Citrus Market Access Solutions for FRW and Island Fly

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South Australia Research & Development Institute (SARDI)

Project Number: CT11002

CT11002

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Market access solutions for Fuller's rose weevil and Island fly

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South Australian Research and Development Institute

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Statement about the purpose of the report
This report details the research and extension delivery undertaken in Project CT11002 on Market access solutions for Fuller's rose weevil and Island fly. Main findings, industry outcomes and recommendations to industry along with suggested areas of future research are discussed.

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April 2013

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The generosity of growers who co-operated with field trials, Graham and Michael Ingerson, Richard and Judy Bertalli, and Dick Ashby is gratefully acknowledged, as is the assistance of Scott Dix from Costa Exchange, Tony Filippi from Mildura Fruit Company and staff at the Loxton Research Centre farm. Thanks also to Nilesh Chand for conducting the Island Fly "Constant Temperature Development Rate" experiments and to Dr Jianhua Mo for the statistical analysis of that data and assistance with developing the Day Degree model. Identification of bacteria was conducted by Ahmed Al-Hashimi with assistance from Dr Kelly Bailey. Kala Bhandari Pradhan assisted with Island Fly and bacterial culture maintenance and carried out trials on the effects of the bacteria on Island fly fitness and attraction of Island fly to bacterial volatiles.

Project Team Members:
Greg Baker, Dr Peter Crisp, Mark Barrett, Lakshmi Nacey, Dr Latif Saleh and Rob Weppler.

5. MEDIA SUMMARY

Project CT11002 has furthered the significant progress achieved in CT07045 towards achieving the suppression of Fuller's Rose Weevil (FRW) required for market access to China and other east Asian countries with similarly stringent quarantine protocols. Field research findings have been used to "fine tune" the integrated Best-Practice management program and scouting protocols. This assures that the Best-Practice Program is effective and remains so for a number of seasons. This will encourage the already widespread uptake of the FRW program, thereby providing more growers with the option of expanding their export markets and improving the industry's security of fruit supply for export to FRW-sensitive markets.

Understanding of the biology of Island fly (*Dirioxa pornia*) was improved in CT07045, however, breakthrough in project CT11002 allowing their successful laboratory culturing has led to the development of a Degree Day model and improved understanding of the relationship between Island fly and their symbiotic bacteria. This relationship between fruit fly and the bacteria is providing opportunities to improve the Sterile Release Technique for pest fruit flies such as *Ceratitis capitata* and *Bactrocera tryoni*. The bacteria also provide opportunities to develop improved lures for monitoring programs and lure and kill management of fruit flies.

The key findings were:

- Updated Best-Practice guide for FRW.
- Establishment of rearing method for Island fly.
- Confirmation that citrus is a conditional non-host of Island fly.
- Island fly populations are correlated with fruit on the orchard floor.
- Island fly constant temperature development model.
- Bacteria isolated from the gut of Island fly have potential for development as a lure and some species may be pathogenic to other Tephritid fruit fly species.

In summary,

While initial research conducted as part of CT07045 and CT11002 has provided an effective field management protocol for FRW, future R&D is required to evaluate other chemical options in the field to ensure ongoing success of the export programme. The area that needs most research and development in the future is post-harvest disinfestation and further development of vital stain technologies available to increase the efficiency and verification of fumigation and other post harvest technologies.

Further R&D is required for Island fly, particularly with regards to possible use of bacteria as a control option which may also transfer to other species of Tephritid fruit fly such as *C. capitata* and *B. tryoni*. The highly attractive nature of some of the identified bacteria should be further investigated for development of attractants for Tephritid species for which we have no or only poorly performing lures.

6. TECHNICAL SUMMARY

6.1 The Problem

The Australian citrus industry produces an estimated $446 million of fruit annually, of which about 25% is exported (Anon, 2007). To maintain and develop the industry, new export markets need to be sought and developed. Australian citrus was recently granted access into the potentially lucrative Chinese market, provided strict phytosanitary requirements could be met. This has brought a renewed focus onto Fuller's Rose weevil (FRW), *Naupactus cervinus* Boheman (Coleoptera: Curculionidae), a citrus pest of which both China and Korea are highly sensitive. FRW is also a quarantine issue for some other Australian citrus export markets (Anon 2007). Eggs laid under the fruit calyx are the most common stage of FRW reported on export citrus. The eggs are not easily detected during routine packing operations. Although hatched eggs are easily distinguished from unhatched eggs, there is no easy way to distinguish between most viable and non-viable unhatched eggs (Buchanan 1988b).

The field based systems approach developed as part of CT07045 has been widely adopted in some regions and facilitated the successful export of 170 containers of oranges to these sensitive markets in 2012. Despite these successes the occasional detections of FRW eggs continues to threaten the viability of the entire citrus export program to Thailand, China and Korea. Detection of FRW before export results in rejection of the fruit. Detection on arrival in China will result in rejection of the shipment, which must be destroyed, re-exported or fumigated with methyl bromide.

