using the 'nlsLM' function from the package 'minpack.lm' in R (R Development Core Team, 2012). Once λ and k are determined, the timing at which a given proportion has been reached can be estimated by the inverse of Eq-1;

$$DD(P) = \lambda(-\log(1 - P))^{1/k} \quad (2)$$

When $P = 0.5$, *DD*(P) gives the median predicted emergence DD. Goodness-of-fit of Equation-1 can be measured by the proportions of variations in P that can be explained by the equation (R^2) (Motulsky and Christopoulos 2004). For a given dataset, a more direct measure of the goodness-of-fit is the difference between predicted and observed median DD. In this study, observed median DD was estimated by linear interpolations of the data.

DD calculation requires the knowledge of the lower (T_0) and upper (T_1) development threshold temperatures and a starting date for DD accumulation (DD_0) (Young and Young, 1998). Mo and Stevens (2014) showed that $T_0 = 15$℃, $T_1 = 40$℃, and DD_0 = *April 1st* provided the best prediction of the timing of median adult wasp emergence. These estimates were based on data collected from the Coomealla Irrigation District (S34°05.369', E142°07.230') in the Sunraysia during 2010-2013. Considering DD was least sensitive to upper developmental threshold (Mo and Stevens 2014), T_1 is kept at 40°C in this study. Values for the other two parameters, T_0 and DD_0, were updated with new datasets collected in this study by cross-validation over a series of candidate values (Geisser 1993). In each step of cross-validation, one dataset was set aside for validation and the remaining datasets for parameter estimation (training datasets). The process is repeated until all datasets have been used as the validation dataset.

Candidate values for T_{lower} were 0, 1, 2, … , 15°C and those for DD_0 January 1st, February 1st, March 1st, … , October 1st. The chosen range of candidate T_0 values covers most reported lower threshold temperatures for insects. Candidate DD_0 values covers the period from egg hatch to the emergence of next generation of adult wasps (Mo and Stevens 2014). The process of cross-validation consisted of eight steps: (1) calculate DDs for given T_0 and DD_0 values in all datasets; (2) select a dataset for validation and keep the remaining datasets for training; (3) pool the DDs and observed accumulated proportions in training datasets and fit the pooled data to Eq-1to estimate the Weibull parameters of λ and k; (4) calculate the observed median DD in the validation dataset and estimate the predicted median DD in the validation dataset using Eq-2 by setting $P = 0.5$; (5) calculate the squared differences between the predicted and observed median DD in the validation dataset; (6) repeat steps (2) to (5) to get the sum of squared differences over different partitions between the training and validation datasets (SSD); (7) repeat steps (1) to (6) to get the sums of squared differences for all candidate values of T_0 and DD_0; and finally (8) find values of T_0 and DD_0 that gave the lowest of SSD (T_{0A} and DD_{0A}).

After the optimal T_0 and DD_0 were found, DDs in all datasets (training and validation) were re-calculated and the updated DDs and the corresponding accumulated proportions were re-fitted to the Weibull function to get the final estimates of λ and k (λ_A and k_A). By now, all parameters of the DD model have been estimated: T_{0A}, DD_{0A}, λ_A and k_A.

Analysis of variance (ANOVA) was conducted to analyse the effects of region and citrus variety on observed median DD after optimal T_0 and DD_0 had been found using general linear models. Where a significant effect was found, pairwise comparisons between different factor levels were conducted using Fisher's LSD test.

Cross-validations, model fitting, and ANOVA were conducted in R (R Core Team 2012).

Results

Adult wasp emergence

Median date of adult wasp emergence occurred between October 27 and November 20 in the 22 datasets (Table A10-2). In most datasets (16), the median emergence date fell in November. The emergence process appeared relatively fast. Adult wasps took only an average of ca. 8 days from completing 5% emergence to completing 95% emergence.

Cross-validations identified August 1 as the optimal starting date for DD accumulations and 8°C as the optimal lower development threshold temperature (Figure A10-2). Other starting dates resulting in similar sums of squares of difference between predicted and observed median DDs were May 1, June 1, July 1, and September 1. Other lower threshold temperatures resulting in similar sums of squared differences were 6, 7, 9, and 10°C.

Observed median DD calculated from the optimal DD starting date and lower threshold temperature did not differ significantly between sites in the Riverina and sites in the Sunraysia ($F = 2.87$, $d.f. = 1, 20$, $P = 0.1059$) (Figure A10-3). However, it differed significantly among citrus varieties ($F = 3.69$, $d.f. = 4, 14$, $P = 0.0298$) (Figure 3). The significant difference was due to lemon trees having lower median DD than trees in all orange varieties and grapefruit ($P < 0.05$). Median DD was similar among all orange varieties and grapefruit ($P > 0.05$).

