Managing citrus gall wasps in southern citrus regions

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NSW Department of Primary Industries

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MANAGING CITRUS GALL WASP IN SOUTHERN CITRUS REGIONS

Jianhua Mo

NSW Department of Primary Industries

Final Report of Project CT10021

October 2013

Project Description

Horticulture Australia Project Number: CT10021

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Project purpose: Develop management strategies for citrus gall wasp in southern Australia

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Disclaimer

Table of Contents

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Media Summary

Citrus gall wasp (CGW) is a native pest of citrus in Australia. Heavily infested trees are covered with unsightly galls of up to 30 cm long, resulting in very little leaf or fruit production and severe dieback. Until recently, CGW was confined to Queensland and central- to northern-New South Wales (NSW), with a noticeable absence in the southern citrus production regions. In the late 1990s CGW was first reported in isolated commercial orchards in Sunraysia, in far southwest NSW. Today, hundreds of hectares of citrus are infested with CGW in Sunraysia and the neighbouring Riverland in northeast South Australia.

Currently, only methidathion is registered for CGW control. This is a broad-spectrum insecticide and not compatible with integrated pest management (IPM) which has been the cornerstone of citrus pest management in Australia.

During 2010-2013, we studied the biology of CGW and investigated alternative management options for its control in the Coomealla Irrigation District, in far southwest NSW.

Two alternative chemicals have been identified as having potential for CGW control: petroleum spray oil (PSO) and imidacloprid. PSO deters the oviposition of CGW adults, and imidacloprid kills CGW larvae inside the galls. Both are less disruptive to populations of beneficial insects than the currently registered methidathion.

Timing is critical for CGW control. PSO targets the adult wasps and should be applied when CGW wasps are most abundant in the orchard. Imidacloprid and the currently registered insecticide target CGW larvae and should be applied around the time of egg hatching. Based on biological investigations, we have developed CGW phenology models that predict the timing of adult wasp emergence and egg hatching. Guidelines have been developed to help citrus growers time their sprays to ensure best results.

It is encouraging to note that significant numbers of the two major parasitic wasps of CGW were recovered in this study, confirming their establishment in the Sunraysia region after several introductions. Emergence of the parasitic wasps lags behind CGW by 2-3 weeks. Releases of the parasitic wasps are usually made by bringing galls from regions where they are well established and distributing them at identified release sites. The time lag provides a window of opportunity to allow most CGW to emerge from the galls before distributing the galls to the release sites, thus minimising the unwanted side-effect of introducing additional CGW wasps to the release area.

Technical Summary

Citrus gall wasp (CGW), *Bruchophagus fellis* (Hymenoptera: Eurytomidae), is an endemic citrus pest in Australia. Female wasps lay eggs inside current-year spring shoots. After hatching, the larvae burrow into the soft bark tissue and feed there until pupation. As the season progresses, the feeding areas gradually swell, eventually forming the characteristic galls, each housing multiple larvae/pupae. When the density is high, galls of up to 30 cm long may form. Heavily infested trees can be covered with galls, resulting in very little leaf or fruit production and severe dieback. All citrus varieties are attacked by CGW.

Until recently, CGW was confined to Queensland and central- to- northern-New South Wales (NSW), with a noticeable absence in the southern citrus production regions of the Riverina, Sunraysia, and the Riverland, where the bulk of Australian oranges are grown. The situation changed in the late 1990s, when CGW was first reported in isolated commercial orchards in Sunraysia, in far southwest NSW. The infestation quickly spread and today hundreds of hectares of citrus are infested with CGW in Sunraysia and the neighbouring Riverland in northeast South Australia. Elsewhere, the wasp has been found in backyard citrus trees in the Riverina in southern NSW and in the outskirts of Perth in Western Australia.

In its natural habitat, CGW is normally kept below damaging levels by several parasitic wasp species. After several releases, the parasitic wasps have established in the Sunraysia but their numbers are not yet high enough to effectively control CGW, leaving chemical control as the only management option. Currently, only methidathion is registered for CGW control. Methidathion is a broad-spectrum insecticide and not compatible with citrus IPM. Timing is critical in CGW control. The adult wasps are the only exposed stage of the life cycle and they are active for only a few weeks each year. CGW larvae can be killed by systemic insecticides or those with trans-laminar activity. Methidathion has some trans-laminar activity and is best used to target newly hatched larvae before the bark tissue has hardened. To time chemical applications against the adults and larvae, we need to know when the adults emerge and eggs hatch. Such information is not yet available for CGW populations in the southern states.

In an effort to develop effective management options against CGW for the southern citrus production regions, we investigated (1) temporal distributions of adult wasp emergence, (2) reproduction parameters of the wasp including egg development, and (3) new chemical control options in the Coomealla Irrigation District in the Sunraysia during 2010-2012.

Emergence of CGW adults at the study sites in 2010-2012 occurred during October-November. Median adult emergence date ranged from 26 October to 20 November depending on year and site. Most adult wasps had emerged by mid-late November. The complete emergence process took about three weeks. Degree-days (DD) accumulated since 1 April using a lower threshold temperature of 15°C and an upper threshold temperature of 35°C or 40°C gave the best predictions of median emergence dates in the three years. The required DD to achieve 5, 50, and 95% emergence were 336, 403, 447 DD, respectively. CGW adult emergence in future years can be predicted using these DD parameters and a combination of observed and average historical temperature data for the target site. As a quick guide, median emergence occurs about three weeks after wasp emergence commences.

It is encouraging to note that significant numbers of the two major parasitic wasps of CGW, *Megastigmus brevivalvus* and *M. trisulcus*, were recovered in this study, confirming their establishment in the Sunraysia region. Emergence of the parasitic wasps lagged behind that of their host by 2-3 weeks. Releases of the parasitic wasps are usually made by bringing galls from regions where they are well established and distributing them at identified release sites. The time lag provides a window of opportunity to allow most CGW to emerge from the galls before distributing the galls to the release sites, thus minimising the unwanted side-effect of introducing additional CGW wasps to the release area.

After emergence from the galls, adult wasps lived 3.1-11.2 days, depending on temperature. Females were able to lay eggs immediately after emergence regardless of mating status, with peak egg-laying occurring in 1-2 day old females. Median egg development period varied from 11 days at 29 $^{\circ}$C to 25 days at 13 $^{\circ}$C. According to the linear relationship between egg development rate and temperature, the median egg development period at the study sites during 2010-2012 was estimated at 16.8 days. Dissections of shoots from a nearby lemon orchard showed that 50% of eggs had hatched by 4 -13 December and 95% by 24 - 26 December, during 2010-2012.

Three alternative chemicals were investigated for CGW control in this study: paraffinic oil (BioPest®), imidacloprid (Confidor® Guard), and spirotetramat (Movento®). Paraffinic oil (petroleum spray oil, PSO) is widely used in Australia to control sap-sucking insects. Imidacloprid and spirotetramat are both systemic insecticides. Applied at the rate of 0.5L formulated product per 100L water in three 1-2 weekly sprays starting from late October, the paraffinic oil reduced subsequent gall formation by over 50%. Reducing the rate to 0.25L formulated product per 100L was not an effective option. There may be scope to reduce the number of sprays to one, if timed correctly. A single application of imidacloprid in the soil from late October to mid November achieved similar control of CGW as three sprays of the paraffinic oil, however, the exceptionally long residual period and potential negative impact of the chemical on beneficial organisms in citrus need to be considered before it is registered. The efficacy of spirotetramat was not confirmed in this study, however, its dual-pathway trans-laminar property and relative short residual period make it a worthy candidate for further investigations.

As a result of the trials conducted in this project and from our previous understanding of these pesticides, the following can be suggested to provide optimum control of the CGW. Paraffinic oil targets adult wasps and should be applied when the wasps are most abundant. Peak wasp abundance is predicted to occur about a week after the predicted median emergence date. A degree-model has been developed to predict the median emergence date. As a precaution, oil sprays should not be applied during peak flowering. Insecticides that target the larvae, such as imidacloprid, spirotetramat and the currently registered methidathion, are best applied either shortly before (allowing time for the chemicals to be absorbed by the trees), or soon after, most eggs have hatched. For CGW populations in the Sunraysia, completion of egg hatching is predicted to occur by the end of December, so the best application window for these chemicals is between late December and early January. Caution needs to be exercised when timing sprays of methidathion and spirotetramat as they are applied to the foliage and most parasitic wasps also emerge from the galls during this period.

Technology Transfer

Project findings were presented to citrus growers through field days, articles to the Murray Valley Citrus Board newsletter, posters at Australian citrus conferences, and frequent email and phone communications with the local industry body and key growers. A scientific paper has been written on the emergence of adult wasps.

Field days

- 11 October 2011, Farm of Richard Bertalli, Coomealla Irrigation District, NSW.
- 9 October 2012, Farm of Shane Smythe, Coomealla Irrigation District, NSW.
- 21 October 2012, Biological Services, Loxton, SA.

Industry articles

- Knowing your foe – new insights into the biology of citrus gall wasp. January 2011, MVCB Newsletter.
- Timing of citrus gall wasp adult emergence. May 2012, MVCB Newsletter
- Promising chemical alternatives for citrus gall wasp control. March 2013, MVCB Newsletter

Conferences

- New insights into the biology and control of citrus gall wasp. 2011 National Citrus Conference, Nuriootpa, SA, 23-26 October 2011.
- Management of citrus gall wasp in citrus. 2012 National Citrus Conference, Leeton, NSW, 21-24 October 2012.

Scientific paper

Mo J & Stevens MM 2013. A degree-day model for predicting emergence of adult citrus gall wasp, *Bruchophagus fellis* (Hymenoptera: Eurytomidae), in southern Australia. Submitted to J*ournal of Asia-Pacific Entomology*.

Recommendations

Two new alternative chemicals to methidathion have been identified as having potential for CGW control: petroleum spray oil (PSO) and imidacloprid. PSO foliar spray at the rate of 0.5L formulated product per 100L water, and imidacloprid soil drench at 9 mL formulated product per tree, both gave satisfactory control of CGW. PSO is less disruptive to natural enemies of citrus pests than imidacloprid and is recommended as the first choice for registration. Imidacloprid soil drench has demonstrated efficacy against a wide range of sap-sucking insects in addition to citrus gall wasp, and is a worthy candidate for consideration of registration.

For best results, PSO should be applied when adult CGW are most abundant in the orchard. A degree-day model has been developed to predict the peak emergence date. As a quick guide, peak wasp emergence occurs about three weeks after wasp emergence has started. As a precaution, PSO, if registered, should not be applied during peak flowering.

Imidacloprid and the currently registered methidathion both target CGW larvae and should be applied around the time of egg hatching. In the Sunraysia, CGW egg hatching completes by the end of December. Hence the best application window for these chemicals is between late December and early January. Timing is less critical for imidacloprid as it has a relatively long residual period.

Emergence of the parasitic wasps of CGW lags behind CGW adult wasps by 2-3 weeks. Releases of the parasitic wasps are usually made by bringing galls from regions where they are well established and distributing them at identified release sites. The time lag provides a window of opportunity to allow most CGW to emerge from the galls before distributing the galls to the release sites, thus minimising the unwanted side-effect of introducing additional CGW wasps to the release area.

For adoption, a technical note providing best management practices for CGW needs to be produced in consultation with the industry and distributed to Sunraysia citrus growers.

General Introduction

Citrus gall wasp (CGW), *Bruchophagus fellis* (Hymenoptera: Eurytomidae), is an endemic citrus pest in Australia (Noble 1936). The females lay eggs inside current-year spring shoots (Fig 1, left). After hatching, the larvae burrow into the soft bark tissue and feed there until pupation. As the season progresses, the feeding areas gradually swell eventually forming the characteristic galls, each housing multiple larvae/pupae (Fig. 1, right). When the density is high, a single gall of over a foot long may form. Heavily infested trees can be covered with galls, resulting in very little leaf or fruit production, and severe dieback. All citrus varieties are attacked by CGW.

Figure 1. CGW females laying eggs on a current-year spring shoot (left), and typical galls developed around the larval feeding areas late in the season (right).

CGW has a single generation per year in Australia (Noble 1936). There are five development stages: egg (Fig. 2, left), 5 larval instars (Fig. 2, middle), prepupa, pupa, and adult (Fig. 2, right) (Fig. 2). All stages except the adult develop inside the galls, with the larva spending most of its time in its 1^{st} instar. The adults emerge from the galls in spring to mate and lay eggs. Eggs laid as far apart as two months may emerge as adults at a similar time, resulting in synchronized emergence events. This flexibility is due largely to the variable duration of the 1^{st} larval instar.

Figure 2. CGW eggs on the underside of the bark (left), mature larvae inside a gall (middle), and the adult female (right).

CGW was first reported as a pest of citrus in the early 1930s in northern New South Wales (NSW) (McKeown 1898). Subsequent surveys showed it was present only in the coastal districts of southern Queensland and northern NSW (Noble 1936). By the late 1990s, the confirmed distribution range had expanded to include the central to northern Queensland coastal districts and the northern and central tablelands of NSW (Fig. 3) (Smith *et al*. 1997). Noticeably, all three major citrus production regions in southern Australia, the Riverina, Sunraysia, and the Riverland were outside the distribution range at that time. The status quo changed in the late 1990s when CGW infestations were first reported in commercial orchards in Sunraysia (Cannard 2007). Hundreds of hectares of citrus are now infested with CGW in the region. Soon afterwards, CGW infestation was reported in backyard citrus trees in Griffith in the Riverina (Hardy & Creek. 2009). In 2012, CGW was found in commercial citrus orchards in Renmark and Loxton in the Riverland (Kim Thiel, personal communication, 18 October 2012). In 2013, galls similar to that caused by CGW were found on the outskirts of Perth in Western Australia (Andras Szito, personal communication, 1 May 2013).

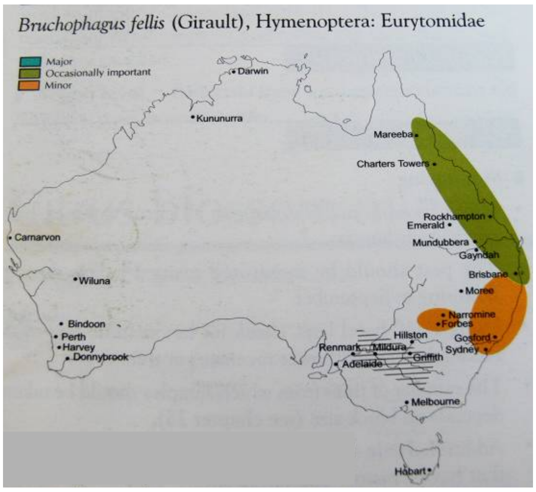

Figure 3. Current distribution of CGW in Australia. Colored regions show its distribution in 1997 and the hatched area shows regions of recent CGW incursions. Base map taken from Smith et al. (1997).

While the impact of CGW on fruit production is widely acknowledged, there have been no attempts to quantify the impact. In the worst case scenario, CGW-infested trees produce no marketable fruit. For an infestation area of 100 ha, this amounts to an annual loss of \$500,000-\$750,000 depending on market price. Assuming a more modest impact of 25% reduction of yield in CGW-infested trees, the annual loss would be \$125,500-\$187,500. The real cost of taking no action is likely to be much higher as the infestation area is still increasing, threatening citrus across the entire Murray Valley, which has an annual value of production of over \$40 million.