In recent years Island fly larvae have been detected in Australian citrus exports to New Zealand, Japan and the USA. The interception of Island fly larvae in citrus exported from the Fruit Fly Exclusion Zone causes significant concern to importing countries' quarantine authorities, until larval identification confirms that it is not *Bactrocera tryoni* or *Ceratitis capitata*. Any increase in frequency of these detections has the potential to cause importing countries to focus on the species and require more evidence to demonstrate the secondary pest status of the species. For this reason, it is in the interests of the Australian export fruit industry to support bio-ecological research into Island fly biology and ecology and to apply this to identify host preferences and control strategies.

The native Island fly, *Dirioxa pornia*, which occurs in eastern Australia from Queensland to Victoria, and in parts of South Australia and Western Australia, has not been considered a primary pest as it is reported to only attack damaged or fallen fruit. Although only rarely trapped in commonly-used cue-lure trapping grids, field scouting observations and larval infestation records indicate that in recent years Island fly has increased in abundance across much of the Fruit Fly Exclusion Zone.

6.2 The Project Science

Fullers Rose Weevil

- Compared field scouting results with packing shed inspections.
- Conduct field trials to verify recommendations for field systems approach.
- Updated and evaluated recommended cultural control practices.

Island Fly

- Conducted constant temperature development studies on Island fly to develop a day degree model to assist the development of management programs.
- Verified the status of citrus as a conditional non-host of Island fly.

6.3 The Key Research Findings, Extension Highlights and Industry Outcomes

- Verification that the integrated management approach for FRW developed in CT07045 is effective and, with minor modifications, provides reliable control in orchards with low initial populations.
- Discovery that failure to maintain weed control, skirting and spray schedules can lead to FRW egg detection in the harvest.
- Establishment of an *Island fly* culture using enterobacteria isolated from the gut of adult flies. These bacteria are highly attractive to Island fly and *Bactrocera tryoni* adults and potentially could lead to the development of lures for monitoring or lure-and-kill technologies.
- Confirmation that citrus is a conditional non-host for Island fly and does not infest sound fruit. However, pinprick damage to the rind is sufficient for Island fly females to lay eggs in fruit.
- Production of a Day Degree model for Island fly has been produced.
- Demonstration that there is a positive correlation between populations of Island fly and discarded and fallen fruit.

6.4 Recommendations

The recommendations in CT07045 the integrated management approach using cultural, chemical and sanitary practices have been modified to read as follows:

1. **Skirt trees to ensure that low foliage does not touch the ground or weeds.**
    - Trees should be skirted high enough to prevent foliage or fruit touching the ground at any time.
    - Skirt height must take into account the future sagging of branches as a result of fruit growth.
    - Skirts should be at least 50cm high to allow for easy trunk treatment and inspection for weeds.

2. **Maintain good weed control to prevent weeds acting as a bridge into the Canopy.**
    - Even single blades of grass have been observed to allow FRW access into a tree canopy
    - Groves should be inspected frequently enough to detect and combat weed regrowth before weeds contact the tree foliage

3. **Spray a band of insecticide onto tree trunks to repel or kill FRW that try to climb the trees.**
    - The synthetic pyrethroids Karate®, Trojan®, and Matador® are registered for trunk band application control of FRW in lemon and orange orchards in all States.
    - The insecticide band should be at least 20cm wide and completely encircle the trunk.
    - Kaolin should be added to the tank to make it easier to ensure complete coverage is achieved; this also will highlight any spray drift onto fruit which may result in fruit rejection due to high reside levels.
    - The band should be in a position where it is not 'washed' regularly by sprinkler irrigation.
    - Insecticide should be reapplied frequently enough to the band to maintain its effectiveness – reapplication every two to three months may be necessary (refer to product labels).
    - If FRW control is required in other citrus varieties, eg mandarins and grapefruit, several carbaryl products are registered (refer to product labels).

Treatment timing

For maximum impact, skirting, weed management and trunk sprays should be maintained from December to harvest. This takes advantage of the seasonally very low numbers of FRW in trees around December. Field management of FRW in export groves should be maintained year-round until populations of the pest have been reduced to low levels. This may take several years.

4. Maintain a good level of grove hygiene and cleanliness

- Light prunings, tumbleweeds, polystyrene boxes etc should be kept out of the grove as they are easily blown under trees where they create bridges between the ground and foliage.

5. Monitor the treated trees regularly to ensure that:

- Weed control is effective.
- Tree skirts are well clear of the ground, weeds and cover crop.
- Insecticide bands are reapplied regularly as per the label instructions.

6. Sanitation and exclusion

- If other orchards adjoin the orchard to be treated, either directly or where divided only by a farm access track, it is recommended that one or two rows of that orchard are treated as above as a buffer.
- FRW adults are quite limited in their capacity to disperse unaided. Hence dispersal between orchard blocks, properties and districts is largely reliant on human intervention. Dispersal can occur either amongst soil with new plantings, or on clothing, machinery and equipment moving into established orchards from infested blocks. Hence simple quarantine and cleaning methods can be used to help prevent FRW from entering non-infested orchards.

The risk of fruit being infested with FRW eggs will be much higher if any of these aspects of management are compromised, even for a short period.