Based on the optimal DD starting date and lower threshold temperature, the two Weibull parameters estimated from the pooled 22 datasets were $\lambda = 749.85$ and $k = 10.17$. The fitted Weibull function explained 94% of all variations in the accumulated proportions of adult wasp emergence across the datasets (R^2) (Figure A10-4). According to the fitted model, 5, 50, and 95% emergence of adult wasps occurred at 560, 723, and 835 DD, respectively.

Table A10-2. Observed dates when 5, 50, and 95% of adult wasps had emerged

Site	Season	Variety	Observed dates of emergence at			5 to 95% (days)
			5%	50%	95%	
BB1	2015-16	Salustiana	24/10/2015	31/10/2015	10/11/2015	17
BB2	2016-17	Salustiana	08/11/2016	20/11/2016	12/12/2016	34
BG	2015-16	Lemon	25/10/2015	31/10/2015	10/11/2015	16
BG	2016-17	Lemon	08/11/2016	17/11/2016	26/11/2016	18
BG	2017-18	Lemon	29/10/2017	08/11/2017	15/11/2017	17
MS	2015-16	Mixed	28/10/2015	06/11/2015	12/11/2015	15
MS	2016-17	Mixed	09/11/2016	19/11/2016	29/11/2016	20
MS	2017-18	Mixed	03/11/2017	11/11/2017	16/11/2017	13
CK	2015-16	Valencia	26/10/2015	01/11/2015	11/11/2015	16
CK	2016-17	Valencia	11/11/2016	18/11/2016	27/11/2016	16
CK	2017-18	Valencia	20/10/2017	06/11/2017	13/11/2017	24
NK1	2015-16	Navel	20/10/2015	29/10/2015	07/11/2015	18
NK1	2016-17	Navel	06/11/2016	16/11/2016	23/11/2016	17
NK1	2017-18	Navel	24/10/2017	08/11/2017	13/11/2017	20
NK2	2015-16	Grapefruit	26/10/2015	31/10/2015	09/11/2015	14
NK2	2016-17	Grapefruit	14/11/2016	19/11/2016	27/11/2016	13
NK2	2017-18	Grapefruit	29/10/2017	08/11/2017	13/11/2017	15
BT	2010-11	Navel	08/11/2010	20/11/2010	26/11/2010	18
BT	2011-12	Navel	20/10/2011	01/11/2011	09/11/2011	20
BT	2012-13	Navel	18/10/2012	30/10/2012	06/11/2012	19
BV1	2012-13	Valencia	29/10/2012	05/11/2012	17/11/2012	19
UK	2012-13	Lemon	20/10/2012	27/10/2012	01/11/2012	12

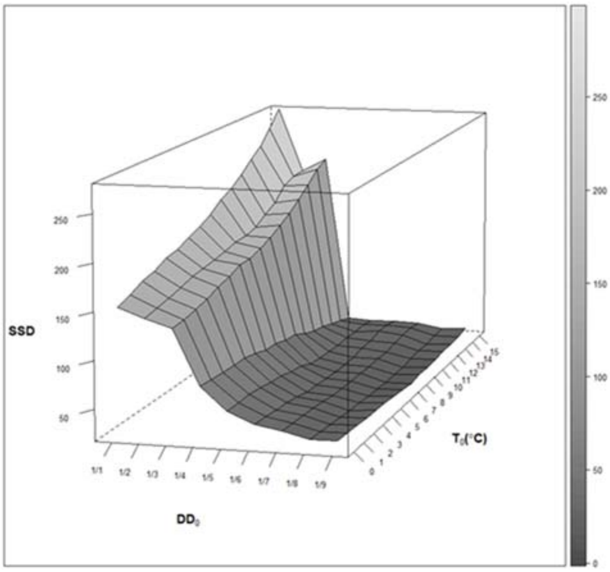


Figure A10-2. Mean sum of squares of difference between predicted and observed median dates (SSD) for egg hatch under different degree-day starting dates (DD_0) and lower threshold temperatures (T_0).