In its natural habitats, CGW is attacked by several parasitic wasp species, the major species being *Megastigmus brevivalvus* and *M. trisulcus* (Hymenoptera: Megastigminae) (Noble 1938) (Fig. 4). Over 90% of CGW larvae can be parasitized (Smith *et al*. 1997). Where the parasitic wasps are active, CGW populations and damage levels are greatly reduced (Smith et al. 1997). After several releases, the parasitic wasps have established in the Sunraysia but numbers are not yet high enough for effective control of CGW (Flett 2011). It is believed that annual releases for 3-5 years may be needed to establish local populations of the parasitic wasps (Hardy *et al*.

2009). Ants may also play a role in the natural regulation of CGW populations (Hely 1982).

Figure 4. Two parasitic wasps of CGW, *Megastigmus brevivalvus* (left) and *M. trisulcus* (right). Images from Smith *et al*. 1997.

Methidathion is the only registered insecticide for CGW control in citrus. Its use is constrained by its high mammalian toxicity and broad spectrum activity against a wide range of invertebrates including natural enemies of citrus pests. Timing is critical for it to be effective against CGW. According to Papacek and Smith (1989), methidathion should be applied after CGW females have finished laying eggs and before the current-year citrus shoots have hardened. In central Queensland the timing corresponds to early December (Papacek & Smith 1989). However, the same timing cannot be assumed for all locations due to differences in temperature and photoperiod.

Noble (1936) and Hely (1982) provided some qualitative descriptions of CGW phenology, including timing of adult emergence and stage-specific development periods. However, most of their estimates were made from uncontrolled experiments. To provide reliable estimates of CGW phenological events in a given location in a given year, we need to quantify the distributions of the phenological events under different temperatures. Such information is currently lacking for CGW.

This project aimed to achieve better control of CGW in southern production regions of Australia based on a better understanding of the phenology of local CGW populations, and effective and IPM-compatible chemical control options. Specifically, distribution models of key phenological events such as adult wasp emergence and egg hatching were developed, and the relationship between phenological stages and degree-days determined. Forecast models were then developed that predict the timing of these events. For alternative chemical control options, the project concentrated on those that pose minimal health risk to humans and are non-disruptive to current citrus IPM programs.

References

Cannard M. 2007. Native parasitic wasps released to control citrus gall wasp. Citrep 51, 14-15.

Hardy S & Creek A. 2009. Final Report - Citrus Gall Wasp Technical Forum. Dareton, 5th May 2009.

Hely PC, Pasfield G & Gellatley JG. 1982. Insect pests of fruit and vegetables in NSW. Inkata Press, Melbourne.

Flett H. 2011. Establishment of Citrus Gall Wasp Parasites in the Murray Valley Region. Final Report of Project CT08000, Horticulture Australia Ltd.

McKeown GM. 1898. Fruits at the Richmond River Experiment Farm. Agric. Gaz. N.S.W. 9, 190-194.

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

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

Papacek DF & Smith D. 1989. Insectidal control of citrus gall wasp in Queensland. General and Applied Entomology 21, 2-4.

Smith D, Beattie GAC & Broadley R. 1997. Citrus pests and their natural enemies. DPI Queensland, Brisbane.

Chapter-1   Emergence of Adult Wasps

Abstract Emergence of adult citrus gall wasp (CGW), *Bruchophagus fellis* Girault (Hymenoptera: Eurytomidae), in citrus orchards in the Coomealla Irrigation District in far west New South Wales was monitored with sticky traps for three seasons from 2010 to 2012. Depending on year, site, and trap type, detection of emergence started from early October to early November, peaked from late October to mid November, and was mostly finished by mid-late November. Most emergence took place during a period of only 19 days. There were some site and trap differences in detection of emergence timing, however the largest differences were observed between years. The role of temperature in emergence timing was investigated with degree-days (DD). DD accumulated since 1 April using a lower threshold temperature of 15 °C and an upper threshold temperature of 35 °C or 40 °C gave the best predictions of median emergence dates in the three years. The required DD to achieve 5, 50, and 95% emergence were 336, 403, 447 DD, respectively. CGW adult emergence in future years can be predicted using these DD parameters and a combination of observed and average historical temperature data for the target site. As a quick guide, median emergence occurs about three weeks after wasp emergence is first observed. Peak wasp abundance in the orchard is predicted to occur about a week after the median emergence date. Two parasitic wasps, *Megastigmus brevivalvus* and *M. trisulcus, attack CGW.* Emergence of the parasitic wasps lagged behind CGW adult wasps by 2-3 weeks.

Introduction

Citrus gall wasp (CGW), *Bruchophagus fellis* Girault (Hymenoptera: Eurytomidae), completes most of its lifecycle inside woody galls (Smith et al. 1997). The only stage directly exposed to contact insecticides is the adult stage. Contact insecticides usually have limited residual activity. For best efficacy, their application should be timed when the adults are most abundant. Knowledge of the timing of adult emergence is also needed in the prediction of timing of egg hatching, which, in turn, is needed to time control actions against newly hatched larvae (Papacek & Smith 1989). Noble (1936) described CGW phenology on the NSW north coast, including timing and pattern of adult emergence. His results were based mostly on observations and some non-controlled experiments.

In its natural habitats, CGW is attacked by several parasitic wasp species, the major species being *Megastigmus brevivalvus* and *M. trisulcus* (Hymenoptera: Megastigminae) (Noble 1938). Over 90% of CGW larvae can be parasitized (Smith et al. 1997). Where the parasitic wasps are active, CGW populations and damage levels are greatly reduced (Smith et al. 1997). After several releases, the parasitic wasps have established in the Sunraysia but numbers are not yet high enough for effective control of CGW (Flett 2011).

In this chapter, we report for the first time statistical distributions of the emergence of CGW adults and its parasitic wasps in a citrus orchard as a function of degree-days based on 3 year's trapping data. Knowledge of such distributions is needed to develop forecast models to predict the timing of wasp emergence and egg hatching in the field.

Materials and Methods

Data collection

CGW adult emergence was monitored with sticky traps during 2010-2012 on citrus farms near Dareton in far west NSW. Most data were collected from a farm in the Coomealla Irrigation District (S34°05.369', E142°07.230'), where CGW infestation in the region was first noted. There, field emergence of adult wasps was monitored annually from 2010 to 2012 in a block of 'Autumn Gold' orange trees (block-1). In 2012, CGW adult emergence was also monitored at two other sites in the region, an abandoned block of lemon (*Citrus* x *limon*) trees (block-2) within 1 km of block-1, and a block of 'Valencia' orange trees on a separate farm in the region (block-3) (S34°04.392', E142°07.943'). Monitoring was conducted either weekly or twice weekly starting sometime before the first adult wasps had emerged from the galls (late September to early October) and finishing after wasp emergence had completed (mid December- early January).

Three types of sticky traps were used in the study: cup traps, rolled yellow sticky traps (RYST), and flat yellow sticky traps (FYST). Cup traps were made from disposable clear plastic cups (480 mL). The interior cup surface was coated with a thin layer of Tangle-Trap® (The Tanglefoot Company, Grand Rapids, MI 49504, USA) to trap emerging wasps. The traps were placed around the target galls through a 1-cm hole in the centre of the cup base and an L-shaped slit linking the cup opening to this hole. Two twist-and-tie wires attached to the opposite sides of the rim of the cup opening were tied around the gall bearing stem to prevent galls directly touching the sticky surface (Fig. 1).

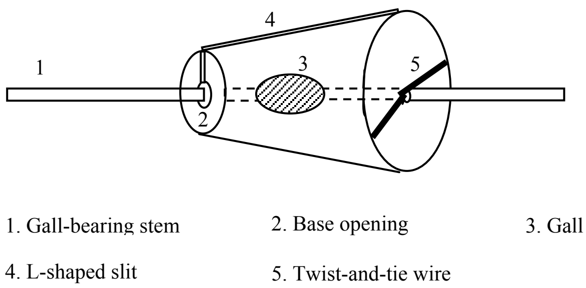

Figure 1. An illustration of the cup trap.

Double-sided yellow sticky traps (75 x 110 mm, Bugs for Bugs, Mundubbera, QLD 4626, Australia) were used either unmodified (FYST) or rolled to form a tube (RYST). In the former case, two RYST traps were made from one double-sided yellow sticky trap. RYST were wrapped around galls as for the cup traps. FYST were hung on twigs in the lower canopy.

Cup traps and RYST traps were each placed around a randomly selected current-year CGW gall with no exit holes. FYST traps were hung on twigs in the lower canopy where galls were relatively more abundant.

In 2010, 50 cup traps were placed on 25 trees and 10 FYST and RYST traps each were placed in pairs in 10 trees in block-1. The traps were replaced weekly. In 2011, 30 cup traps were placed in 15 trees and 10 FYST traps in 10 trees in block-1. The traps were replaced twice weekly. In 2012, 10 cup traps and 10 FYST traps were placed in pairs in 10 trees in each of block-1, block-2, and block-3. The traps were replaced twice weekly.

Replaced traps were wrapped individually in Glad-Wrap® and taken back to the laboratory, where they were checked under a stereo microscope and the numbers of adult wasps were counted. Any parasitic wasps found were also recorded.

Hourly temperature and humidity during the study period in block-1 were monitored with dual-channel data-loggers (Gemini Data Loggers, West Sussex, UK). Daily maximum and minimum temperature data in Mildura airport were obtained from the Australian Bureau of Meteorology website (http://www.bom.gov.au). Mildura airport is 15-20 km away from the monitoring sites.

Data analysis

For each season, monitoring block, and trap type, wasps caught by all traps were summed for each inspection date. The sums were then added sequentially by inspection dates to give the cumulative numbers of wasps caught by each date since monitoring started. Assuming trapping was by passive interception and the probability of a wasp being trapped was not influenced by the number of wasps already caught in the trap, the cumulative numbers of wasps caught by an inspection date would be proportional to the cumulative numbers of wasps emerged by that date. Hence, the cumulative proportions of wasps emerged by a given inspection date can be estimated by the proportion of wasps caught by the date over the total number of wasps caught during the entire monitoring period.

To predict the timing when a given proportion of the wasps have emerged, the cumulative proportions were fitted to the following Weibull distribution function (Weibull 1961):

$$p(t) = 1 - \exp(-(t/\lambda)^k) \qquad (1)$$

where $p(t)$ is the proportion of wasps emerged by time t, and λ and k are parameters to be estimated. The time unit t was expressed either as days after 1st September (DAS) in the corresponding season, or as degree-days (DD) accumulated since a given date in the season. The former unit is independent of temperature and suited only for describing the emergence process at specific sites in individual seasons. The latter unit is temperature-dependant and ideal for describing emergence processes across sites and seasons, and for emergence prediction. Parameters λ and k in Equation-1 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 of the wasps have emerged can be estimated by the inverse of Equation-1,

$$t = \lambda(-\log(1-p))^{1/k} \qquad (2)$$

When $p = 0.5$, t gives the median predicted emergence date. Goodness-of-fit of Equation-1 in fitting adult emergence data was measured by the variation in emergence proportion explained by the equation (R^2). A simpler and more intuitive measurement of goodness-of-fit used was the difference between predicted and observed median emergence dates, with predicted median dates estimated from fitted Weibull distributions and observed median dates from linear interpolations of emergence data.

Assuming the cumulative distribution of wasp emergence follows the Weibull distribution, and that every wasp lives for L days, the relative abundance of live wasps alive at a given date t, $Z(t)$, can be estimated by the following equation:

$$Z(t) = \sum_{i=0}^{L} [\exp(-((t-i-1)/\lambda)^k) - \exp(-((t-i)/\lambda)^k)] \qquad (3)$$

where λ and k are parameters of the Weibull distribution.

To predict emergence with temperature data, we need to describe the emergence process as a function of DD. This can be done by fitting observed cumulative emergence to the Weibull function in Equation-1. DD calculation requires the knowledge of the lower (T_{lower}) and upper (T_{upper}) development threshold temperatures, and the starting date for DD accumulation (D_{start}). Neither T_{lower} nor T_{upper} is known for CGW. D_{start} can be any dates after eggs are laid. In this study, a series of combinations of candidate values of T_{lower}, T_{upper}, and D_{start} were evaluated to determine which set of values resulted in the least sum of squares of the differences between predicted and observed median emergence dates (SS_{median}) in multiple datasets. Specifically, T_{lower} was tested at all temperatures from 0 to 15 °C in 1 °C increments. T_{upper} only affects DD calculations at high temperatures, so it was tested at 35 and 40 °C only. The tested threshold temperatures are within the ranges reported for most insects. D_{start} was tested at the first day of each month from January to September in the same season. This range of D_{start} was chosen considering CGW egg-laying normally finished in December and adult emergence normally started in October in the study region. Three season's adult emergence data from cup traps in monitoring block-1 were used to determine the best values for T_{lower}, T_{upper}, and D_{start}.

DD were estimated directly from hourly temperatures logged in block-1, or indirectly from daily maximum and minimum temperatures in Mildura airport using the single-sine method with horizontal cut-off (Roltsch *et al.* 1999) when hourly temperature data were not available.

Finally, the best-fit Weibull function was used to predict the time when 5, 50, and 95% of the wasps had emerged.

Results

Traps of all three types caught sufficient CGW adults (more than 100 wasps per trap site per season) for analyses of CGW emergence patterns. Cup traps and the FYST traps were similarly efficient (417-2197 wasps vs. 266-3302 wasps per trap per season). RYST traps were the least efficient of the three (106 wasps per trap site per season). Cumulative wasp emergence data were well fitted by the Weibull function, which explained over 99% of the variations in the proportions of wasps emerged (Fig. 2-5; Table 1). The fittings were particularly good around the median emergence dates.

Table 1. First, 5%, 50%, and 95% emergence dates of adult CGW wasps and parasitic wasps as observed/estimated from trap data [1].

				Emergence dates				Fitted Weibull		
Wasp species	Trap	Block	Year	First	5%	50%	95%	λ	k	R^2
CGW	Cup	1	2010	56	70	80	87	82.00	19.42	0.9998
CGW	Cup	1	2011	46	47	61	70	63.24	10.31	0.9964
CGW	Cup	1	2012	31	46	57	64	58.29	12.43	0.9949
CGW	FYST	1	2010	63	77	84	88	85.05	28.44	0.9977
CGW	FYST	1	2011	32	48	61	70	63.29	10.48	0.9988
CGW	FYST	1	2012	34	50	60	67	61.69	14.17	0.9951
CGW	RYST	1	2010	63	73	83	89	84.09	21.44	0.9964
CGW	Cup	2	2012	45	44	53	58	54.03	14.67	0.9865
CGW	FYST	2	2012	35	42	58	70	60.87	8.24	0.9953
CGW	Cup	3	2012	45	53	64	71	65.83	13.33	0.9924
CGW	FYST	3	2012	35	52	65	74	67.34	11.34	0.9931
Parasitic wasps[2]	FYST	1	2011	54	64	75	82	76.49	16.65	0.9978
Parasitic wasps	Cup	1	2012	56	66	78	85	79.40	16.10	0.9938
Parasitic wasps	FYST	1	2012	62	68	77	83	78.52	20.79	0.9908
Parasitic wasps	FYST	3	2012	70	69	81	89	82.89	16.72	0.9966

[1] Dates are given as days since 1 September in respective years. First emergence dates are given as the median dates between the last negative and first positive sampling dates. Dates for 5, 50, and 95% emergence were estimated from the fitted Weibull function.

[2] *Megastigmus brevivalvus* and *M.trisulcus*.