We recommend that further research be undertaken to:

1. evaluate other chemical options that may be suitable alternatives to the current APVMA approved options;
2. evaluate other formulation options which may improve the longevity of chemicals in the field;
3. assess post-harvest options for removal of egg masses from under the fruit calyx;
4. develop control protocols for Island fly;
5. assess the potential of new fruit fly lures derived from bacterial volatiles;
6. assess the potential of pathogenic bacteria as a management option for fruit fly.

7. BACKGROUND

7.1 Fuller's Rose Weevil

The Australian citrus industry produces an estimated $446 million of fruit annually, of which about 25% is exported (Anon, 2007). To maintain and develop the industry new export markets need to be sought and developed. Australian citrus was recently granted access into the potentially lucrative Chinese market, provided strict phytosanitary requirements could be met. This has brought a renewed focus onto Fuller's Rose weevil (FRW), *Naupactus cervinus* Boheman (Coleoptera: Curculionidae), a citrus pest of which both China and Korea are highly sensitive. FRW is also a quarantine issue for some other Australian citrus export markets (Anon 2007). Eggs laid under the fruit calyx are the most common stage of FRW reported on export citrus. The eggs are not easily detected during routine packing operations. Although hatched eggs are easily distinguished from unhatched eggs, there is no easy way to distinguish between most viable and non-viable unhatched eggs (Buchanan 1988b).

Project CT11002, which concluded in December 2012, has made significant progress towards providing the information and tools required to suppress Fuller's Rose Weevil (FRW) to orchard levels (i.e. nil detect) required for market access to China and other countries with similarly stringent quarantine protocols. Further, based on new knowledge of the behaviour of the FRW, particularly the timing of key life-cycle events, an integrated Best-Practice management program and improved scouting protocols have been devised.

In summer 2011-12 large plot and whole-block scale trials were established in commercial orchards in SA to assess the performance of the protocols in a 'real world' scenario. The assurance that these trials will provide, that the Best-Practice Program is effective over number of seasons, will improve the uptake of the Program and provide more growers with the option of expanding their export markets and improve the industry's security of fruit supply for export to FRW-sensitive markets.

Key FRW research.

- Assess the Best Management Practice protocols recommended in the outcomes of CT 07045 and fine tune to address apparent weak points and reduce costs if possible, and
- Compare field monitoring detections of FRW with pack house sampling.

7.2 Island fly

Island fly (*Dirioxa pornia* Walker) is a native Australian tephritid which has historically been considered a species of no economic significance due to its apparent preference for over-ripe, damaged or rotting fruit. In recent years, Island fly larvae have been detected by quarantine authorities in Navel and Valencia oranges exported

to New Zealand, USA and Japan. As the larvae are morphologically similar to tephritids of economic significance, the detection of Island fly larvae becomes a quarantine issue until molecular identification is completed. Further, ongoing interceptions of Island fly may trigger trading partners to re-categorize the quarantine status of Island fly, with serious consequences for the FFEZ citrus production regions. Export markets are critical to the viability of the Australian citrus industry, and research efforts are currently underway to investigate basic biological questions with a view to elucidating how Island fly are contaminating export fruit.

Previously, the scientific literature on Island fly was scant, with morphological descriptions of the larval, puparial and adult stages, a description of the mating behaviour and a number of host records comprising the bulk of the information available. A preliminary study as part of Project CT07045 has contributed some valuable findings about Island fly behaviour and seasonal abundance, which will help guide the direction of future research.

A major question about the biology of Island fly was whether females are capable of piercing sound citrus fruit in order to lay eggs. Studies on the physical appearance of the ovipositor (egg laying structure) reveal that females do not have the adaptation needed to pierce sound fruit. Tephritids of major pest significance have evolved bare, sharp or serrated ovipositor tips which can be used to pierce the intact skin of sound fruit, thereby enabling the insertion of the ovipositor into the fruit in order to place eggs. The ovipositor of Island fly has a relatively broad tip and long sensory hairs that extend beyond the tip. This form of ovipositor is common in species that lay their eggs in rotting plant material. In addition, in experimental oviposition preference trials, eggs were primarily laid in peel damage, and to a lesser extent, the navel of a damaged orange. Under these conditions eggs were not found in the navel of sound, intact oranges. These findings support the long-held Australian hypothesis that this species is not a true economic fruit fly, but has a biology analogous to ferment flies which use natural crevices in rotting plant material, including fruit, as the egg laying substrate. This information and laboratory observations that eggs can be laid in the crevices formed by touching fruit, suggests that crevices formed by the touching surfaces of packed fruit may be sites for oviposition. This has implications for the role of post-harvest processes (in-field collection sites and post-packing storage) in infestation risk, and will be a focus of proposed new research.

In summary, this project has completed the development and delivery of a FRW field Management Program for Australian citrus growers to successfully meet Chinese phytosanitary requirements, including the reduction of well-established FRW infestations to nil-detect levels. It has also made progress towards the development of a systems approach for citrus growers and packing sheds to successfully manage Island fly and minimize further detections in overseas markets, thereby minimizing the significant risk of our international trading partners re-assessing the biosecurity status of this native fruit fly species.