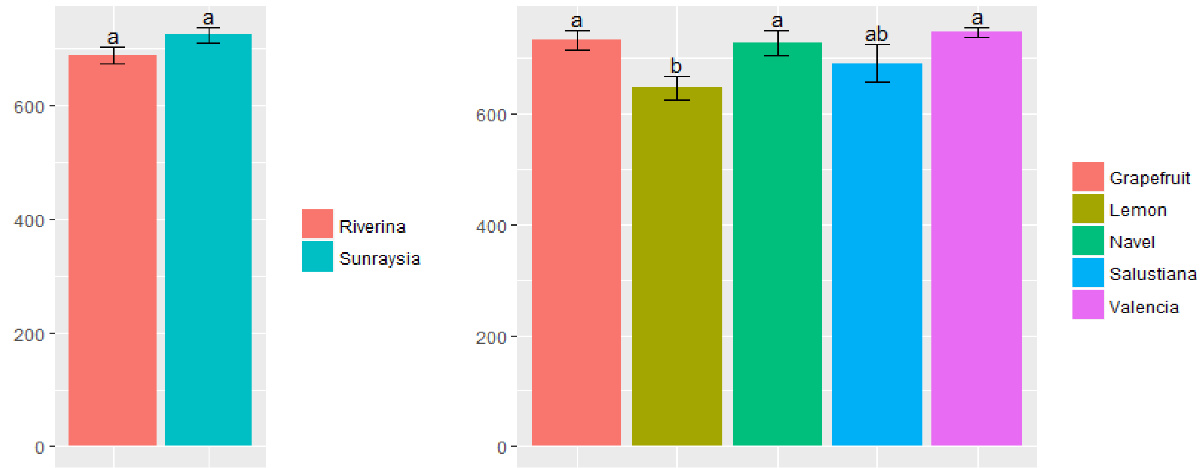


Figure A10-3. Effects of region and variety on median observed DD in adult wasp emergence. Error bars show the standard errors. Bars labelled with different letters were significantly different (*P < 0.05*) by Fisher's LSD tests following the detection of an overall significant effect by ANOVA.

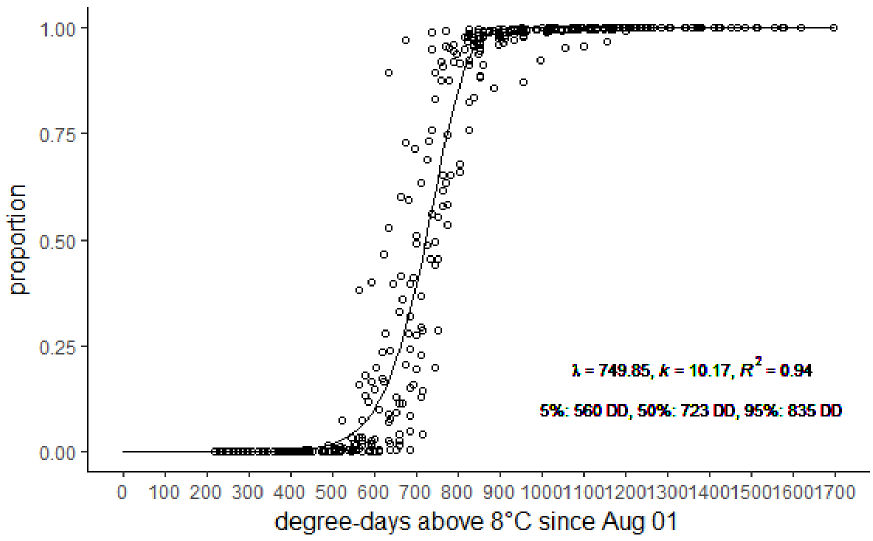


Figure A10-4. Fitted (line) and observed (circle) accumulated proportions of adult wasp emergence as a function of degree-days in the pooled 22 datasets.

Predicted median date from the model differed from the observed median date by an average of 3 days in the 22 datasets (Table A10-3). In 15 datasets the difference was within 3 days. Corresponding mean differences for 5% emergence was 5 days and that for 95% emergence 4 days. Mean positive bias of the model (predicted dates later than observed dates) in median date was 4 days and mean negative bias (predicted dates earlier than observed dates) was 2 days.

Table A10-3. Differences between predicted and observed dates for 5, 50, and 95% of adult wasp emergence in individual datasets. Positive differences indicate the predicted dates were later than observed dates and negative differences indicate the predicted dates were earlier than observed dates. Model parameters used to get the predicted values: *DD_0 = August 1, T_0 = 8C, λ = 749.85, k = 10.17.*