<u>CGW emergence in block-1 in 2010</u>

CGW adult emergence was first recorded around 56 DAS in cup traps, and 63 DAS in FYST traps and RYST traps (Table 1). The cumulative distributions of emergence in the three trap types were similar with only small differences in the middle sections showing slightly earlier emergence in cup traps than the other two trap types (Fig. 2). According to the fitted distributions, 5%, 50%, and 95% emergence occurred at 70-77, 80-84, and 87-89 DAS, respectively (Table 1). Difference in timing between the three trap types decreased from 7 days at 5% emergence, to 4 days at 50% emergence, and 2 days at 95% emergence.

CGW emergence in block-1 in 2011

Adult emergence was first recorded around 46 DAS in cup traps and 32 DAS in FYST traps, 10 and 31 days later than in 2010 by the two trap types respectively (Table 1). Cumulative distributions of emergence by the two trap types were almost identical (Fig. 3). According to the fitted distributions, 5%, 50%, and 95% emergence occurred at 47, 61, and 70 DAS, respectively, in cup traps, and 48, 61, and 70 DAS, respectively, in FYST traps (Table 1).

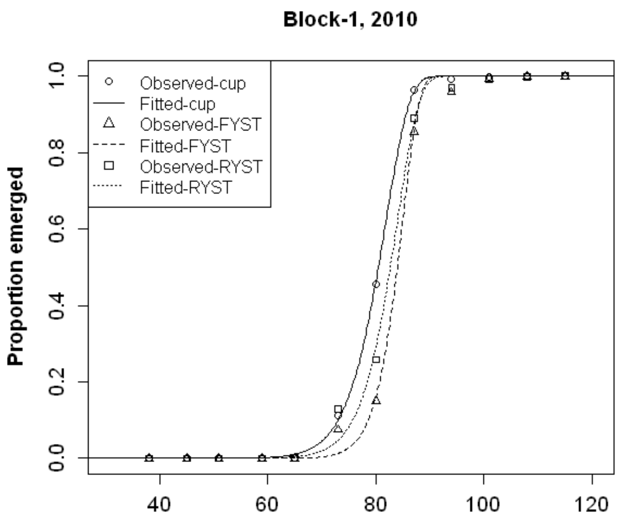

Figure 2. Observed and fitted cumulative distribution of CGW adult emergence in monitoring block-1 in 2010.

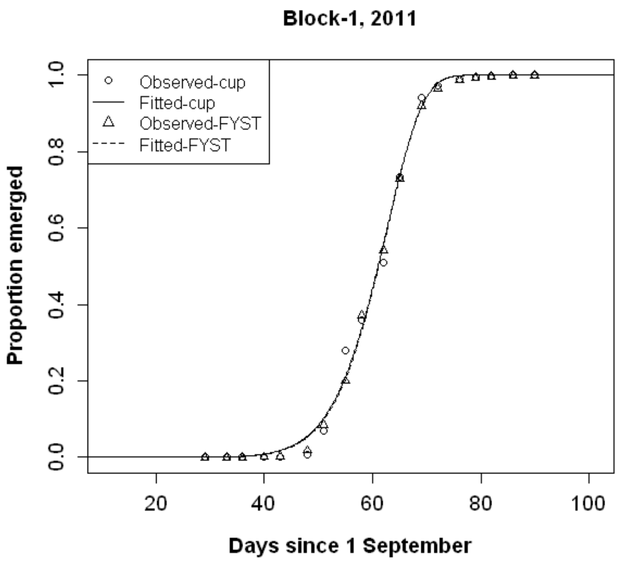

Figure 3. Observed and fitted cumulative distribution of CGW adult emergence in monitoring block-1 in 2011.

CGW emergence in block-1 in 2012

This season saw the earliest emergence of adult CGW wasps in block-1 in all three years. The first recorded emergence by cup traps was 25 days earlier than 2010 and 15 days earlier than in 2011 (Table 1). The FYST traps showed a similar difference in the first emergence date between 2012 and 2010, however, the difference between 2011 and 2012 was negligible (2 days). Cumulative distributions of emergence recorded from the two trap types were similar in shape, but emergence from cup trap data was slightly earlier than in FYST traps (Fig. 4). According to the fitted distributions, 5%, 50%, and 95% emergence occurred at 46, 57, and 64 DAS, respectively, in cup traps, and 50, 60, and 67 DAS, respectively, in FYST traps (Table 1).

CGW emergence in block-2 in 2012

Adult emergence was first recorded around 45 DAS in cup traps and 35 DAS in FYST traps (Table 1). The first emergence date, as shown by cup traps in this block, was 14 days later than in block-1 in 2012 but similar to that in block-1 in 2011. First emergence in FYST traps occurred at a similar time in this block as in block-1 in 2012. Cumulative distributions of emergence in cup traps showed a much more rapid emergence pattern than in FYST traps (Fig. 4). This was reflected in the characteristic emergence dates. According to the fitted distributions, 5%, 50%, and 95% emergence occurred at 44, 53, and 58 DAS, respectively, in cup traps, and 42, 58, and 70 DAS, respectively, in FYST traps (Table 1). The median emergence dates in this block were similar to those in block-1.

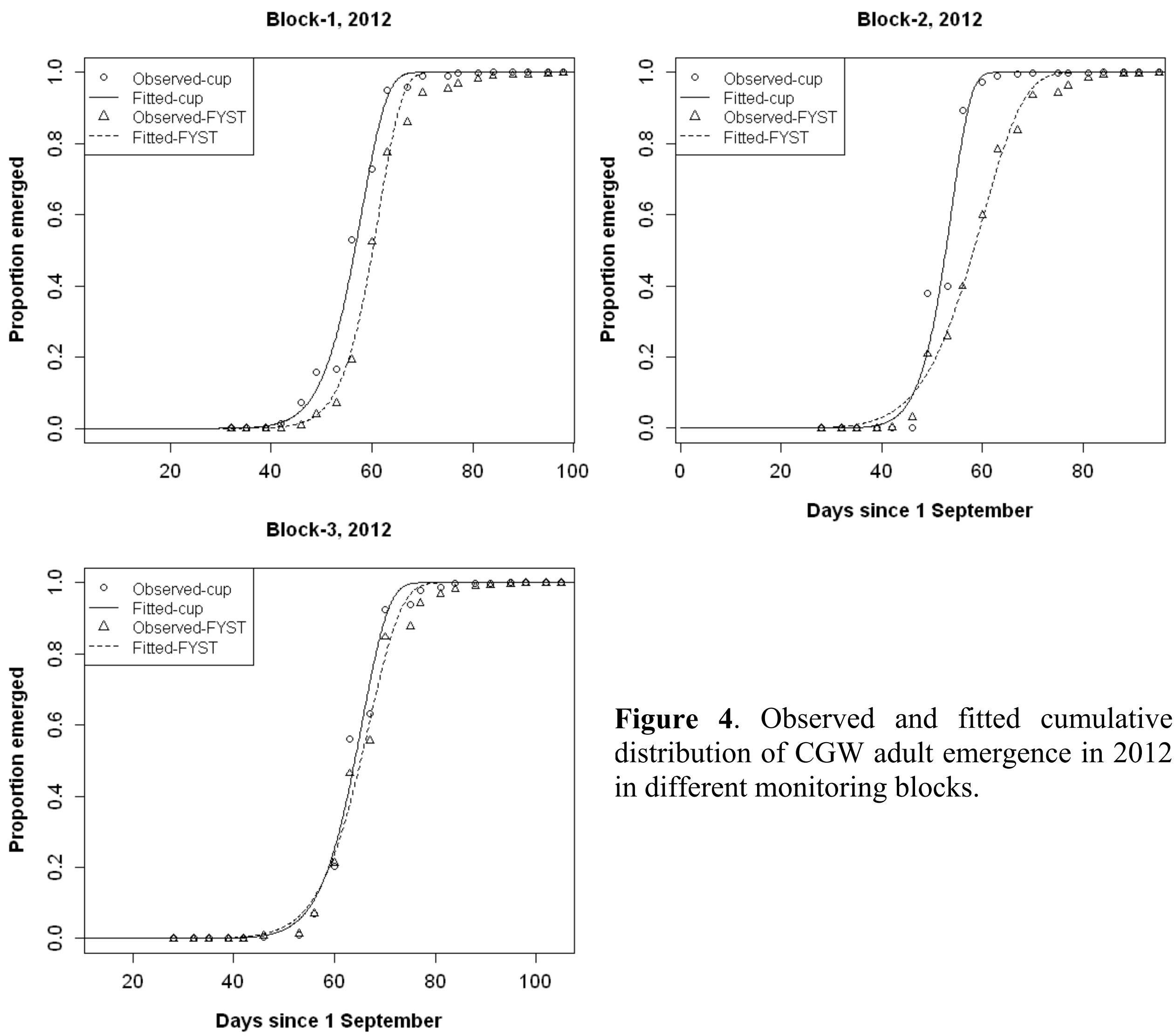

Figure 4. Observed and fitted cumulative distribution of CGW adult emergence in 2012 in different monitoring blocks.

CGW emergence in block-3 in 2012

The first emergence dates in this block were identical to those in block-2 in 2012 from both cups traps and FYST traps data (Table 1). Cumulative distributions of emergence in the two trap types were almost identical during the early half of the emergence period, but emergence in cup traps finished earlier than in FYST traps (Fig. 4). According to the fitted distributions, 5%, 50%, and 95% emergence occurred at 53, 64, and 71 DAS, respectively, in cup traps, and 52, 65, and 74 DAS, respectively, in FYST traps (Table 1). Median emergence in this block occurred 7-11 days later in cup traps and 5-7 days later in FYST traps than in the other two blocks in 2012.

Wasp abundance

Based on the fitted Weibull distribution parameters for the cup trap data in 2010-2012 (Table 1) and Equation-3, abundance of live wasps in block-1 peaked 2-8 days after the median wasp emergence date given the hypothesised wasp longevity range of 1-15 days (Table 2). The time lag increased with increasing longevity, however, as a proportion of longevity, the time lag actually decreased.

Table 2. Difference in days between the date of peak abundance of live wasps and the date of median emergence of CGW under different values of adult longevity (days) using fitted parameters of the Weibull distribution for cup traps in block-1 in 2010-2012.

Longevity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2010	2.2	2.7	3.2	3.6	4.0	4.4	4.8	5.1	5.5	5.8	6.2	6.5	6.8	7.0	7.4
2011	2.6	3.0	3.5	4.0	4.4	4.9	5.3	5.7	6.1	6.5	6.9	7.3	7.7	8.0	8.4
2012	2.2	2.7	3.2	3.6	4.1	4.5	4.9	5.3	5.7	6.0	6.4	6.7	7.0	7.4	7.7

Emergence of parasitic wasps

In addition to CGW, the two main parasitic wasps of CGW in Queensland and northern NSW, *Megastigmus brevivalvus* and *M.trisulcus*, were also recovered in the traps. Sufficient numbers were caught by the FYST traps in block-1 in 2011 and in block-1 and block-3 in 2012, and by the cup traps in block-1 in 2012, to enable estimates of the characteristic emergence dates. First emergence dates of the parasitic wasps were 19-30 days later than the first emergence dates for CGW. The cumulative distributions of emergence of the parasitic wasps were largely parallel to the corresponding distributions of CGW in the same block, year and trap type, with a time lag of 2-3 weeks in both the median emergence dates and the 95% emergence dates (Fig. 5; Table 1).

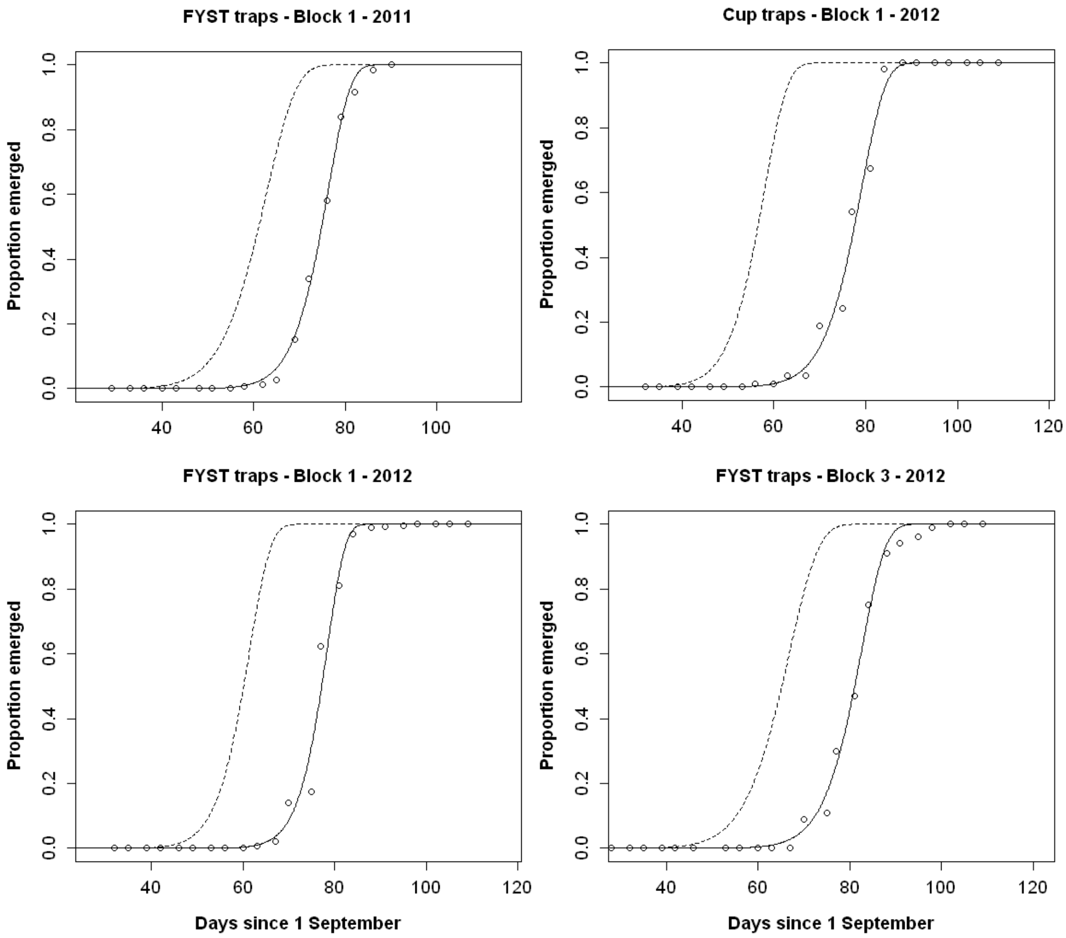

Figure 5. Observed (circles) and fitted (solid line) cumulative distribution of the emergence of parasitic wasps in comparison to that of CGW by the same trap type and in the same year (dotted line).

<u>Degree-day Model</u>

Three season's CGW adult emergence data from cup traps in block-1 were fitted to the Weibull function using DD estimated from different lower (T_{lower}) and upper (T_{upper}) development threshold temperatures and starting months (D_{start}) to see which DD parameter values resulted in the best-fit based on the sum of squares of differences between observed and predicted median emergence dates (SS_{median}). SS_{median} varied considerably and nonlinearly with both T_{lower} and D_{start} but was relatively unaffected by T_{upper} (Fig. 6). For both T_{upper} = 40 and 35 °C, SS_{median} reached its minimum at T_{lower} = 15 °C and D_{start} = 1 April. Other T_{lower}/D_{start} values yielding relatively low SS_{median} values were: 14 °C /1 April, 11-15 °C/1 May, and 12 °C/1 July.