8. MATERIALS AND METHODS

8.1 Fullers Rose Weevil

8.1.1 Renmark field trial

A large scale field trial was established at Renmark in South Australia's Riverland to evaluate the Best Practice recommendations developed in CT07045 on a whole block commercial scale. Six blocks were selected with a known history of FRW detections of 1-2% infestation of sampled fruit. Three blocks were subjected to the best practice of tree skirting, weed control and six weekly applications of a 30 cm wide trunk band of Karate Zeon® (250 g L^{-1} Lambda-cyhalothrin) 300ml $100L^{-1}$ (approximately 200 ml $tree^{-1}$) commencing 7 December 2010 until 17 July 2011 using the SARDI butt sprayer (6 applications).

The trial was assessed by fruit sampling as part of the grower's normal scouting program, tree shaking in April and July and a 600 fruit sample at harvest. The remaining three blocks were managed as per normal for non-export blocks; weed control but no skirting or trunk banding and were assessed as for the treated blocks.

8.1.2 Waikerie field trial

In the 2011-2012 season the pesticide butt-treatment was applied on a 6 weekly basis from December until June to an orchard in Waikerie with a history of low-moderate densities of FRW. All plots were skirted and effective weed management was maintained throughout the trial. Trunk banding sprays were applied to trees in 4 replicated plots either 6 times commencing in December 2011 or 3 times commencing in March 2012, and unsprayed plots were used as controls. The treatments, 30 cm wide trunk band of Karate Zeon® (250 g L^{-1} Lambda-cyhalothrin) 300ml $100L^{-1}$ (approximately 200 ml $tree^{-1}$), were applied to the trees using the SARDI trunk spray unit. The trial was assessed by fruit sampling as part of the grower's normal scouting program, and tree shaking in April and July.

8.1.3 Export orchard survey

In addition, in collaboration with Mildura Fruit Company several commercial orchards in Sunraysia that adopted the recommended best practice program (including butt-spraying) were monitored throughout the 2011-2012 production season. The orchards were monitored using;

1. Tree shaking as described in CT07045 (to assess numbers of FRW successfully reaching the tree canopy)
2. Fruit assessment (routine assessment of 10 fruit per bin at arrival at the packing shed to assess numbers of fruit with egg masses).

Data collected from the various trial orchards will be collated to verify the orchard monitoring protocols recommended in CT07045.

8.1.4 Long term trials

It was planned to monitor orchards treated as part of CT07045, however, major changes in the management practice at these orchards, such as severe hedging at the Colignan and Albemerle sites and use of broad spectrum insecticides at Dareton, would have been likely to confound the results, and potentially result in poor advice to growers and financial losses. As a result these activities were not conducted.

8.2 Island Fly

8.2.1 Laboratory culture

A laboratory culture was established at Waite Campus using wild flies, captured from various areas of the South Australian Riverland region in McPhail traps baited with Ammonium Acetate (Biolure® FRUIT FLY FFA, Suterra LLC USA) and Putrescine (Biolure® FRUIT FLY FFP, Suterra LLC USA) lures. These flies were transported to the Waite Insectary, and placed in 47.5cm x 47.5cm x 90 cm bugdorm (Megaview Science Co., Taiwan) mating cages kept at 27^{o}C in natural light supplemented by fluorescent light timed to come on approximately 1 hour after sunrise and turn off prior to sunset. The wild flies were provided with *ad libitum* diet (Appendix 1), water source from gels crystals (Brunnings Easy Wetta water storage crystals; Bunnings Warehouse, Australia) and supplementary bacteria isolated from adult wild Island fly and cultured on yeast extract agar (Amyl Media Pty Ltd) (Appendix 3). Subsequent generations of adults were maintained in the same manner.

Egg collection

Island fly eggs were collected in a 75 mL plastic container which had approximately ten equi-distant holes punctured with a heated needle towards the upper-third section of the container. The container was lined on the inside with lime green paper (3.5 cm by 10 cm) and a Vileda® Wettex sponge cloth of the same dimensions to keep the green paper moist. The lime green liner for the egging container was selected in colour preference trials conducted prior to the experiments described here (Data not presented). 12 mL of orange juice (Just Juice/Golden Circle) was then poured into the container, which was then placed inside the insect cage. Eggs were laid through the holes of the egging device onto the green paper. The device was collected from the cages every 24 hours between Tuesday and Friday and after 72 hours on Monday. The green paper was extracted from the device and washed using a water squirt bottle onto a sieve mesh that collected the eggs.

Rearing of Island fly larvae

Eggs from the sieve mesh were placed on a piece of paper which was placed on a container of larval diet (modified from Baker *et al.* 2011), so that as the larvae eclosed from the eggs they could easily access the diet (Appendix 2).

During experiments with Island fly the larval diet was placed in a 90 mm Petri dish. A Vileda® Wettex® sponge cloth was cut into the size of a 9 mm Petri dish, rinsed with running hot water, and placed in a sterile Petri dish of the same dimension. 15-18 mL of larval diet was poured onto the Vileda® Wettex® sponge cloth in the Petri plate. Eggs of Island fly were counted and placed on the wet cloth. The Petri plate was then sealed with Parafilm M (Pechinay Plastic Packaging Inc., Chicago, USA) and placed in an incubator at 30°±2 C. If the number of eggs exceeded 100, an additional Petri plate was used for the additional eggs. Larval diet was topped up as required.