			Differences in days		
Site	Season	Variety	5%	50%	95%
BB1	2015-16	Salustiana	0	6	4
BB2	2016-17	Salustiana	0	1	-13
BG	2015-16	Lemon	0	6	5
BG	2016-17	Lemon	2	4	5
BG	2017-18	Lemon	-4	2	2
MS	2015-16	Mixed	-3	0	3
MS	2016-17	Mixed	1	2	2
MS	2017-18	Mixed	-9	-1	1
CK	2015-16	Valencia	-7	-1	-3
CK	2016-17	Valencia	-12	-2	-5
CK	2017-18	Valencia	-1	-2	0
NK1	2015-16	Navel	-7	0	-1
NK1	2016-17	Navel	-15	-3	-5
NK1	2017-18	Navel	-10	-4	0
NK2	2015-16	Grapefruit	-1	2	1
NK2	2016-17	Grapefruit	-7	0	-1
NK2	2017-18	Grapefruit	-5	-4	0
BT	2010-11	Navel	-2	-1	0
BT	2011-12	Navel	1	3	2
BT	2012-13	Navel	5	7	9
BV1	2012-13	Valencia	-6	1	-2
UK	2012-13	Lemon	3	10	14

Egg hatch

Median date of egg hatch occurred between November 30 and December 24 in the 18 datasets analysed (Table A10-4). In all but one dataset, the median date fell in December. Most eggs (95%) had hatched by the end of December in all datasets. The period from median adult wasp emergence to median egg hatch averaged 33 days (25-49 days).

Cross-validations identified October 1 as the optimal starting date for DD accumulations and 2 or 3°C as the optimal lower threshold temperatures (Figure A10-5). Other starting dates produced >40% larger sums of differences between predicted and observed median dates (Figure A10-6). On the other hand, lower threshold temperatures of 0-5°C produced similar sums of differences values as 2 or 3°C. In the follow-up analyses, 2°C was chosen as the optimal lower threshold temperature.

Table A10-4. Observed dates when 5, 50, and 95% of egg hatch.

Site	Season	Variety	Observed dates of egg hatch at			A to E *
			5%	50%	95%	(days)
BB1	2015-16	Salustiana	19/11/2015	30/11/2015	18/12/2015	30
BG	2015-16	Lemon	19/11/2015	01/12/2015	15/12/2015	31
BG	2016-17	Lemon	03/12/2016	13/12/2016	22/12/2016	26
BG	2017-18	Lemon	28/11/2017	07/12/2017	15/12/2017	29
MS	2015-16	Mixed	17/11/2015	06/12/2015	19/12/2015	30
MS	2016-17	Mixed	10/12/2016	20/12/2016	29/12/2016	31
MS	2017-18	Mixed	28/11/2017	14/12/2017	29/12/2017	33
CK	2015-16	Valencia	24/11/2015	07/12/2015	14/12/2015	36
CK	2016-17	Valencia	11/12/2016	20/12/2016	31/12/2016	32
CK	2017-18	Valencia	05/12/2017	12/12/2017	24/12/2017	36
NK1	2015-16	Navel	26/11/2015	05/12/2015	14/12/2015	37
NK1	2016-17	Navel	09/12/2016	20/12/2016	28/12/2016	34
NK1	2017-18	Navel	29/11/2017	10/12/2017	20/12/2017	32
NK2	2015-16	Grapefruit	24/11/2015	02/12/2015	10/12/2015	32
NK2	2017-18	Grapefruit	01/12/2017	07/12/2017	21/12/2017	29
BV2	2012-13	Lemon	10/12/2016	24/12/2016	31/12/2016	49
BT	2010-11	Navel	02/12/2010	15/12/2010	22/12/2010	25
BT	2012-13	Navel	30/11/2012	15/12/2012	26/12/2012	46

* Days from median adult wasp emergence to median egg hatch.

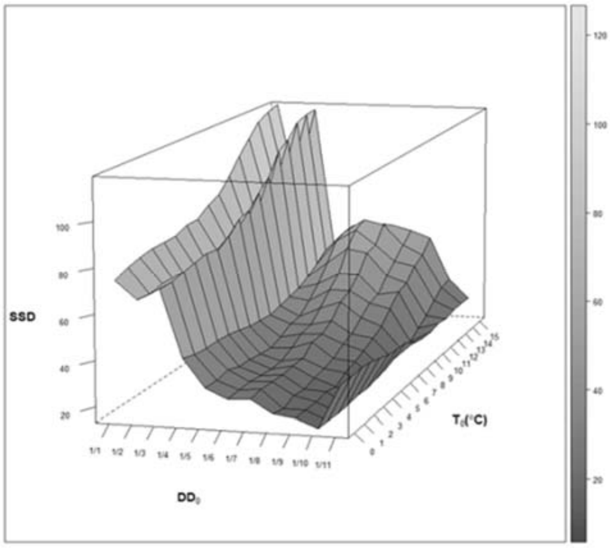


Figure A10-5. Mean sum of squares of difference between predicted and observed median dates (SSD) for egg hatch under different degree-day starting dates (DD_0) and lower threshold temperatures (T_0).