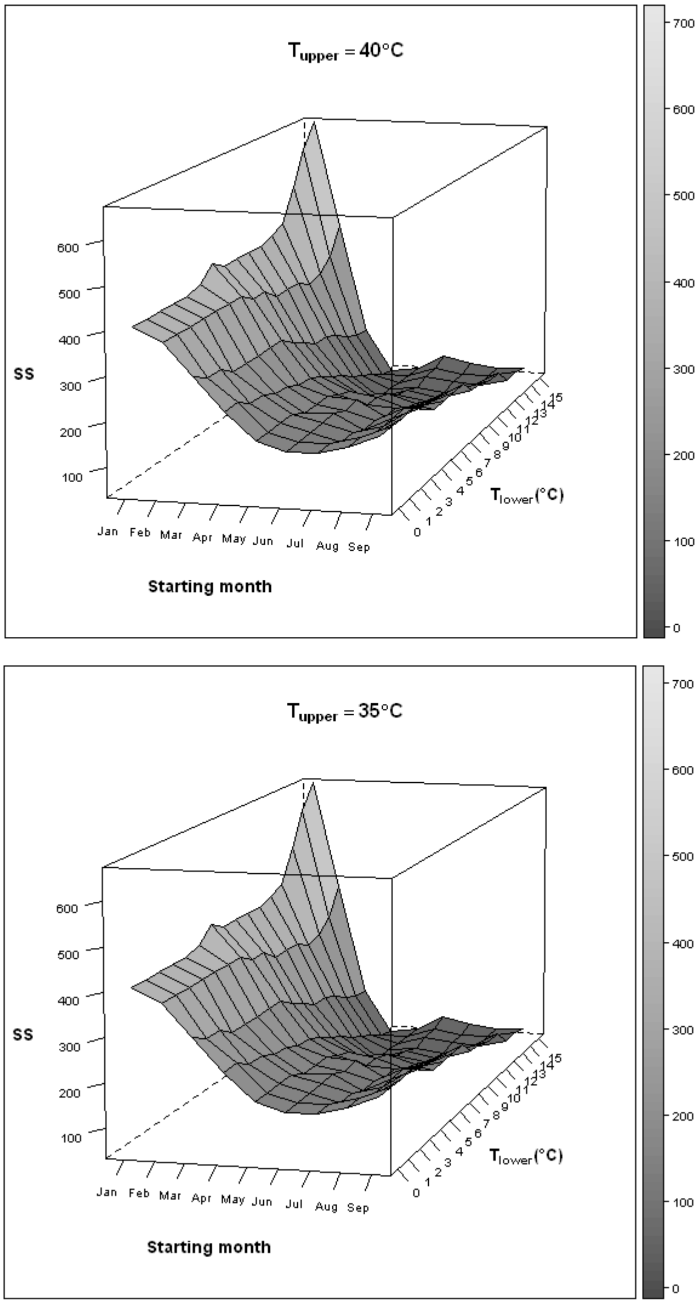

Figure 6. Sum of squares of difference between observed and predicted median emergence dates in three season's CGW emergence data in block-1 (SS_{median}) for degree-days calculated under different starting month and lower threshold temperatures.

With the DD parameters set at the values giving the minimum S_{median}, the emergence patterns of CGW adults in block-1 in relation to DD in all three seasons were positioned relatively close to each other, especially in the middle and latter part of the emergence processes (Fig. 6). For the purpose of prediction, data over the three seasons were pooled and fitted to the Weibull function to describe the general CGW adult emergence process. According to the pooled fitted cumulative distribution (Fig. 7), 5, 50, 95% emergence occurred at 336, 403, and 447 DD after 1 April respectively. The corresponding dates for median emergence (50%) in the three seasons were 16 November 2010, 1 November 2011, and 30 October 2012, which differed from the observed median dates by no more than four days (Table 2). Differences between predicted and observed dates for 5% and 95% were similarly small (less than 3 days) (Table 3).

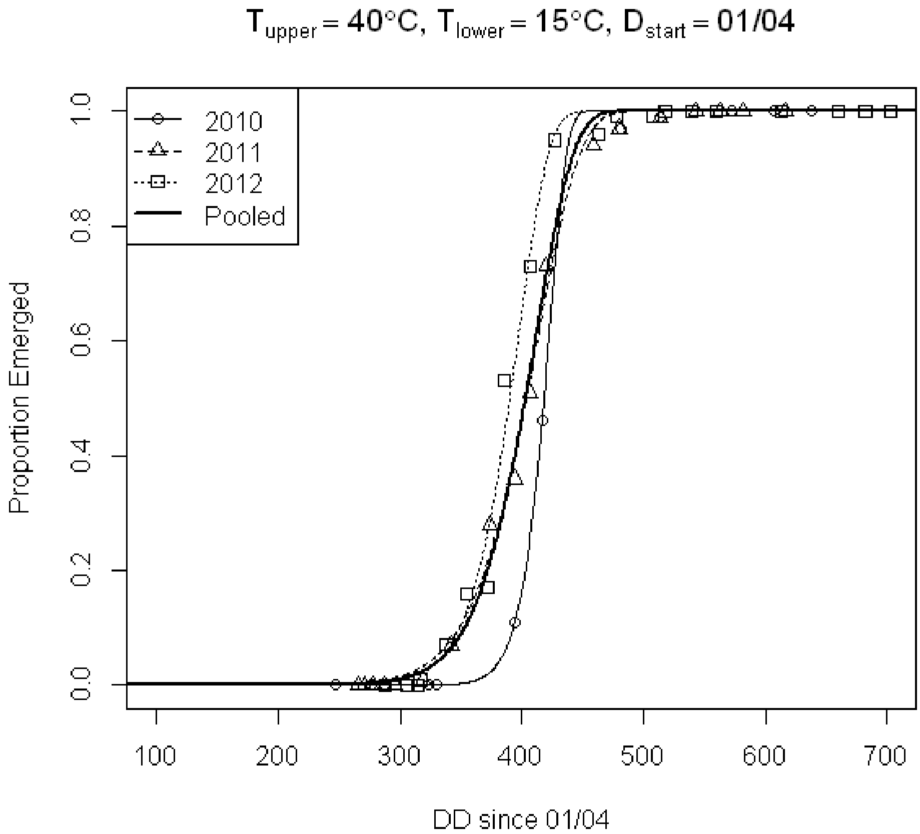

Figure 7. Cumulative emergence of CGW adults in block-1 during 2010-2012 as a function of DD calculated based on values of T_{upper}, T_{lower}, and D_{start} that gave the minimal sum of squares of differences between predicted and observed median emergence dates over the three seasons (SS_{median}).

The required DD of 336, 403, and 447 can be used to predict dates for 5, 50, 95% emergence of CGW adults in future years. First, DD needs to be accumulated daily from 1 April of the target year using local temperature data and with the lower threshold temperature set at 15°C and the upper threshold temperature at 40°C. From 1 April to the day before the prediction is to be made, observed local temperature data can be used. For days when temperature data has not yet been recorded, long-term average daily temperature data can be used. The predicted date will be the date when the accumulated DD first reaches or exceeds the required DD. Predicted dates for the emergence of parasitic wasps can be obtained by adding 17-21 days to the predicted dates for CGW adult emergence.

Table 3. Observed and predicted dates* of 5, 50, and 95% emergence in block-1 during 2010-2012.

	5% (336 DD)		50% (403 DD)		95% (447 DD)	
Year	Observed	Predicted	Observed	Predicted	Observed	Predicted
2010	8 Nov	6 Nov	20 Nov	16 Nov	26 Nov	23 Nov
2011	20 Oct	20 Oct	1 Nov	1 Nov	9 Nov	7 Nov
2012	15 Oct	16 Oct	26 Oct	30 Oct	2 Nov	5 Nov

* Predicted dates were estimated by the dates when the required DD were reached in the respective years. Required DDs were estimated from the fitted Weibull function for the pooled emergence data over the three seasons. DD accumulation started at 1 April in the respective years with the lower threshold temperature set at 15 °C and the upper threshold temperature set at 40 °C.

Discussion

Noble (1936) provided the first and only detailed description of the biology of CGW, including adult emergence. In this study, we investigated the statistical properties of the adult emergence process in order to develop a guide on its timing in the future in the Sunraysia region.

Adult emergence was monitored for three years during 2010-2012 in citrus orchards in the Coomealla Irrigation District in the Sunraysia. Three types of sticky traps were used for the monitoring: cup traps, flat yellow sticky traps, and rolled yellow sticky traps. All three trap types yielded sufficient data for analyses of CGW adult emergence patterns. However, the rolled yellow sticky traps were significantly less efficient than the other two trap types and hence were only used in the first year.

CGW adult emergence in the Coomealla Irrigation District started between 1st October (2012) and 3rd November (2010). The median emergence date varied from 23rd October in 2012 to 23rd November in 2010. Most wasps (95%) had emerged by 13th November (2012) and 28th November (2010). Within the same year, there were some differences in emergence timing between different trap types and monitoring sites, although the extent of the differences were not as great as those between years. In general, the cup traps recorded earlier emergence than the flat yellow sticky traps, and the maximum difference was five days. This was probably due to differences in trap design. The cup traps were tubular in structure and enclosed the CGW galls from all sides except one, and are ideally suitable for catching wasps soon after their emergence. By contrast, the flat yellow sticky traps were open and the wasps they caught were those already in the air and of mixed ages.

Variations of emergence timing between different monitoring sites were investigated in 2012. The lemon block (block-2) recorded the earliest emergence, followed by the 'Navel' orange block (block-1) and the 'Valencia' orange block (block-3), with a largest between-site difference of seven days in median emergence date as measured by both the cup traps and the flat yellow sticky traps. Due to the unreplicated nature of the data, it is not known whether the site differences were due to the different citrus varieties, the existence of separate CGW populations in the three monitoring sites, or variations in site microclimate.

Based on the timing of 5 to 95% emergence, most wasps (90%) emerged within a period of 11-28 days, with an average of 19 days. In 11 of 12 datasets, the first half of the emergence phase (5-50%) was longer than the second half of the emergence phase (50-95%), suggesting an asymmetrical distribution of daily emergence rate.

Emergence of the wasps signals the end of their development inside the galls and the duration of the development is influenced by temperature. In this study, we used degree-days to predict the timing of CGW adult emergence. Development of such a prediction tool requires prior knowledge of the lower and upper development threshold temperatures, the starting date of development, and the required number of degree-days since the starting date for the development. The starting date can be set as the date when eggs were laid. However, this is inconvenient to use as egg-laying occurs on different dates in different years, as seen from the adult emergence dates. For this reason, we decided to use the first of a month after the egg-laying has finished. In Chapter-2 we show that CGW egg-laying finished by late December at the study sites in all years, so 1st January would be a good candidate. However, other months may also be suitable. Noble (1936) dissected galls in late winter and early spring and noticed larvae at similar developmental stage despite the egg-laying dates being several months apart, suggesting that later starting dates may be more suitable. To find the best starting date, we tested all months from January to September, the latter being the month before wasp emergence was observed at the study sites. Neither the lower nor the upper development threshold temperatures are known for CGW. We tested all temperature from 0 °C to 15 °C as the lower threshold temperature and 35 °C and 40 °C as the upper threshold temperature.

Best predictions of median emergence timing at the study sites during 2010-2012 was achieved by setting the starting date at 1st April, the lower threshold temperature at 15 °C, and the upper threshold temperature at either 35 °C or 40 °C. With these degree-day parameter values, the required number of degree-days for 5, 50, and 95% emergence was 336, 403, and 447 DD, respectively. The maximum difference between predicted and observed median dates for 2010-2012 was only four days. Independent determinations of the upper and lower temperature thresholds are needed before our emergence model can be further refined. However, in the interim, we can use 1st April as the starting date and 15 °C as the lower threshold temperature to achieve sufficiently accurate estimates of peak emergence to facilitate CGW control operations. To predict timing of CGW adult emergence in future years, we can use a combination of observed and historical local temperature to calculate the daily degree-days since 1st April and then accumulate the daily degree days until the required amount is reached, for example 403 DD for the median emergence date. As a quick guide, median emergence can be predicted from the date when wasp emergence is first observed. Data from this study suggests an average lag time of around three weeks.

With the median emergence date known, the date for peak abundance of the wasps in the orchard can be predicted by adding another time lag to the median emergence date. The length of the time lag depends on wasp longevity. In Chapter-2 we show that the average longevity of adult wasps is approximately 8.5 days at 19 °C. This temperature is close to the average daily temperature in November when most wasp emergence at the study sites occurs. At this temperature the time lag was 5-6 days or about one week.

In addition to CGW wasps, two of its known parasitic wasps, *Megastigmus brevivalvus* and *M.trisulcus*, were also recovered in our traps. After CGW was detected in Sunraysia, several releases of the two parasitic wasps were made around the study sites (Cannard 2007). The recovery confirms the establishment of the parasitic wasps, although the populations are not yet at the level reported in Queensland (Smith *et al.* 1997). This study shows that emergence of the parasitic wasps occurred 2-3 weeks after that of their unparasitised hosts. When the emergence of the parasitic wasps was still in its early phase (5%), CGW emergence had already passed its peak (50%). Releases of the parasitic wasps are usually made by bringing galls from regions where they are well established and distributing them at identified release sites. The time lag provides a window of opportunity to allow most CGW to emerge from the galls before distributing the galls to the release sites, thus minimising the unwanted side-effect of introducing additional CGW wasps to the release area.

References

Cannard M. 2007. Native parasitic wasps released to control citrus gall wasp. Citrep 51, 14-15.

Flett H. 2011. Establishment of Citrus Gall Wasp Parasites in the Murray Valley Region. Final Report of Project CT08000, Horticulture Australia Ltd.

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

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

Papacek DF & Smith D. 1989. Insectidal control of citrus gall wasp in Queensland. General and Applied Entomology 21, 2-4.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Roltsch WJ, Zalom FG, Strawn AJ, Strand JF & Pitcairn MJ. 1999. Evaluation of several degree-day estimation methods in California climates. International Journal of Biometeorology 42, 169-176.

Smith D, Beattie GAC & Broadley R. 1997. Citrus pests and their natural enemies. DPI Queensland, Brisbane.

Weibull W. 1961. Fatigue Testing and Analysis of Results. Pergamon Press.

Chapter-2 Reproduction and Egg Development

Abstract The sex ratio of adult wasps varied during the emergence process, from male biased in the early phase to female biased thereafter. The overall M/F ratio was about 0.7. Longevity was similar between the two sexes, varying from 3.1 to 11.2 days, depending on temperature. Provision of water or honey did not significantly lengthen longevity. CGW females were able to lay eggs immediately after emergence and peak oviposition occurred in newly hatched females except when temperature was low (below 15°C). Both the length of the oviposition period and peak oviposition age decreased with temperature. At the time when female wasps were active during 2010-2012 at the study sites, approximately three quarters of all CGW eggs would have been laid by females within three days of their emergence. Median egg development period varied from 11 days at 29°C to 25 days at 13°C. According to the linear relationship between egg development rate and temperature, the median egg development period at the study sites during 2010-2012 was estimated at 16.8 days. Dissections of field collected shoots showed that 50% of eggs had hatched by 4-13 December and 95% by 24-26 December over the three years. There appears to be considerable variations in egg development rates among local CGW populations in different citrus blocks.