8.2.2 Rate of Development Study

The wild flies were provided with *ad libitum* diet (Appendix 1), water source from gel crystals (Brunnings Easy Wetta water storage crystals; Bunnings Warehouse, Australia), supplementary bacteria grown on agar gel and egg laying devices. The first generation (F1) offspring used in this experiment were obtained from the wild flies and the eggs from the F1 were used in constant rate of development study.

Experimental Design

The experiment was designed as completely randomized, having five constant temperature treatments and at least 12 replicates for each temperature range. Each replicate consisted initially of 10 eggs and a success rate of at least 25% was used as a benchmark for continuing observations into the next life cycle stage.

Incubator Settings

Westinghouse refrigerated incubators (Model No. RP432V-R; Electrolux Home Products Pty Ltd, Australia) were used in this study. These were thoroughly cleaned and calibrated with 5 constant temperatures of 15, 20, 25, 30 and 35^{o}C (±1^{o}C), 70 ± 10% relative humidity and 12:12 L:D photoperiod. The incubators were equipped with temperature control units (Model SR1-81-10, Shimaden Co. Ltd, Japan) and were monitored daily. The temperature was also monitored with temperature data loggers (Tinytag Talk 2, Model TK-4014, Gemini Data Loggers (UK) Ltd, UK) placed in each incubator and checked weekly. The automatic readings taken by data loggers were of the actual, minimum and maximum temperatures at 15-second intervals.

Egg Stage

Freshly laid eggs were obtained within a four hour period (0900-1300 hours) from the F1 generation of Island fly in the 45cm^3 rearing cages (Bug Dorm, MegaView Science Co., Ltd; Taiwan) at SARDI Entomology rearing rooms at Waite Campus,

Urrbrae, South Australia. Preliminary observations indicated that the flies were most active during this period for oviposition, in accord with other studies by Ero *et al*. (2011) and Muthuthantri and Clarke (2012). The temperature of the rearing rooms was maintained at 27^{o}C (± 1^{o}C), 12:12 L:D photoperiod (2 sets of 3x40 watts Phillips fluorescent lights on timer control) and 70 ± 10% relative humidity. Eggs were collected in egg laying devices as described in section 8.2.1 Laboratory culture. After four hours the egging devices were retrieved and the eggs laid on the green paper were removed by washing with water from a wash bottle. The solution was strained through a fine mesh cloth and sieved to collect the eggs. Ten eggs each were placed evenly onto a strip of moist black blotting paper 50mm x 20mm using a small camel's hair brush. Black blotting paper was selected to make it easier to observe the eggs and whether or not they had hatched. Vileda® material was cut to the size of a 90mm Petri dish, washed in hot water and placed in a sterile Petri dish that contained fruit fly larval diet (Appendix 2). The black paper with the ten eggs on it was placed in the centre of the Vileda® material, the Petri dishes were immediately closed and sealed using paraffin wax tape (Parafilm®) and placed in incubators.

Larval Stage

Daily observations were recorded of the developmental changes of eggs hatching into larvae observed under a microscope. The larval diet food supplement (Appendix 2) with 4% Pen-Strap® (Penicillin Streptomycin Solution (100x) **(**10,000 I.U. ml^{-1} Penicillin 10,000 µg ml^{-1} Streptomycin), Sigma-Aldrich, USA) was added every third day to reduce the chances of microbial contamination in the Petri dishes until the active feeding period of third instar larvae at the jumping stage was reached.

Pupal Stage

When the larvae were ready to jump, the Petri dish was opened (lid removed) and placed in a 750ml plastic jar of 15cm height, containing 2.5cm of moist Vermiculite ® (Exfoliators (Aust) Pty Ltd, Australia) as base. The jars were closed with lids that had punctured holes for airflow but prevented larvae from escaping. The Vermiculite® base was lightly moistened twice a week with water from a spray bottle. The pupae were counted and observed daily.

Eclosion Stage

The development from third instar stage for jumping, to pupation and eclosion were observed and recorded daily. Eclosions were recorded according to constant rearing temperatures, removed daily from the jars and transferred to the rearing cages in the laboratory. A similar rearing and egg extraction method was used by Ricalde *et al*. 2012.

Statistical Analyses

The development time for each stage of life was replicated at least 12 times. The effect of treatment on development times was tested using Standard Analysis of Variance (ANOVA). Comparison of means was done where necessary for a Least

Significant Differences (LSD) test (P=0.05). GenStat software (Version: Release 14.1 for PC/Windows 7; VSN International Ltd., Hemel Hempstead, UK.) was used to statistically analyse all the data.

Temperature Summation Model

It was assumed that the temperature to development rate relationship was linear above a certain lower threshold for development (Fletcher 1989). Thus, in order to complete the development stages a constant number of heat units (usually expressed as day-degrees) were required (Wagner *et al*. 1984; Fletcher 1989). A series of constant temperatures were used to determine the development time of individual life stages where 25% of the individuals completed a biological stage, establishing a temperature-time relationship. The development rate (100/development time) was plotted against temperature.