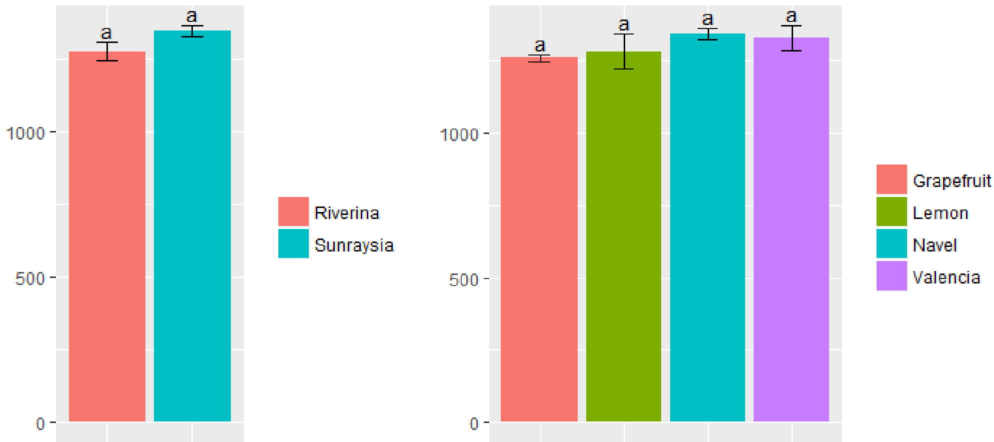


Figure A10-6. Effects of region and variety on median observed DD in egg hatch. Error bars show the standard errors. Bars labelled with different letters were significantly different (*P < 0.05*) by Fisher's LSD tests following the detection of an overall significant effect by ANOVA.

Observed median DD calculated from the optimal DD starting date and lower threshold temperature did not differ significantly between sites in the Riverina and sites in the Sunraysia (*F = 4.35, d.f. = 1, 16, P = 0.0535*) (Figure A10-6). Observed median DD was also similar between citrus varieties (*F = 0.74, d.f. = 3, 11, P = 0.0.5480*) (Figure A10-6).

Based on the optimal DD starting date and lower threshold temperature, the two Weibull parameters estimated from the pooled 18 datasets were $\lambda = 1372.84$ and $k = 10.67$. The fitted Weibull function explained 92% of all variations in the accumulated proportions of adult wasp emergence across the datasets (R^2) (Figure A10-7). According to the fitted model, 5, 50, and 95% emergence of adult wasps occurred at 1039, 1327, and 1521 DD, respectively.

Predicted median date from the model differed from the observed median date by an average of 3 days in the 18 datasets (Table A10-5). In 11 datasets the difference was within 3 days. Corresponding mean differences for 5% egg hatch was 4 days and that for 95% egg hatch 3 days. Mean positive bias of the model (predicted dates later than observed dates) in median date was 4 days and mean negative bias (predicted dates earlier than observed dates) in median date was 2 days.

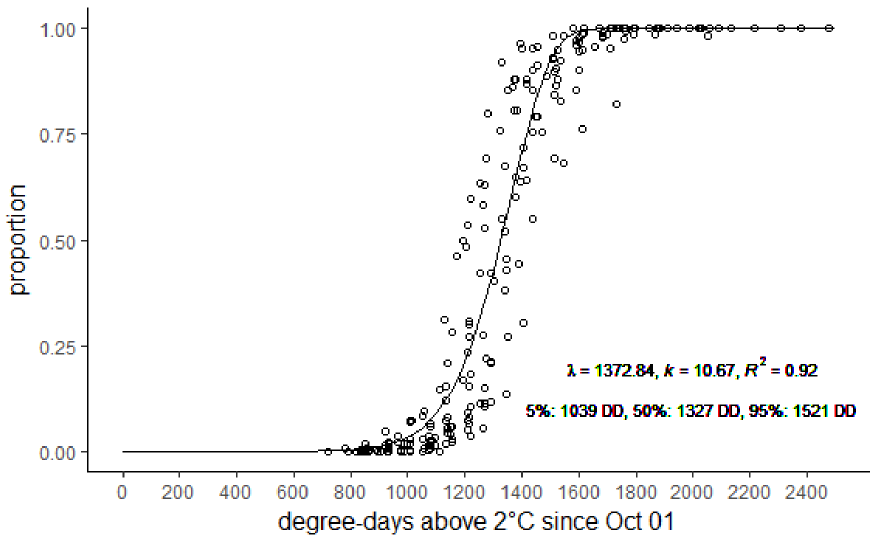


Figure A10-7. Fitted (line) and observed (circle) accumulated proportions of egg hatch as a function of degree-days in the pooled 18 datasets.