Introduction

The biology of citrus gall wasp (CGW), *Bruchophagus fellis* Girault (Hymenoptera: Eurytomidae), was first described in detail by Noble (1936). Smith *et al*. (1997) provided an updated summary of CGW biology in their book on the IPM of citrus pests in Australia. Both descriptions were based on studies of CGW populations in humid, subtropical regions of northern New South Wales (NSW) or Queensland. In this chapter, we report for the first time, adult sex ratio, longevity, and oviposition and egg duration of CGW populations in the semi-arid Sunraysia region in southwest NSW, where CGW has recently established (Cannard 2007). Combined with the knowledge of the timing of adult emergence reported in Chapter-1, this biological data will help us predict the timing of CGW egg hatching in the field. The latter information is needed to time sprays of methidathion, currently the only registered insecticide for CGW control, against newly hatched larvae. Methidathion is a foliar insecticide but it also has some trans-laminar activity and is able to penetrate young plant tissue. Absorption of the insecticide by the shoots is greatly reduced after the bark has hardened (Papacek & Smith 1989), so methidathion application for larval control should be timed soon after egg hatching.

Materials and Methods

<u>Adult source</u>

CGW adults were sourced annually during 2010-2012 from galls collected from an abandoned block of lemon trees in the Coomealla Irrigation District in Sunraysia. The galls were collected during August-September when CGW were mature larvae or pupae. The exercised gall-bearing shoots were placed in an insulated cooler with ice packs and taken to the laboratory within 24 hours. In the laboratory, the gall-bearing shoots were sealed at both ends with wax and then placed in a shaded area in a 25°C controlled temperature room until adult emergence.

Sex ratio

Sex ratio was estimated from wasps that emerged from galls collected in 2011. Live CGW wasps were sexed as they emerged from the galls by the relative size of the abdomen to the thorax. In male CGW wasps, the abdomen is considerably smaller than the thorax, whereas in the females the abdomen is of similar size to the thorax (Noble 1936). Dead wasps from longevity/oviposition experiments were sexed under a stereo microscope by examining them for the presence/absence of an ovipositor.

Adult longevity

Adult longevity was studied in 2010 and 2011. In 2010, newly emerged wasps were placed in glass petri dishes (7 cm diameter) in groups of 20 (10 females and 10 males) in a 25°C constant temperature room. Light period was fixed at 14-hour light and 10-hour darkness. Humidity was not controlled. The wasps were fed with either water, 10% honey solution, or were unfed. Water and honey were provided in cotton balls soaked with the respective solution. Five replicates were implemented for each food source. The wasps were checked daily until all had died. Two duplicate experiments were conducted.

In 2011, newly emerged wasps were placed in groups of 10 (5 F: 5 M) in 70-mL clear plastic containers (43 mm diam. x 55 mm) covered with a piece of mesh fabric. Water was suppled to the wasps through a cotton wick inserted though a hole cut at the base of the container. The other end of the wick was dipped inside a water reservoir in another container of the same size below the wasp container above. The experiment was conducted at 5 constant temperatures: 13.3, 19.0, 24.1, 27.0, and 29.0°C in refrigerated incubators, with 10 replicates for each treatment. Light period was fixed at 14-hour light and 10-hour darkness. Humidity was not controlled. The wasps were checked daily until all had died.

Oviposition

Experiments were conducted in 2011 to determine the effect of the age of female wasps on the number of eggs laid. Newly emerged (less than 24-hour old) CGW adults were transferred daily to separate 1.9-L clear plastic containers (12 x 8 x 19 cm). For water supply, a small hole was cut at the base of each container, through which a dental wick was inserted with one end dipping in a water source and another inside the container (Fig. 1). Separate containers were used to keep wasps of different ages as the source of test wasps, and each container was provisioned with 10% honey in a cotton ball. Two testing containers and one source container were kept at the same temperature in the same incubator. Each of the testing containers was provided with three current year lemon shoots of around 15-cm in length. To reduce water loss, the exposed ends of the shoots were sealed with wax. Initially, 40 female wasps and 20 male wasps less than 24-hour old were introduced to each testing container. After each 24-h of exposure, shoots from the testing containers were taken out and new shoots were provided. The numbers of dead male and female wasps in each container were counted. The removed shoots were individually labelled with wasp age (days after emergence), date of shoot removal, temperature, and number of surviving male and female wasps. Dead wasps from each testing container were replaced with live ones of the same age and sex from the source container. The experiments were conducted at three constant temperatures: 13.3, 19, and 24.1°C, in refrigerated incubators. Light period was fixed at 14-h light and 10-h darkness. Humidity was not controlled. A minimum of two experiments were done for each adult age and temperature.

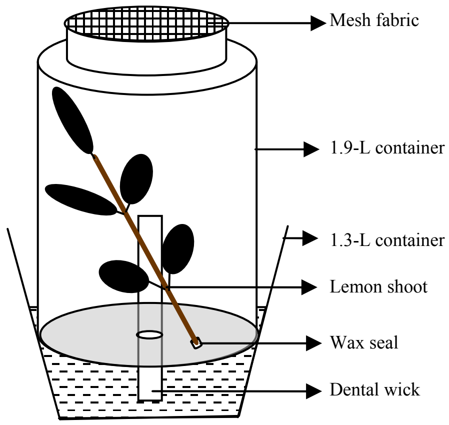

Figure 1. Test arena for oviposition experiments.

Shoots exposed to the wasps were dissected and examined under a stereo microscope at 18-25x magnification to count the number of eggs in each shoot. CGW eggs were recognised by their oval shape and long 'tails' (Noble 1936).

<u>Egg duration in the laboratory</u>
Current-season lemon shoots were placed in 1.9-L clear plastic containers (120 x 80 x 190 mm) containing over 100 CGW adult wasps. After 24-h exposure, the shoots were taken out and the bark of the shoots was carefully peeled off to reveal the eggs. Eggs were then individually transferred to dots of fluffy fabric (7-mm diameter) cut out from an Artwrap™ ribbon roll in groups of 10-20 with a pair of fine-tipped tweezers under a stereomicroscope at 18-25x magnification. The egg-loaded fabric dots were individually placed in the centre of a moistened filter paper in a glass Petri dish (30 x 10 mm). The dishes were covered with lids, put in opaque cardboard boxes, and placed in an incubator at a set temperature. Development status of the eggs was checked daily under a stereomicroscope at 18-25x magnification. To minimise disruption of egg development, checking started from the 10th day after the eggs were laid. Both Noble (1936) and our own previous observations had shown that CGW egg development required more than 10 days. Egg development was studied under five constant temperatures: 13.3, 19, 21, 24.1, and 29°C under red light.

<u>Egg hatching in the field</u>
To estimate the date of CGW egg hatching in the field, current-season lemon shoots of about 15-cm length were collected twice weekly each year from 2010 to 2012 from CGW infested citrus orchards in the Coomealla Irrigation District in the Sunraysia in southwest NSW. In 2010 and 2011, shoots were collected from the same block of lemon trees where adult wasps were sourced for laboratory investigations of CGW biology. In 2012, shoots were collected from this lemon block, as well as from two other citrus blocks in the region. One was a block of 'Autumn Gold' navel orange trees within 1 km of the lemon block and the other a block of 'Valencia' orange trees about 2 km away. Depending on year, shoot collection started in early to mid November when few eggs had hatched, and ended in late December to late January

when most eggs had hatched. The numbers of shoots collected per collection date were 20-70 in 2010, 20 in 2011, and 10 in 2012. Collected shoots were immediately placed in automotive radiator coolant containing ethylene glycol (65 g/L) to stop egg development. In the laboratory, the 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.

Data analysis

Comparison of longevity between male and female CGW adults was made with ANOVA for the completely randomized design. The relationship between temperature and longevity was described by the inverse of the Logan type III rate model (Herrera *et al.* 2005) by treating mortality as the completion of the adult stage. The relationship between temperature and egg development rate (inverse of egg development period) was described by the Logan type III rate model and the linear rate model, the latter providing direct estimates of development threshold temperature and the amount of heat units (degree-days) required for completion of development. The cumulative proportions of eggs laid by age in the laboratory and the cumulative proportions of eggs hatched by date in field collected lemon shoots were fitted to the Weibull distribution function (Weibull 1961). All analyses were done in R (R Development Core Team 2012).

Results

Sex ratio

Males were generally more abundant than females in the first six days since the start of wasp emergence from the galls (Fig. 2). From then until the end of emergence, more females emerged than males. The male to female ratio dropped below 1/3 toward the final stage of emergence. Overall, close to twice as many females than males emerged during the entire emergence period (M/F ratio = 0.57). A total of 4109 dead wasps from experiments of adult longevity and oviposition were sexed and the male to female ratio was about 0.70.

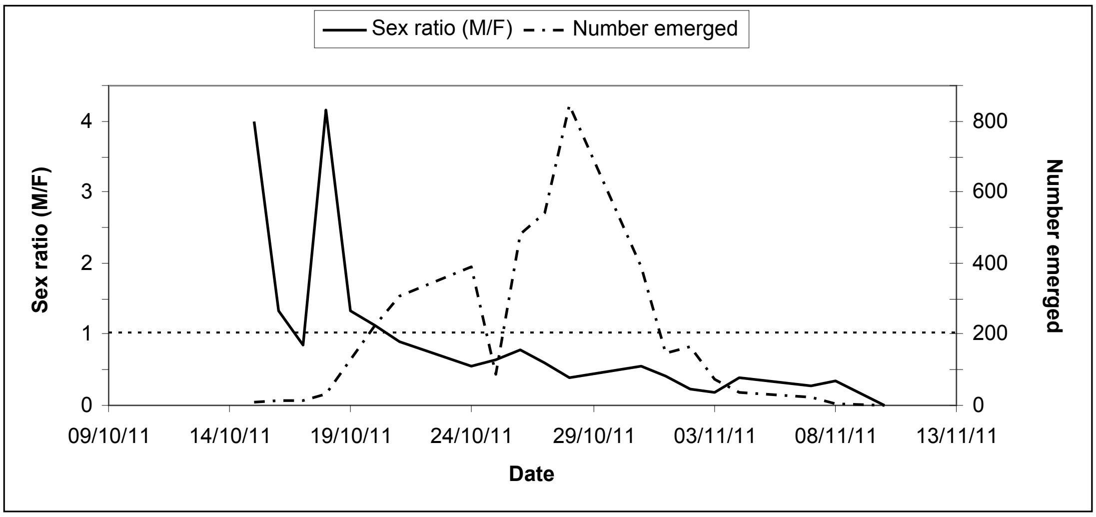

Figure 2. Sex ratio (M/F) and number of wasps emerged by date from CGW galls collected from a block of lemon trees in the Coomealla Irrigation District in the Sunraysia in 2011. Dotted line shows a sex ratio of 1:1.

<u>Adult longevity</u>

In 2010, the average median longevity (mean ± SE) of adult wasps fed with water, 10% honey, and nothing (control) was 6.2 ± 0.4, 5.5 ± 0.5, and 4.7 ± 0.2 days, respectively. There were no significant differences between the three treatments (*F = 3.39; DF = 2, 12; P = 0.0682*). In 2011, the average median longevity of adult wasps fed with water, 10% honey, and nothing (control) was 5.1 ± 0.1, 6.0 ± 0.3, and 5.6 ± 0.2 days, respectively. Again, there were no significant differences between the three treatments (*F = 3.59; DF = 2, 12; P = 0.0600*).

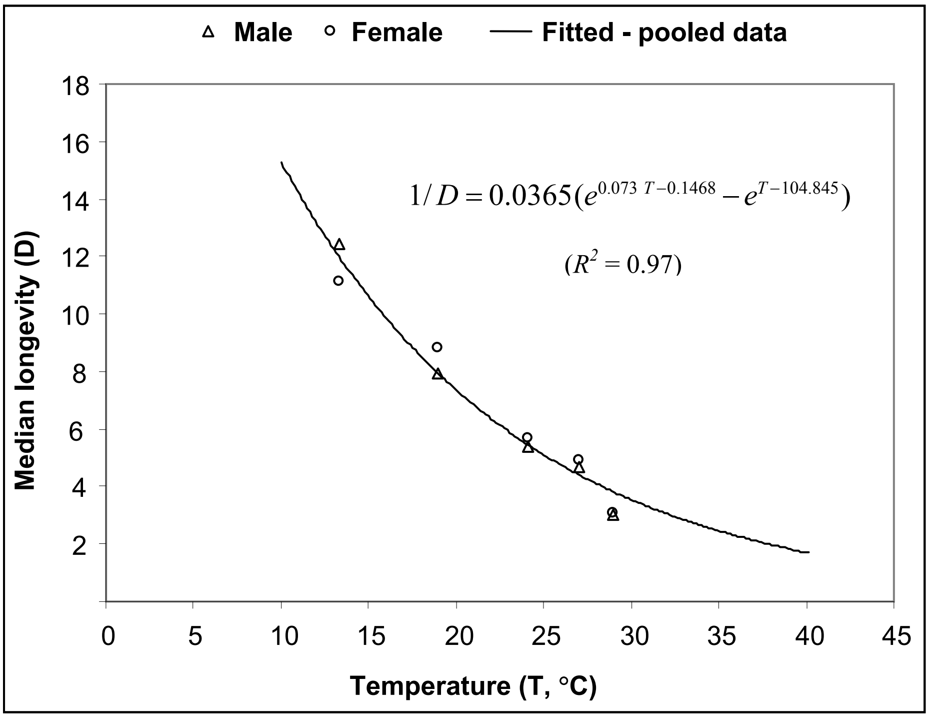

Figure 3. Median longevity of CGW adult wasps under different constant temperatures. Solid line shows nonlinear fitting by the inverse of Logan type III rate model.

The 2011 experiment investigated CGW longevity under different constant temperatures. Median longevity of the adults varied from 3.0 days at 29°C to 12.4 days at 13.3°C in males, and from 3.1 days at 29°C to 11.2 days at 13.3°C in females (Fig. 3). The longevity was almost identical between the sexes. The observed relationship between median longevity and temperature from the pooled data of males and females was well described by the inverse of the Logan Type III rate model (R^2 =

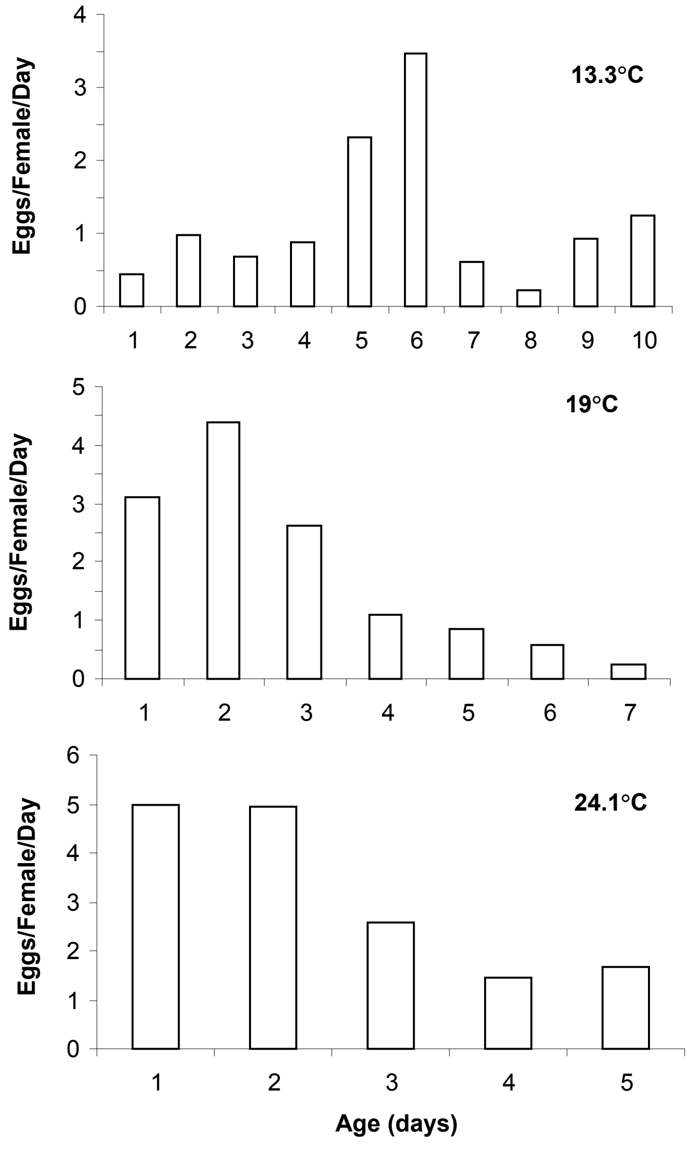

Figure 4. Oviposition rates of CGW females of different ages under constant temperatures.