Logan Type III model was used for linear and non-linear regression graphs. This was considered an improvement on earlier non-linear models of regression graphs. It has four parameters (Pr 1= scaling parameter, Pr 2= scaling parameter, Pr 3= lower threshold temperature and Pr 4= Upper threshold temperature) and approximates a sigmoid curve.

The regression line of the graph was extrapolated to the x-axis to determine the lower development threshold, where the development rate was zero. The number of day-degrees above the lower threshold needed to complete development is known as thermal constant K. K was calculated from the regression equation using the relationship y=K/(x-t) (Fletcher 1989). A similar model was also used by Duyck and Quilici (2002).

Survival Rate

The total number of individuals alive at the end of each stage was divided by the initial number to obtain the stage-specific survival rate. This same calculation was used by Duyck and Quilici (2002). The rate was converted to a percentage for egg to larvae and egg to eclosion stages for comparisons in the five temperature ranges.

They were fed with adult Island fly diets (Modified from Baker *et al*. 2011; Appendix 1) every Tuesday and Friday. Water was provided in gel form (Bunnings *Easy Wetta Water Storage Crystals*) as per label instructions of the manufacturer, and was changed twice a week, along with the food (*ad libertum)*. Both food and water were placed together on circular lids of 750 mL plastic containers (10 cm diameter) inside the cage for each culture.

Pupation and eclosion

When 50% or more of the surviving larvae in the Petri dish were ready for pupation, the larvae were transferred from the Petri plate into a pupation device (750 mL plastic container) that contained a 1 cm high layer of moist vermiculite. The lid of this

container was made of either meshed nylon (0.2 mm by 0.25 mm), or plastic that was punctured with more than 30 small holes, for aeration purposes.

8.2.3 Host Status Verification (Navel Oranges)

The status of citrus as a host of Island Fly was assessed using FAO guidelines (Anon. 2005). A laboratory cage trial was established using five cages of 10 punctured (50 1mm diameter x 10mm deep pin holes per fruit) navel oranges, 10 male and 10 female mature Island fly were added to each cage with food and water ad libitum. The adult flies used in this trial were from a culture maintained at Waite Campus. The flies were removed from the cages after 24 hours. The oranges were then incubated at approximately 25^{o}C on a 2cm deep bed of vermiculite and monitored for larval development and adult emergence.

The second stage of the laboratory trial was established as above except that the oranges were carefully inspected using a dissecting microscope at 10x magnification and only undamaged fruit placed in the cages. This trial was duplicated; in the first trial fruit were placed in the cage so that they were not touching, and in the second trial the fruit were piled into a pyramid so that they were touching providing potential egg laying sites, thus emulating fruit touching in the field.

A field trial was established at Loxton in South Australia where ten 40 cm insect rearing sleeves (Megaview Science Co Ltd, Taiwan) were placed on branches of Navel orange trees so that there were five ripe fruit in each cage. In 5 of the sleeve cages the fruit were punctured as for the laboratory trials and the fruit in the remaining 5 cages were left undamaged. Ten female and 10 male flies from the Waite Campus culture were added to each sleeve cage and provided with adult diet, and were removed after 48 hours. The fruit was then harvested and returned to the laboratory for incubation at 25^{o}C on a bed of vermiculite and inspected for larval development and adult emergence.

8.2.4 Fallen fruit and Island fly population density

Eight orchards with either a low (average <5 fruit tree^{-1}) or high (average >50 fruit tree^{-1}) number of discarded fruit under the trees (Figure 1) were selected for monitoring *Island fly* population densities throughout an 18 month period (August 2011 – January 2013). The number of fallen fruit per tree was calculated as an average from a count of 10 trees. Counts of fruit were taken in August 2011 when orchards were selected, fallen fruit numbers were also counted in August 2012 when all orchards were considered to have low numbers (average <5 fruit tree^{-1}). The populations were monitored by placing three McPhail traps in each orchard with Ammonium Acetate (Biolure® FRUIT FLY FFA, Suterra LLC USA), Putrescine (Biolure® FRUIT FLY FFP, Suterra LLC USA) lures and Dichlorvos as a toxicant (1 cm^2 section of a Kill-Master Zero pest strip 186 g kg^{-1} Dichlorvos. Barmac Industries Pty Ltd). Traps were monitored three weekly in peak population periods (October to April) and four weekly when populations were at their lowest (May to September).

The sites were divided into two groups; Group A – High density in 2011 and low density in 2012, and Group B – Low density in 2011 and 2012.

Figure 1: Fallen and discarded fruit under trees in orchards used to assess relationship between population density of *Dirioxa pornia* and fruit on the ground. Images A & B low density (<5 fallen fruit per tree), C & D high density (>50 fallen fruit per tree).

8.2.5 Packing Shed Surveillance

In 2011 Island fly larvae were detected in a Navel orange shipment exported to Tasmania and second grade Navel oranges being marketed in Adelaide. Both shipments were traced back to one packing shed. A network of McPhail traps with Ammonium Acetate (Biolure® FRUIT FLY FFA, Suterra LLC USA), Putrescine (Biolure® FRUIT FLY FFP, Suterra LLC USA) lures and Dichlorvos as a toxicant (1 cm^2 section of a Kill-Master Zero pest strip 186 g kg^{-1} Dichlorvos. Barmac Industries Pty Ltd) were established in surrounding orchards (Citrus and Avocado), ornamental street trees adjacent to the packing shed and in the breezeway where fruit was stored prior to packing. Traps were checked on a three weekly basis for 12 months from detection of the larvae.