Table A10-5. Differences between predicted and observed dates for 5, 50, and 95% egg hatch in individual datasets. Positive differences indicate the predicted dates were later than observed dates and negative differences indicate the predicted dates were earlier than observed dates. Model parameters used to get the predicted values: *DD_0 = October 1, T_0 = 2°C, λ = 1372.84, k = 10.67.*

			Differences in days		
Site	Season	Variety	5%	50%	95%
BB1	2015-16	Salustiana	3	6	-3
BG	2015-16	Lemon	3	6	0
BG	2016-17	Lemon	3	7	6
BG	2017-18	Lemon	-1	4	4
MS	2015-16	Mixed	5	1	-4
MS	2016-17	Mixed	-4	0	-1
MS	2017-18	Mixed	-1	-3	-10
CK	2015-16	Valencia	-3	-1	0
CK	2016-17	Valencia	-7	-1	-4
CK	2017-18	Valencia	-9	-1	-5
NK1	2015-16	Navel	-5	1	0
NK1	2016-17	Navel	-5	-1	-1
NK1	2017-18	Navel	-3	1	-1
NK2	2015-16	Grapefruit	-3	4	4
NK2	2017-18	Grapefruit	-5	4	-2
BV2	2016-17	Lemon	-6	-5	-4
BT	2010-11	Navel	1	3	7
BT	2012-13	Navel	-1	-3	-5

Discussion

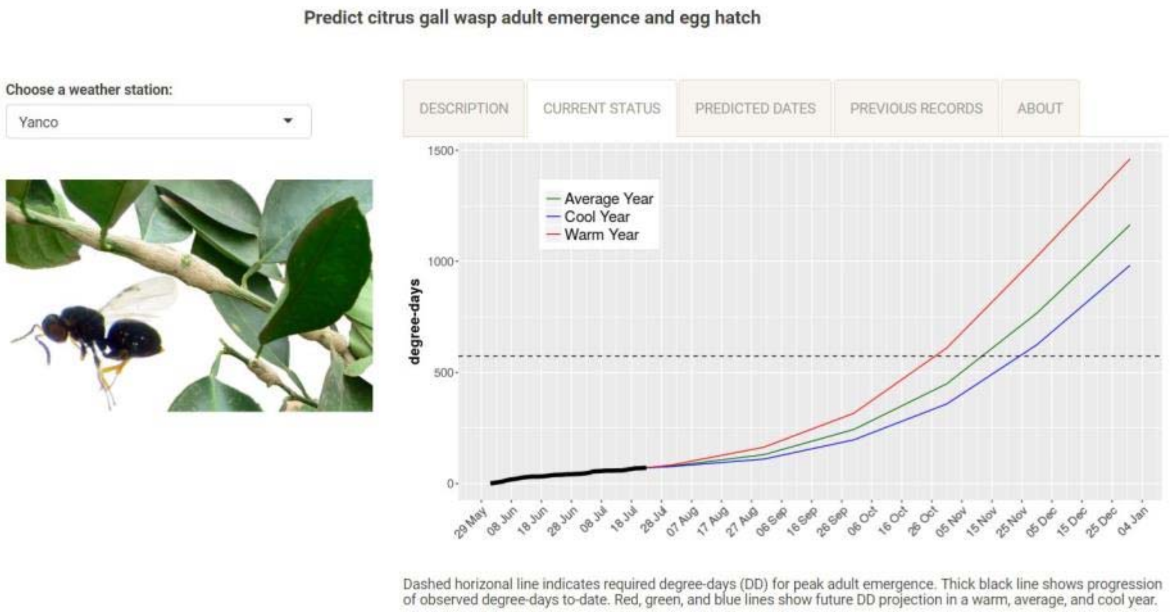
Project CT10021 developed a degree-day model for adult wasp emergence based on data collected in the Sunraysia during 2010-2012. Using the biofix date of April 1 and the lower threshold temperature of 15°C in degree-day calculations, the model predicted 5, 50, and 95% adult wasp emergence at 336, 403, 447 degree-days respectively. In this study, we have updated the adult model with 17 new datasets collected from the Riverina and the Sunraysia during 2015-2017.

The updated model has four parameters, two for degree-day accumulations and two for describing the relationship between degree-days and accumulated proportions of adult wasp emergence (Weibull function). The two-degree day parameters are August 1 as the biofix date and 8°C as the lower threshold temperature. Using the pooled data of degree-days and accumulated proportions, the two Weibull function parameters were estimated as $\lambda = 749.85$ and $k = 10.17$. The updated model predicted 5, 50, and 95% adult wasp emergence at 560, 723 and 835 degree-days, respectively. On average, the predicted dates for 5, 50, and 95% adult wasp emergence differed from the corresponding observed dates by 5, 3 and 4 days, respectively, across the 22 datasets. The prediction precision was similar to that by the original model (Mo and Stevens 2014). However, the updated model is more robust and should be more representative of CGW emergence patterns than the original model as it was developed with more datasets from more regions, seasons, and citrus varieties and the model parameters have been validated with independent datasets. Analysis of observed median emergence dates showed that there was no need to develop separate models for adult wasp emergence for different regions or citrus varieties, although the adult wasps appeared to have emerged slightly earlier from lemon trees than from trees of other citrus varieties.