0.90) (Fig. 3).

<u>Oviposition</u>
Egg-laying was observed by females of all ages from 1 to 10-day old at 13.3°C, from 1 to 7-day old at 19°C, and from 1 to 5-day old at 24.1°C. The peak egg-laying age was 6 days post emergence at 13.3°C and 1-2 days post emergence at 19 and 24.1°C (Fig. 4). The cumulative proportions of eggs laid by age were well described by the

Weibull distribution function (R^2 > 0.97) (Fig. 5). According to the fitted distributions, 50% of all eggs were laid by females within 6 days of their emergence at 13.3°C, within 3 days of their emergence at 19°C, and within 2 days of their emergence at 24.1°C. By the age of 14, 8, and 5 days post emergence, the females had

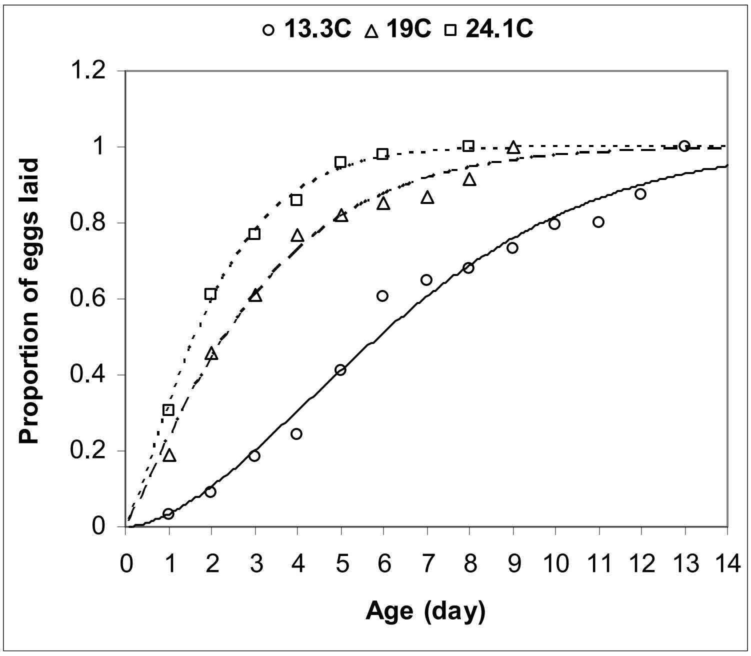

Figure 5. Cumulative proportions of eggs laid by age at three constant temperatures. Lines were drawn from fitted values of the Weibull distribution function

laid most their eggs (95%) at 13.3, 19, and 24.1°C, respectively.

<u>Egg development in the laboratory</u>
Median egg duration increased with decreasing temperature, from 11 days at 29°C to 25 days at 13.3°C (Fig. 6). The relationship between development rate and temperature was well fitted by both the linear rate model (R^2 = 0.87) and the nonlinear Logan type III rate model (R^2 = 0.91) (Fig. 6).

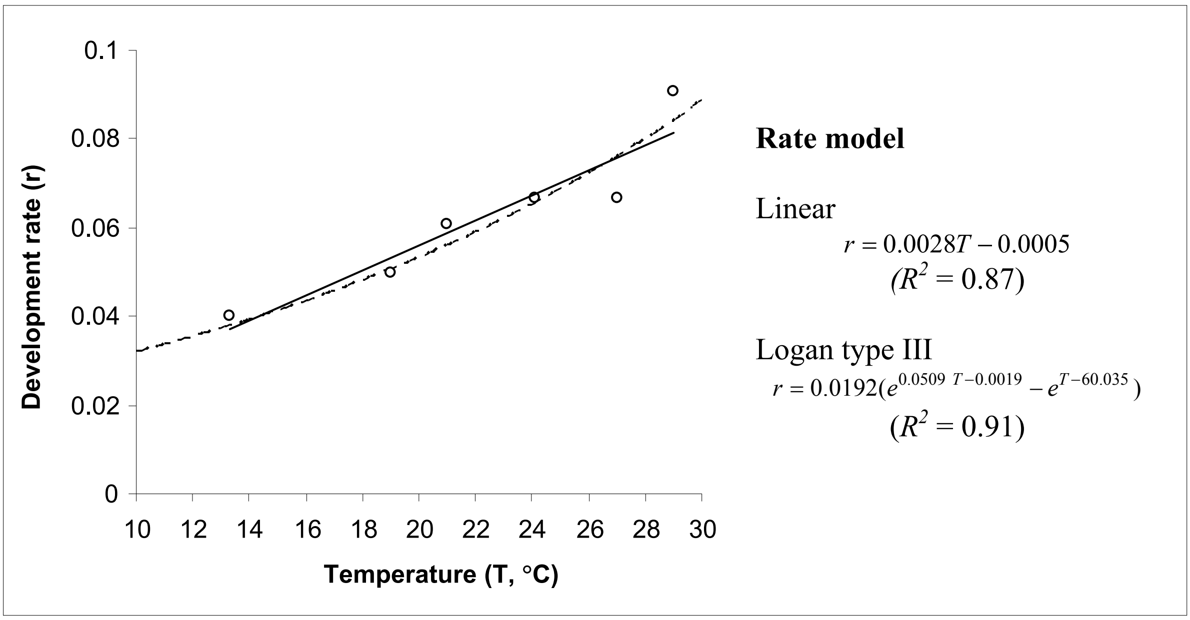

Figure 6. The relationship between development rate and temperature in CGW egg development. Solid line shows linear fitting of the data and dashed line nonlinear fitting using the Logan type III rate model.

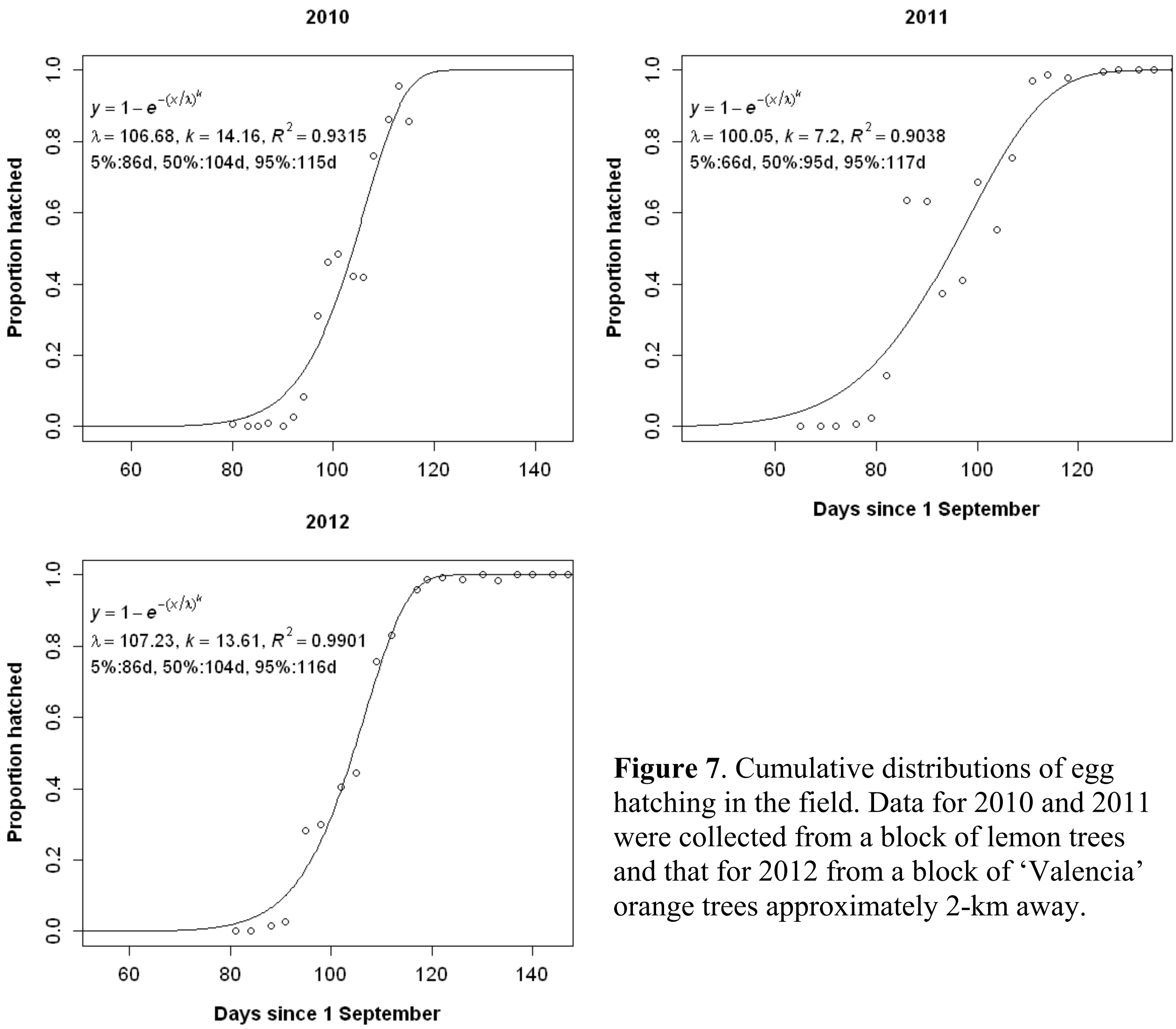

Figure 7. Cumulative distributions of egg hatching in the field. Data for 2010 and 2011 were collected from a block of lemon trees and that for 2012 from a block of 'Valencia' orange trees approximately 2-km away.

Egg hatching in the field

The proportion of hatched eggs in field-collected shoots ranged from less than 1% to about 85% in 2010, and from 0% to 100% in the latter two years. Cumulative distributions of hatched eggs during the sampling periods in all three years were well fitted by the Weibull distribution function ($R^2 > 0.90$). In comparison, a noticeably better fit was obtained from data in 2010 and 2012 than from data in 2011, as seen from the levels of scattering of observed proportions of hatched eggs around the fitted lines (Fig. 7). In general, better fitting was obtained during the middle and the late phases of egg hatching. According to the fitted distributions, timing of median hatching (50%) occurred at 95-104 days since 1 September (4th - 13th December). Timing for 95% egg hatching was almost identical in all three years at 115-117 days since 1 September (24th -26th December).

Discussion

In this chapter, we reported our investigations of CGW adult sex ratio, longevity, oviposition, and egg development based on laboratory and field data. Knowledge of these biological attributes is needed in the prediction of timing of CGW egg hatching in the field, which, in turn, is needed to time control actions against the vulnerable young CGW larvae.

The sex ratio of adult wasps varied during the emergence process, from male biased in the early phase to female biased thereafter. The overall M/F ratio was about 0.7, slightly higher than that reported by Noble (1936) (0.51-0.59). The differences may have been due to random variations in CGW populations.

Longevity was similar between the two sexes, agreeing with the findings of Noble (1936). Median longevity decreased from 3.1 days at 29°C to 11.2 days at 13.3°C. Noble (1936) studied CGW adult longevity under three variable temperature ranges (10-13°C, 17.7-22.8°C, and 16-18°C) and two constant temperatures (20°C and 25°C). His data showed a median longevity range of 5-17 days. His results are not directly comparable to ours due to the different temperatures used. Two of his temperatures were close to ours, e.g. 20°C and 25°C versus 19°C and 24.1°C in our study. His median longevity for the two temperatures differed from ours for the corresponding temperatures by less than 3 days. In addition to temperature effect, we also investigated the effect of food provision on CGW adult longevity. Our results show that the provision of water or honey does not lengthen longevity, suggesting that CGW adults are endowed with sufficient energy reserves at emergence for a maximum life span, and do not need to seek out additional food for successful reproduction.

As observed by Noble (1936), CGW females were able to lay eggs immediately after emergence and, except at 13.3°C, peak oviposition occurred in newly hatched females within 48 hours of emergence. Both the length of the oviposition period and peak oviposition age decreased with increasing temperature. At 19°C and 24.1°C, over 73% of all eggs were laid within the first three days of female emergence. Daily average temperatures around the time when female wasps were active during 2010-2012 in the Coomealla Irrigation District were 19.8-21.4°C, within the bounds of 19-24.1°C. Thus it is reasonable to expect that three quarters of all CGW eggs are laid by females within three days of their emergence in the field. Noble (1936) did not study temperature effects on oviposition.

CGW eggs are laid inside the shoots and it is difficult to know exactly when individual eggs have hatched. There are two solutions to this problem. One is to transfer the eggs to an artificial platform so that their development can be directly observed. The other is to regularly dissect shoots containing eggs of known deposition dates and determine the proportions of eggs hatched on different dates. The first approach was used in this study. The results showed a median egg development period of 11-25 days over the temperature range of 13.3-29°C. In Chapter-1 we show that median adult emergence in the study area during 2010-2012 occurred during 26 October - 20 November. The average daily temperature at the study area in 20 days following the median emergence dates over the three years was 21.6°C. Assuming this temperature and the linear rate-temperature parameters, the median egg development period in the field was estimated at 16.8 days, which is within the range estimated from the data of Noble (1936) (15.9-18.2 days) who studied CGW egg development from field collected shoots from the NSW central coast using the second approach. This is encouraging, considering the temperature difference and the large variations of egg development between individuals noted by Noble (1936).

CGW egg development in the field was estimated by dissections of lemon shoots from the same orchard where CGW adults used in this study were sourced, but where the oviposition dates were unknown. The results showed a median egg-hatching date range of 4-13 December and a 95% egg-hatching date range of 24-26 December during 2010-2012. In two of the three years, the median egg-hatching dates occurred over 20 days later than that predicted by simply adding the expected median egg period around the time when egg development was in progress (16.8 days) and a 2-day delay between wasp emergence and egg-laying to the observed median adult emergence dates in a nearby orchard in the respective years (1 November 2011 and 26 October 2012) (Chapter-1). Several factors may have contributed to the anomaly. First, data used for estimating adult emergence and egg hatching dates were collected from different citrus blocks. CGW populations in different locations may not have been entirely synchronised in development. Second, temperature may not have been the only factor affecting CGW egg development. Noble (1936) noted that eggs deposited on the same dates hatched out on quite different dates under the same temperature regime, suggesting genetic variations or the involvement of host-tree related factors in egg development.

References

Cannard M. 2007. Native parasitic wasps released to control citrus gall wasp. Citrep 51, 14-15.

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

Papacek DF & Smith D. 1989. Insectidal control of citrus gall wasp in Queensland. General and Applied Entomology 21, 2-4.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Smith D, Beattie GAC & Broadley R. 1997. Citrus pests and their natural enemies. DPI Queensland, Brisbane.

Weibull W. 1961. Fatigue Testing and Analysis of Results. Pergamon Press.

Herrera AM, Dahlsten DD, Tomic-Carruthers N & Carruthers RI. 2005. Estimating temperature-dependent development rates of *Diorhabda elonga* (Coleoptera: Chrysomelidae), a biological control agent of Saltcedar (*Tamarix* spp.). *Environmental Entomology* 34(4), 775-784.