9. RESULTS

9.1 Fullers Rose Weevil

9.1.1 Renmark Field Trial

There were no FRW detected in the treated orchards in the Renmark trial (Table 1), however, in untreated trees FRW were detected by branch shaking.

Table 1: Fullers Rose weevil detections in Renmark citrus orchards. . 2011 trees either subject to TREATED (skirting, weed control and trunk banding with Karate Zeon® (250 g L^{-1} Lambda-cyhalothrin) 300ml L^{-1} (approximately 200 ml $tree^{-1}$) on 6 occasions at 6 weekly intervals) or UNTREATED (only routine weed control).

Treated/Untreated	2009[1]	2010[1]	2011 Trial[2]
Treated	3%	2%	0
Treated	7%	3%	0
Treated	2%	3%	0
Untreated	0%	5%	5
Untreated	2%	3%	4
Untreated	8%	5%	7

1: Scouting of samples of 70 fruit adjusted to % of fruit with eggs
2: Detections are adult weevils found through branch shaking 20 trees.

9.1.2 Waikerie Field Trial

In the Waikerie trial no FRW were detected in tree shaking assessments of the plots where spray bands of Karate Zeon® had been applied to tree trunks either six times or three times, whereas six FRW were collected from the control trees. The results are not statistically significant largely due to the low numbers of FRW collected.

9.1.3 Export Orchard Survey

The survey of planned export orchards highlights that selecting orchards that are FRW free is a viable option and provides export fruit at a substantially lower cost (Table 2). Once FRW free orchards have been identified maintaining that status through simple orchard hygiene such as ensuring machinery, equipment and vehicles are washed to removed any weevils and making staff aware of checking hair, clothing etc before entry should be a priority.

The detections of FRW eggs on receival at the packing shed and in export shipments were investigated and appeared to be either due to too few trunk band applications or extended periods between treatments. In all cases where eggs were detected at receival at the packing shed FRW had been detected during field assessments of the source orchards except for two detections at export destinations. In one of these export market detections the adjoining orchard had high populations of FRW and

weevils were collected in the first row of the export orchard where a weed had grown into the canopy.

Table 2: Field and receival detections of FRW adults or eggs in 30 export blocks surveyed. Adult field detection numbers are number of blocks with individual FRW detections in brackets.

No of Sprays	Blocks	Field Adult detections[1]	Field Egg detections[2]	Receival adult detections[3]	Receival egg detections[3]
0	13	0	0	1	0
1	1	0	1	0	1
2	4	1(6)	0	0	0
3	7	1(1)	0	0	1
4	2	2(23)	0	0	0
5	1	1(1)	1	0	0
6	2	0	1	0	1
7	1	0	0	0	0

1: Adult weevils detected by shaking 20 trees and counting adult weevils on $1m^2$ sheet.
2: Sampling 70 fruit and removing calyx.
3: Sampling 10 fruit per bin on receival at packing shed.

9.2 Island Fly

9.2.1 Laboratory Culture

After a number of attempts a method of rearing Island fly, as described in the materials and methods, which resulted in a steady increase in population size from generation to generation was developed. Three critical developments in the development of this successful culturing method were the development of a high protein diet, increased light intensity and the addition of selected bacteria isolated from the gut of wild Island flies to the diet.

- Island fly eggs examined prior to development of the high protein diet (Appendix 1) were infertile and males dissected for inspection appeared to be sterile. While these fertility problems were partially overcome with the introduction of the high protein diet fertility rates were still below 25% and populations continued to decrease from one generation to the next.
- Originally the windows of the culture room were blocked out and artificial lighting was used which was programmed to provide artificial twilight and moonlight. However following the relocation of the culture to a room with large windows and bright, natural sunlight the viability of the culture improved.

- Bacteria isolated from the gut of Island fly were plated and pure cultures isolated. A number of the cultures were placed on the colony cages and the results observed, the adult flies were highly attracted to a number of the bacterial isolates. The bacterial cultures that were most attractive to Island flies were added to the diet and behaviour and reproduction monitored. One bacterium in particular led to noticeably increased vigour, increased fecundity and increase in population in subsequent generations.

9.2.2 Rate of Development Study

The developmental rates of the life stages of *Island fly* are temperature dependent. The shortest mean development time observed was 26.9 days at 30^{o}C for egg to eclosion stage. The 30^{o}C temperature range gave the optimum mean development times for all stages of *Island fly* in this study. At 35^{o}C the development did not progress beyond the third instar larvae jumping stages due to the lethal nature of the upper threshold temperature, and all larvae died (Table 3).

Table 3: Summary of different immature life stages of *Island fly's* mean development time (days) at five constant temperatures for Constant Rate of Development studies.