In addition to the adult emergence model, we've developed a degree-day model for egg hatch. The two degree-day parameters are October 1 as the biofix date and 2°C as the lower threshold temperature. The two Weibull function parameters are $\lambda = 1372.84$ and $k = 10.67$. Estimated degree-day requirements for 5, 50, and 95% egg hatch are 1039, 1327, and 1521 degree-days, respectively. This model showed a similar prediction precision as the adult emergence model, with the predicted median egg hatch dates differing from the corresponding observed median egg hatch dates by an average of only 3 days across the datasets. The same precision was achieved in the prediction of 95% egg hatch date. As in the adult emergence, we found no need to develop separate models for different regions and citrus varieties.

The two degree-day models were developed with field sample data. The biofix dates and lower threshold temperatures estimated in the models may not be the same as those estimated with experimental data. Also degree-day parameters estimated from field data may not be unique, as shown in our analyses. However, together with the two Weibull parameters, they provided adequate descriptions of the relationships between degree-days and accumulated proportions, enabling us to use degree-days to predict the timing of CGW adult wasp emergence and egg hatch. Actual timings in a particular citrus orchard may differ slightly from the predicted timings for four reasons. First, the models were developed using temperature data from a central weather station in each region. Different citrus orchards may have different microclimates, resulting in different rates of degree-day accumulations. Secondly, CGW adult wasps can fly and large incursions of adult wasps from outside may distort local adult wasp emergence and oviposition patterns. Thirdly, individual eggs, larvae, and pupae may develop at different rates. Finally, orchard-specific operations (irrigation, fertilization, pruning, etc) may affect CGW development. Despite the likely differences, the models will provide sufficiently accurate predictions of the average timings of adult emergence and egg hatch in a specific region and year, allowing citrus growers time to plan their CGW management actions.

Based on the models, an interactive, online tool has been developed that predicts the time when adult CGW are likely to emerge from the galls and when the eggs laid by the current season wasps are likely to hatch (https://citrusgallwasp.shinyapps.io/predict). The prediction tool is easy to use. Growers simply select a weather station from a dropdown list and the predictions will are shown (Figure A10-8). A link to the final version will be available on the Hort Innovation website on the citrus.



Current status | Predicted dates | Previous Records | About

Adult emergence

	Start	Peak	Finish
Normal Year	28/10/2018	13/11/2018	24/11/2018
Cool Year	10/11/2018	28/11/2018	08/12/2018
Warm Year	08/10/2018	24/10/2018	04/11/2018

Egg hatching

	Start	Peak	Finish
Average Year	28/11/2018	12/12/2018	22/12/2018
Cool Year	05/12/2018	20/12/2018	31/12/2018
Warm Year	17/11/2018	01/12/2018	09/12/2018

Start date is indicated by 5% emergence, peak date by 50% emergence, and finish date by 95% emergence

Suggest checking egg hatching dates after peak adult emergence

Figure A10-8. Screenshots of the CGW online tool. The top screenshot shows observed degree-days accumulated to date (thick black line) and future predicted degree-days to date under average (green line), warm (red) and cool (blue) temperatures. Dashed line indicate required degree-days for peak adult emergence. The bottom screenshot shows the predicted dates for 5, 50, and 95% adult wasp emergence and egg hatch.