Chapter-3 Chemical Control

Abstract Three field trials were conducted in CGW-infested citrus orchards in the Coomealla Irrigation District in far southwest NSW to evaluate the potential of BioPest®, Confidor® Guard, and Movento® for CGW control. BioPest® is a promising alternative to the currently registered Supracide®. Applied at 0.5% in three 1-2 weekly sprays, BioPest® reduced subsequent gall formation by over 50%. Reducing the rate to 0.25% was not an effective option. For maximum effect, the oil should be applied when adult wasps are most abundant. There may be scope to reduce the number of sprays to one if it is timed correctly, however, no convincing results were obtained regarding the relative efficacy of different spray frequencies in this study due to low control populations. Confidor® Guard is also effective against CGW. A single application of the chemical to the soil from late October to mid November achieved similar control of CGW as three sprays of BioPest®. The exceptionally long residual period and potential negative impact of the chemical on beneficial organisms in citrus need to be considered before it is registered. Confidor® Guard targets the larvae and is best applied shortly before (allowing time for absorption by the trees) or soon after most eggs have hatched. The efficacy of Movento® was not confirmed in this study, however, its dual-pathway trans-laminar properties and relative short residual may justify its further evaluation.

Introduction

In Queensland and northern NSW citrus gall wasp (CGW) populations are normally kept below damaging levels by natural enemies, particularly two parasitic wasp species, *Megastigmus brevivalvus* and *M. trisulcus* (Hymenoptera: Megastigminae) (Papacek & Smith 1989; Smith *et al*. 1997). In the Sunraysia region, however, the parasitic wasps are at their early establishment stage and their numbers are not high enough to effectively control CGW (Cannard 2007), leaving chemical control as the only available control option. Currently only methidathion (e.g. Supracide®) is registered. Its use is constrained by its high mammalian toxicity and broad-spectrum activity against a wide range of invertebrates including the natural enemies of citrus pests. Additionally, it does not always provide satisfactory control of CGW (Richard Bertalli, personal communication, 5 September 2010)

A scoping study identified Confidor® Guard (350 g/L imidacloprid, suspension concentrate) and Movento® (240 g/L spirotetramat, suspension concentrate) as potential alternatives to methidathion (Steven Falivene, personal communication, 21 October 2008). Both are systemic insecticides (Elbert *et al*. 2008; Vermeer & Baur 2008), targeting primarily sap-sucking insects such as aphids, whiteflies, leafhoppers, and thrips. CGW is not a sap-sucking insect but its larvae feed inside the plant tissue and it would be expected to be also affected by the chemicals due to their systemic activity. Petroleum spray oil (PSO) is registered for use in citrus in Australia against scale insects. PSO controls pests either by suffocation, or by altering their behaviour by reducing feeding and egg-laying (Beattie 2005). Considering its potential to deter oviposition by CGW adults, PSO was recommended for testing against CGW (Creek & Hardy 2009).

This chapter reports the results of our efficacy trials of potential chemicals for CGW control in the Sunraysia region.

Materials and Methods

Three annual trials were conducted in citrus orchards in the Coomealla Irrigation District in far southwest NSW during 2010-2012.

<u>2010 Trial</u>

The trial was conducted in a 1.41 ha block of 'Autumn Gold' navel orange trees (root stock: 'Citrange') on a farm in the Coomealla Irrigation District in far southwest NSW. The trees were 16 years old, 2.5 m tall, spaced at 3 m within rows and 6 m between rows. The trial was designed as randomised complete blocks, with five replicates and each block occupying a separate row. A plot was two consecutive trees within the same row. Neighbouring blocks were separated by a buffer row and neighbouring plots within the same block by two trees. Three unregistered chemicals, BioPest® (815 g/L paraffinic oil), Confidor® Guard, and Movento® were tested along with the registered Supracide® and an untreated control. BioPest® was applied to the foliage at 0.5% with 4 L of water/tree on 25 October, 9 November, and 19 November 2010. Confidor® Guard was applied as a soil drench at 9 mL/tree with 1 L of water/tree along the drip lines on 25 October 2010. Movento® was applied to the foliage at 40 mL/100 L with 4 L of water/tree on 9 November and 9 December 2010. Supracide® was applied to the foliage at 125 mL/100 L with 4 L of water/tree on 9 December 2010. For all foliar sprays, Hasten® was added at 50 mL/100 L as the adjuvant.

On 25-26 October 2010, before any treatments were applied, 20 current-year shoots were randomly selected from each tree in each plot and tagged with numbered plastic tags. Half of the tagged shoots were measured for length and diameter. On 4-5 May 2011, all tagged shoots were cut from their bases and taken to the laboratory. Galls on the tagged shoots were counted and individually measured for diameter and length. Finally, galls from the same plot were put together and the total gall weight measured.

<u>2011 Trial</u>

The trial was conducted in a 1.35-ha block of 'Autumn Gold' navel orange trees (root stock: 'Citrange') on a farm in the Coomealla Irrigation District in far southwest NSW, which was adjacent to the farm used in the 2010-2011 trial. The trees were 8 years old, 2.5 m tall, and spaced at 3 m within rows and 6 m between rows. The trial was designed as randomised complete blocks. A block consisted of a row of single-tree plots separated by two trees in the same row. Six blocks were placed in two rows of citrus trees with an in-row buffer of two trees and a buffer row between the two treatment rows.

Five treatments were tested in this trial: BioPest® foliar spray at 0.25 and 0.5%, Confidor® Guard, Movento®, and a water-only control. Biopest® was applied to the foliage with 4 L of water/tree on 21 October, 31 October, and 10 November 2011. Confidor® Guard was applied once as a soil drench at 9 mL/tree with 1 L of water/tree along the drip lines on 21 November 2011. Movento® was applied to the foliage at 40 mL/100 L with 4 L of water/tree on 17 November and 8 December 2011. All foliar sprays used Hasten® as the adjuvant at 50 mL/100 L.

Two sets of efficacy data were collected, one from tagged shoots and the other from frame sampling. On 10-11 October 2011, before treatments were applied, 40 randomly chosen current-year shoots were tagged on each tree and their lengths measured. On 7-8 May 2012, all tagged shoots were cut from their bases and taken to the laboratory. Galls on the tagged shoots were counted and individually measured for diameter and length. Galls from the same plot were put together and the total gall weight measured. With frame sampling, a 50 x 50 x 50 cm frame was placed into a corner of the lower canopy of each tree, with the corner position rotating clockwise at 90° intervals from tree to tree. All galls within the frame were removed and taken back to the laboratory for measurement.

After the first Movento® application, three random samples of 1 kg of current-year shoots and foliage each were collected from Confidor®, Movento® and control plots at approximately 4 week intervals to assess chemical residue levels. The samples were placed in plastic zip bags and stored in a freezer before being sent to Bayer Crop Science for analysis.

<u>2012 Trial</u>

This trial investigated the effects of different timings and frequencies of BioPest® sprays on CGW infestation. It was conducted in the same block as the 2010 trial. BioPest® was applied at 0.5% to the foliage at eight timing / frequency combinations: (1) three sprays applied on 23rd October, 2nd November, and 12th November 2012, (2) two sprays applied on 23rd October and 2nd November 2012, (3) two sprays applied on 23rd October and 12th November 2012, (4) two sprays applied on 2nd and 12th November 2012, (5) one spray applied on 23rd October 2012, (6) one spray applied on 2nd November 2012, (7) one spray applied on 12th November 2012, and (8) an unsprayed control. Timings for the three sprays were set to approximately 3, 4, and 5 weeks after CGW emergence was first observed at the trial site with sticky traps. The trial was designed as complete randomised blocks of eight plots each in a single row of trees. Each plot consisted of two consecutive trees in the same row. A 2 tree buffer was placed between adjacent plots in the same row and a 1 row buffer was placed between neighbouring blocks. It was conducted in the same block as the 2010 trial.

Two sets of efficacy data were collected, one from tagged shoots and the other from frame sampling. On 9 October 2012, before any treatments were applied, 20 randomly chosen current-year shoots were tagged on each tree in each plot and their lengths measured. On 3 June 2013, all tagged shoots were cut from their bases and taken to the laboratory. Galls on the tagged shoots were counted and individually measured for diameter and length. Galls from the same plot were put together and the total gall weight measured. Frame sampling data was conducted on 4th June 2013. A 50 x 50 x 50 cm frame was randomly placed into the lower canopy of each tree in each plot. All galls within the frame were removed and taken back to the laboratory for measurement.

<u>Data analysis</u>

Data from tagged shoots were analysed by ANOVA with respect to total gall weight, number of galls, and the proportion of galled shoots, and that from frame samples in regard to total gall weight and number of galls. Where significant treatment effects were detected ($P < 0.05$), the treatment means were seperated by Fisher's LSD tests. Proportional data were transformed by arcsine√x before analysis. Data from plots with missing shoots were corrected by the respective proportions of tagged shoots recovered to ensure equality of sample size. All analyses were made in R (R Development Core Team 2012).

Results

2010 Trial

Significant treatment effects were detected in total number of galls ($F = 4.30; DF = 4, 16; P= 0.0150$) and the proportion of tagged shoots galled ($F = 5.75; DF = 4, 16; P = 0.0046$) but not in the total gall weight ($F = 1.79; DF = 4, 16; P = 0.1801$). In comparison to the control, Oil (BioPest®) and Confidor® Guard reduced total gall weight by over 60%, total number of galls by over 50%, and proportion of galled shoots by over 40%, however, we were unable to statistically separate the two treatments from the control (Fig. 1). Similarly, the BioPest® treatment produced fewer galls than the control, Supracide®, or Movento® in four of the five experimental blocks but had more galls than Supracide® in block-1.

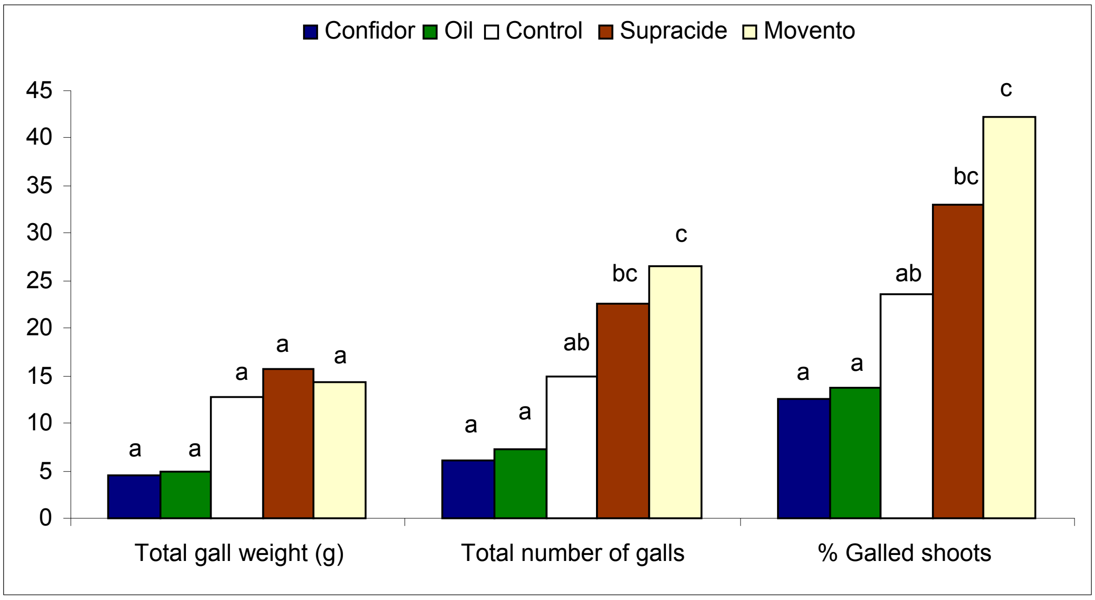

Figure 1. Total gall weight, number of galls, and percentages of tagged shoots in different treatments in the 2010 trial. Forty shoots were tagged in each treatment. Bars in the same group sharing a common letter are not significantly different by LSD test at $P = 0.05$ following detections of significant treatment effects by ANOVA.

Neither Movento® nor Supracide® showed any effects on any of the three gall wasp infestation indices ($P > 0.05$). Interestingly, they performed worse than the control in all three galling indices analysed (Fig. 1) and the difference between Movento® and the control was significant in the total number of galls and in the percentage of galled shoots (*P < 0.05*). It is unlikely that Movento® had actually enhanced the galling activity. This result may have been due to pre-treatment variations of the test trees in their attractiveness to CGW.

2011 Trial

Data from tagged shoots showed significant treatment effects in the total number of galls (*F = 3.80; DF = 4, 20; P = 0.0127*) and the proportion of tagged shoots galled (*F = 3.07; DF = 4, 20; P = 0.0400*) but not in total gall weight (*F = 2.63; DF = 4, 20; P = 0.0649*). Where significant treatment effects were detected, only the high-rate oil can be statistically separated from the control (Fig. 2). On average, the BioPest® 0.5% treatment reduced total gall weight by 72%, total number of galls by 62%, and proportion of galled shoots by 43% in comparison to the control.

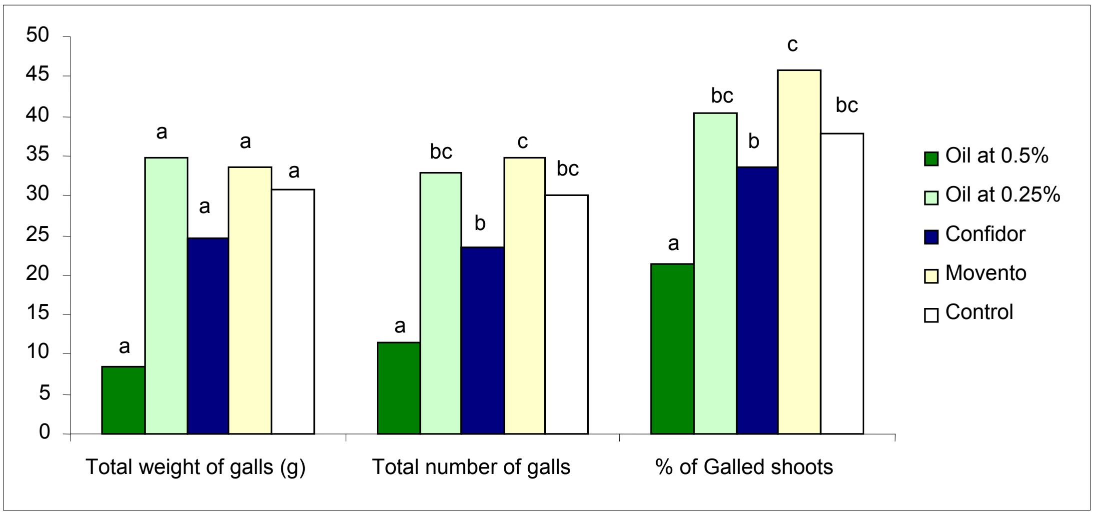

Figure 2. Total gall weight, number of galls, and percentages of tagged shoots galled in different treatment in the 2011 trial. Forty shoots were tagged in each treatment. Bars in the same group sharing a common letter are not significantly different by LSD test at *P = 0.05* following detections of significant treatment effects by ANOVA.

Data from frame samples showed significant treatment effects in both gall weight (*F = 4.20; DF = 4, 16; P = 0.0163*) and number of galls (*F = 3.06; DF = 4, 16; P = 0.0476*). In comparison to the control, BioPest® 0.5%, Confidor® Guard, and Movento® reduced total gall weight by 70, 70, and 55% respectively, and number of galls by 54, 63, and 43% respectively (Fig. 3). The differences between each of the three treatments and the control were all significant (*P < 0.05*), however, there were no significant differences within the three treatments. The low rate BioPest® performed no better than the control.

Movento® residue in treated trees reached over 12 times that of the background level (control trees) on 25 November 2011, within eight days of its first application and 13 days before the second application. The residue level dropped to less than 5 times the background level by 23 December 2011, 46 days after the first application and 15 days after the second application. By 20 January 2012, 64 and 33 days after the first and second applications, respectively, the residue level dropped to background levels.

Confidor® Guard residue levels in treated trees reached 38 times the background level on 23 November 2011, 32 days after the chemical was applied, and was still 24 times the background level on 20 January 2012, 60 days after the application.

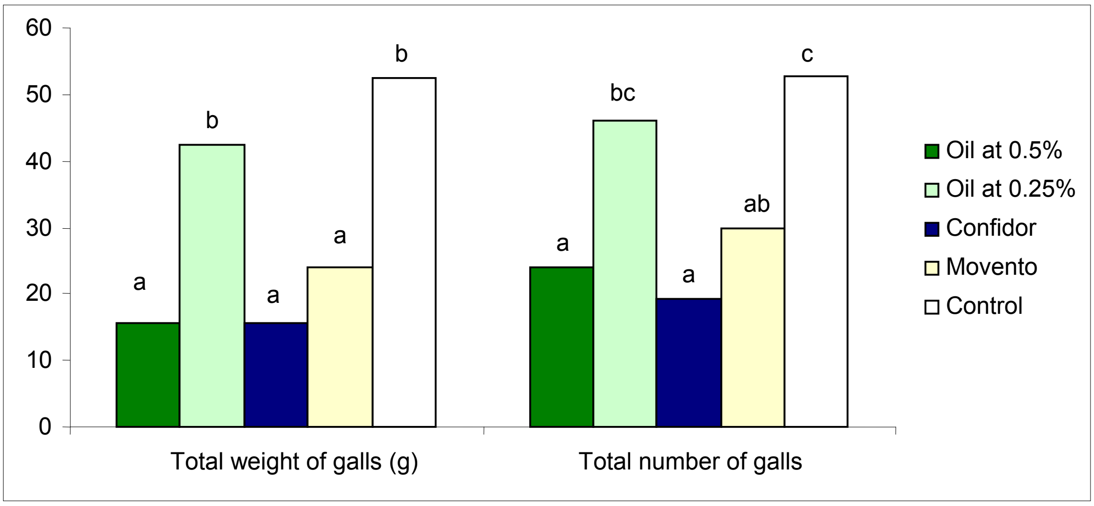

Figure 3. Total gall weight and number of galls in different treatments in frame samples from the 2011 trial. Bars in the same group sharing a common letter are not significantly different by LSD test at $P = 0.05$ following detections of significant treatment effects by ANOVA.

<u>2012 Trial</u>

CGW infestation was much lower in this trial than in the previous two trials. Less than 12% of tagged shoots in the unsprayed control developed galls in this trial as compared to 24% in the 2010 trial and 38% in the 2011 trial. Data from the tagged shoots showed no significant treatment effects in either the total number of galls ($F = 1.62$; $DF = 7, 28$; $P = 0.1698$), total gall weight ($F = 1.16$; $DF = 7, 28$; $P = 0.3569$), or the proportion of tagged shoots with galls ($F = 1.36$; $DF = 7, 28$; $P = 0.2630$), despite the large variations between the different spray frequency / timing treatments (Fig. 4). Numerically, the 1 spray 2nd November treatment performed the best, and the 2 spray, 23rd October / 12th November treatment performed the worst.

Data from the frame samples also showed no significant treatment effects in either the total number of galls ($F = 1.20$; $DF = 7, 28$; $P = 0.3368$) or gall weight ($F = 1.30$; $DF = 7, 28$; $P = 0.2855$). Numerically, it was again the 2 spray, 23rd October / 12th November treatment that performed the worst, however, the best-performing treatment in this case was the 3-spray treatment (Fig. 5).

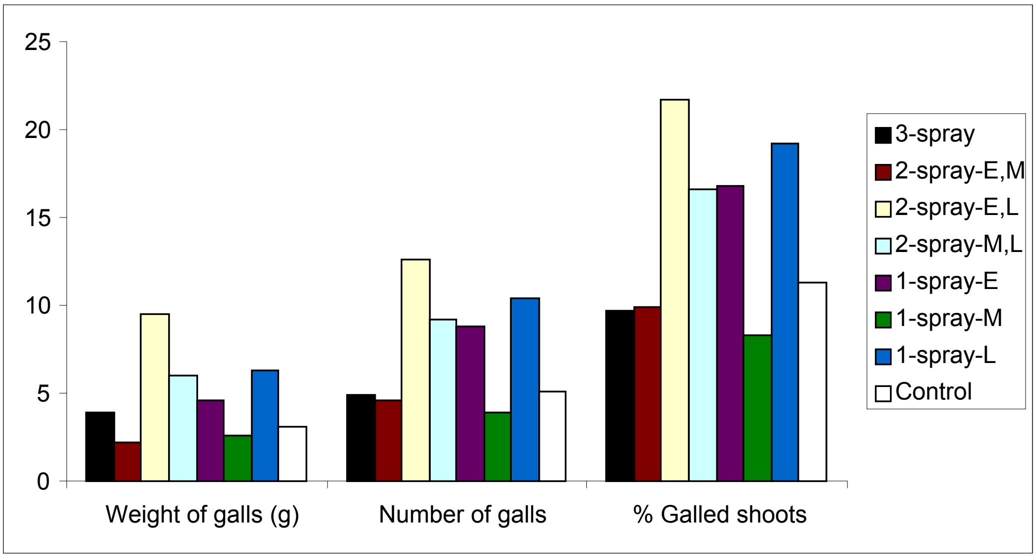

Figure 4. Total gall weight, number of galls, and percentages of tagged shoots galled in different treatments in the 2012 trial. Eighty shoots were tagged in each treatment. E: 23rd October, M: 2nd November, L: 12th November. There were no significant differences among the treatments at *P = 0.05*.

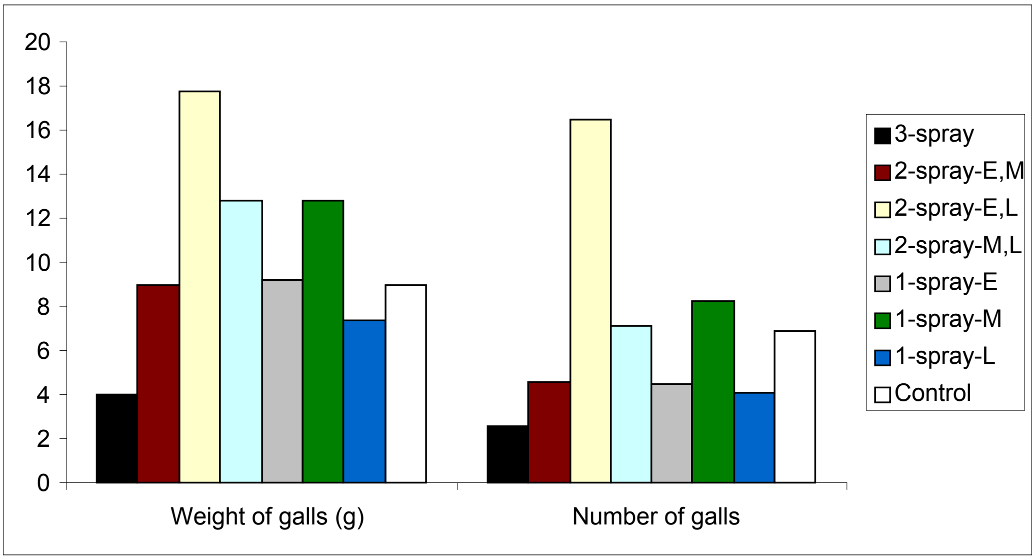

Figure 5. Total gall weight and total number of galls in different treatments in framed samples in the 2012 trial. E: 23rd October, M: 2nd November, L: 12th November. There were no significant differences among the treatments at *P = 0.05*.

Discussion

Three field trials were conducted in CGW-infested citrus orchards in the Coomealla Irrigation District in far southwest NSW to evaluate the potential of BioPest® oil, Confidor® Guard, and Movento® for CGW control. BioPest® was evaluated in all three trials. In two of the trials, three foliar applications of the oil at 0.5% resulted in significant control of CGW infestation, reducing the number of galls by over 50% and the total gall weight by over 60%. However, the effects were less convincing in the 2012 trial due to the low CGW populations in control plots and large, apparently random variations in the data. The 2011 trial investigated if the BioPest® application rate can be reduced to 0.25%. The result was negative. Although more trials are needed to reject the low rate with confidence, we don't believe this is necessary considering that oil is used to control a range of insects in citrus and in most cases the recommended rate is 0.5% or higher. The 2012 trial compared different application frequencies and timings for BioPest®. Due to reasons above, no treatments performed statistically better than the control in this trial. Numerically, however, there were large differences in CGW infestation indices among the different spray frequencies and timings, with the 3-spray treatment and the 1-spray, 2nd November treatment performing best and the 2-spray, 23rd October / 12th November treatment performing the worst. It appears that timing is more important than frequency for oil sprays. CGW adult emergence at the trial site in 2012 peaked on 26 October 2012 and 95% of the wasps had emerged by 2nd November (Chapter-1). The first application was made before the peak and the third application after the 95% emergence date, and both sprays would have missed the bulk of the emerged adults. The second application was made on the same day as the 95% emergence date and seven days after peak emergence. Taking into account longevity of 3-11 days for the adult wasps (Chapter-2), the second application was made around the time when the CGW population at the trial site was at its peak. PSO controls insects by suffocation or by changing their behaviour (Beattie 2005). In the case of CGW control by BioPest®, the underlying mechanism is probably deterrence of egg-laying by CGW females. It has been observed that CGW adults avoided visiting trees that have been sprayed with oil (Richard Bertalli, personal communication, 5 September 2010)

PSO sprays during flowering may affect fruitset (Richard Bertalli, personal communication, 27 March 2013). Peak flowering of Washington navel in Dareton, less than 10 km from the study sites, during 2010-2012 occurred during 4-8 October (Tahir Khurshid, personal communication, 28 March 2013). Assuming similar flowering dates, the first BioPest® sprays in this study were put out 17-18 days after peak flowering, so any negative effects of the oil sprays on fruitset, if present, would have been small. As a precaution, however, it is important that sprays of BioPest® or other PSO products be timed after petal fall.

Confidor® Guard was evaluated as a soil drench in two trials. In both trials the chemical significantly reduced CGW infestation. The level of efficacy was similar to BioPest®, reducing galls by over 50%. Being a systemic insecticide, Confidor® Guard targets only the larval stage and hence its application should ideally be made after peak egg hatching. In this study, Confidor® Guard was applied two weeks to one and a half months before the peak egg hatching dates (Chapter-2) in the two trials, indicating precise timing is not critical for Confidor® Guard application. This is not surprising considering the excellent residual effects of imidacloprid (Elbert *et al.* 2008), the active ingredient of Confidor® Guard. The long residual period also warrants extra caution if the chemical is to be registered. The likely impacts of the chemical on beneficial organisms should also be taken into account when considering registration. Two parasitic wasps attack CGW (Smith *et al.* 1997) and both have been recovered from the study site (Chapter-1). By killing CGW larvae, the chemical also kills the parasitic wasps living inside them. Additionally, imidacloprid may have indirect negative impacts on some predatory insects (Mizell *et al*. 1992) and bees (Bortolotti *et al*. 2003).

Movento® was evaluated as a 2 foliar-spray treatment in two of our trials and failed to provide any significant control of CGW. Residue data collected during the 2011 trial showed that the chemical peaked inside the foliage in late November. Thereafter, the residue level quickly declined, becoming undetectable by mid-January. The peak residue date predated the second spray, suggesting that the residue was mainly from the first spray. Dissections of lemon shoots from a nearby orchard indicated peak egg hatching around mid December 2011. Around this time Movento® residue was still detectable but at a much lower level than the peak (5 versus 12 times background level). It is likely that at this residue level Movento® was not highly effective against CGW larvae. Later applications appear to be a logical solution to the problem, however, this was not supported by our data. In the 2011 trial, the first spray may have been applied too early but the second spray was applied within a week of the peak egg hatching date and so should have been appropriately timed for larval control. Unfortunately, for unknown reasons, the second spray did not result in significant increases of Movento® residue inside the foliage. Movento® residue was not monitored in the 2010 trial but the second spray was put out five days after peak egg hatching and would have targeted an even larger proportion of larvae than in the 2011 trial. It appears that factors other than timing may have been responsible for the poor control. One likely factor is perhaps the variable absorption rate of the chemical by the foliage. Mo *et al*. (2007) evaluated Movento® for controlling onion thrips (*Thrips tabaci*) in onions (*Alium cepa*) and noticed different results in different trials. Poor absorption was suggested as being partly responsible for the unsatisfactory results.

Supracide® was tested in the 2010 trial only and was found ineffective against CGW. Due to its high mammalian toxicity and broad spectrum activity, this chemical was not included in the latter two evaluation trials.

In summary, BioPest® is a promising alternative to the currently registered Supracide®. Applied at 0.5% in three 1-2 weekly sprays, BioPest® reduced gall incidence by over 50%. Reducing the rate to 0.25% was not an effective option. For maximum effect, the oil should be applied when the wasps are most abundant in the orchard. There may be scope to reduce the number of sprays to one if timed correctly, however, no convincing results were obtained regarding the relative efficacy of different spray frequencies in this study due to low control populations in 2012. Confidor® Guard is also effective against CGW. A single application of the chemical in the soil from late October to mid November achieved similar control of CGW as three sprays of BioPest®. The exceptionally long residual period and potential negative impact of the chemical on beneficial organisms in citrus need to be considered before it is registered. Confidor® Guard targets the larvae and is best applied shortly before (allowing time for absorption by the trees) or soon after most eggs have hatched. The efficacy of Movento® was not confirmed in this study.

References

Beattie GAC. 2005. Using petroleum based spray oils in citrus. Agfact H2.AE.5, NSW Department of Primary Industries.

Bortolotti L, Montanari R, Marcelino J, Medrzycki P & Maini S. 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bulletin of Insectology 56(1), 63-67.

Cannard M. 2007. Native parasitic wasps released to control citrus gall wasp. Citrep 51, 14-15.

Elbert A, Haas M, Springer M, Thielert W & Nauen R. 2008. Applied aspects of neonicotinoid uses in crop protection. Pest Management Science 64(11), 1099-1105.

Hardy S & Creek A. 2009. Final Report - Citrus Gall Wasp Technical Forum. Dareton, 5th May 2009.

Mizell RF & Sconyers MC. 1992. Toxicity of Imidacloprid to Selected Arthropod Predators in the Laboratory. The Florida Entomologist 2, 277.

Mo J. 2007. Development of management options for onion thrips in onion. Final report of project VN04004, Horticulture Australia Ltd, Australia..

Papacek DF & Smith D. 1989. Insectidal control of citrus gall wasp in Queensland. General and Applied Entomology 21, 2-4.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Smith D, Beattie GAC & Broadley R. 1997. Citrus pests and their natural enemies. DPI Queensland, Brisbane.

Vermeer R & Baur P. 2008. Movento® product development: custom-made formulations for an exceptional active ingredient. Bayer CropScience Journal 61(2), 141-158.