Appendix-11 Communications

Grower meetings/field days

Region	Place	Date	Organiser	Attendants	Presenter(s)[1]
Riverina	Leeton NSW	15/09/2015	NSW DPI	23	J Mo, A Creek
Riverina	Griffith NSW	15/09/2015	NSW DPI	18	J Mo & A Creek
Sunraysia	Mildura VIC	16/09/2015	NSW DPI	>20	J Mo, S Falivene, C Swanbury
Riverina	Griffith NSW	24/02/2016	NSW DPI	16	J Mo & A Creek
Riverina	Griffith NSW	30/08/2016	NSW DPI	14	J Mo & A Creek
Sunraysia	SS Citrus NSW	13/09/2016	NSW DPI	25	J Mo, S Falivene
Riverland	Loxton SA	20/09/2016	CASAR	70	C Swanbury
Riverland	Loxton SA	7/09/2017	CASAR	45	C Swanbury
Riverina	Griffith NSW[2]	16/10/2017	NSW DPI	110	J Mo
Sunraysia	Mildura[2]	18/10/2017	NSW DPI	72	J Mo
Riverland	Loxton SA[2]	19/10/2017	CASAR	>33	J Mo
QLD	Gayndah[3]	29/11/2017	CAL[5]	>50	J Mo
Sunraysia	Mildura VIC	21/08/2018	NSW DPI	35	J Mo
WA	Perth[2]	12/09/2017	WA Citrus	20	J Mo & B Walsh
Riverland	Winkie SA	25/09/2018	CASAR	40	C Swabury, S Falivene
Riverina	Griffith NSW	28/09/2018	NSW DPI	34	J Mo, A Creek
Riverina	Leeton NSW	28/09/2018	NSW DPI	28	J Mo, A Creek

[1]Person(s) who presented CGW project findings; [2]NSW DPI 2017 Roadshow; [3]QLD Post-season regional forum 2017; [4]CASAR – Citrus Australia SA region; [5]CAL – Citrus Australia Ltd;

The R&D roadshow toured Perth WA, Riverland, Sunraysia, and Riverina during September-October 2017. Scientists and industry experts presented practical technical information on citrus production and protection to citrus growers and industries in a series of workshops during the roadshow. Jianhua Mo presented up findings of the CGW project.

Industry publications

Positive signs of citrus gall wasp trials. *Australian Citrus News*, Autumn 2016, pp. 25-25.

Plan now for citrus gall wasp. *Australian Citrus News*, Spring 2016, pp. 19-19.

Clay sprays drive the citrus gall wasp away. *Good Fruit & Vegetables*. https://www.goodfruitandvegetables.com.au/story/4154613/clay-spray-drives-wasps-away/?cs=4917

Protective spray reduces gall wasp. *Australian Citrus News*, Winter 2017, pp. 14-15. https://www.citrusaustralia.com.au/news/latest-news/protective-spray-reduces-gall-wasp

New tool makes predicting citrus gall wasp activity easy. *Australian Citrus News*, Winter 2018, pp. 28-28.

Gall wasp research. *Australian Tree Crop*, April/May 2017, pp. 15-15.

Online tool to predict citrus gall wasp. *Australian Tree Crop*, October/November 2018, pp. 37-37.

New project to strike citrus gall wasp. *Citrus Connect**, October 2015. http://www.dpi.nsw.gov.au/agriculture/horticulture/citrus/citrus-connect/oct-2015?a=581743

Update on the gall wasp project. *Citrus Connect*, December 2015. https://us11.campaign-archive.com/?u=59ba43482b8c913efe7355823&id=17a1eb3387

CGW project update: Adult control trials. *Citrus Connect*, March 2016. https://us11.campaign-archive.com/?u=59ba43482b8c913efe7355823&id=69da562618

Clay deters gall wasp egg lay. *Citrus Connect*, June 2016. https://us11.campaign-archive.com/?u=59ba43482b8c913efe7355823&id=825b81f0a4

Citrus Gall Wasp emergence soon: new control strategies. *Citrus Connect*, September 2016. https://us11.campaign-archive.com/?u=59ba43482b8c913efe7355823&id=7ae9410c4b

Potential chemical options for citrus gall wasp control in Valencia trees. *Citrus Connect*, July 2017.

Gall wasp autumn control. *Citrus Connect*, September 2017.

Other communications

- Project findings were regularly sent to citrus growers and industries in the Riverina and Sunraysia through *Citrus Connect,* which is a quarterly electronic newsletter of NSW DPI.
- Two project workshops were held in Mildura, one on 15 June 2016 and another on 14 June 2017, to review project findings and discuss project plant for next year. David Daniels (Citrus Australia), Andrew Harty (Costa Group), Mal Wallis (Citri Care, QLD), and Scott Dix (Costa Group), Ben Bring (Costa Group), Colin Nankivell (Grower), Frank Gallucio (Sumitomo), Peter Cole (Sumitomo), and Aedan Gorman (Bayer) represented industries at the 2017 workshop. David Daniels (Citrus Australia), Andrew Harty (Costa Group), Justin Lane (Mildura Fruit Company), and Scott Dix (Costa Group) represented citrus industries at the 2017 workshop. Jianhua Mo presented findings from R&D activities conducted by NSW DPI. Craig Swanbery presented findings from R&D activities by Fruit Doctors.
- Jianhua Mo did a live interview on CGW management in the Gardening program of ABC Radio Adelaide on 19 September 2015.
- WA members of the project regularly sent CGW information to growers by email.