Temperature (^{o}C)	Mean development time (days)			
	Egg oviposition to hatch	Egg to Larval Jump	Egg to Pupa	Egg to Adult
15	19.6	33.3	36.1	96.6[1]
20	12	17.5	19	61.4
25	6.4	10.4	11.4	30.9
30	5.9	10.3	11.3	26.9
35	6.6	9.5	All dead	All dead

[1] The figure stated for 15^{o}C was ongoing when this trial was completed and represents only two of 12 replications that were set up in the experiment.

The Logan Type III model was used for linear and non linear regression graphs. This model was considered an improvement from other non-linear models and had a higher accuracy in predicting results than linear models.

The egg to hatching stage had a thermal constant (K) of 153.85 degree-days and a lower development threshold (T_O) of 4.83^{o}C (
Figure 2). The optimum temperature for egg development was 33^{o}C at which hatching would take place in 4 days at a maximum rate of 0.25 times. Beyond that, the development rates decreased. The lethal high temperature was estimated to be over

35.6°C when all developments ceased. The R^2 value of 0.6837 indicated a positive linear relationship between development rate and temperature.

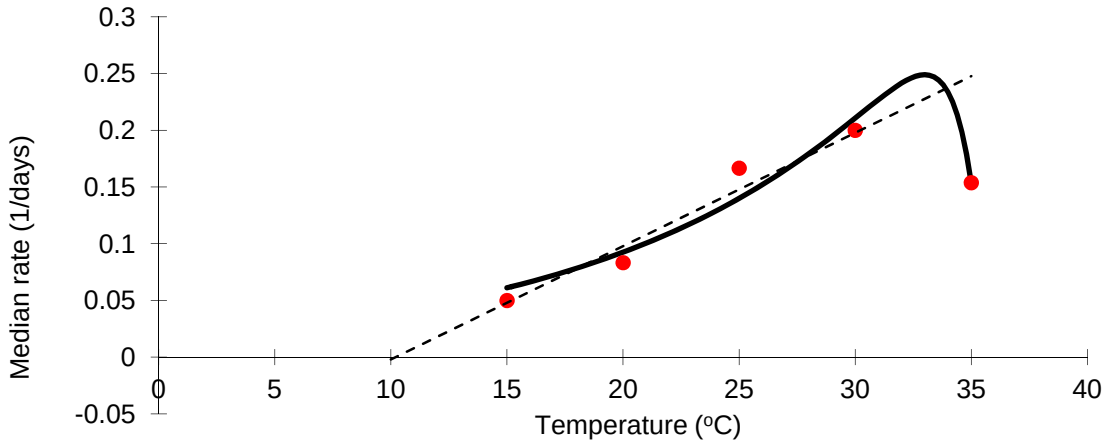

Figure 2: Influence of temperature on the egg to hatching development time of Island fly. The dotted line represents linear regression and the solid line represents non-linear regression.

The optimum temperature for egg to third instar jumping stage was 33.4°C at a maximum rate of 0.13 and a shortest median development time of 7.9 days (Figure 3). The egg to jumping K was 263.16 degree-days; the lower development threshold was 4.03°C, while the upper was 36.2°C. There was a positive linear relationship between development rates and temperature with 0.8429 as R^2 value.

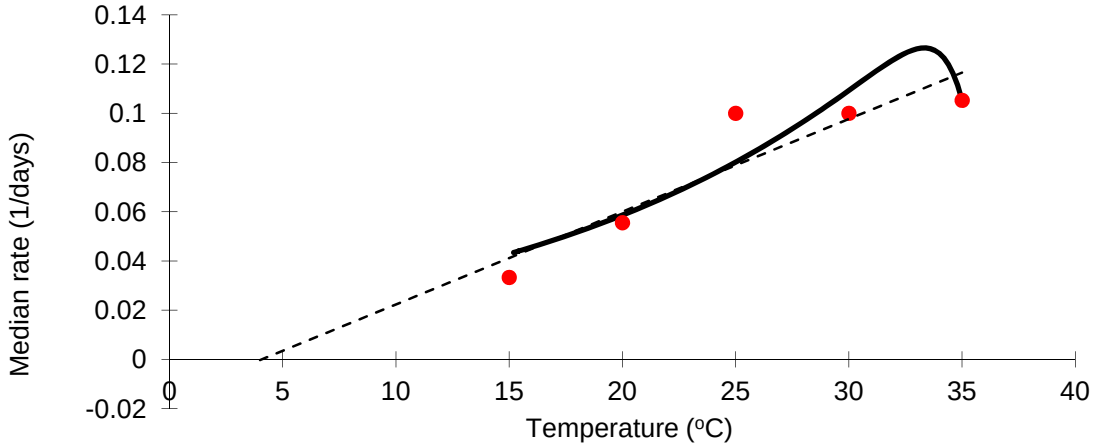

Figure 3: Influence of temperature on the egg to larval jumping development time of *Island fly*. The dotted line represents linear regression and the solid line represents non-linear regression.

The median development rate for egg to pupation stage was 0.12 times at an optimum temperature of 28.4°C and median time of 8.3 days (Figure 4). The T_O was 7.62°C and K was 222.22 degree-days. The lethal high temperature was estimated to be over 30.8°C. R^2 value of 0.8996 indicated a positive linear relationship between development rates and temperature.

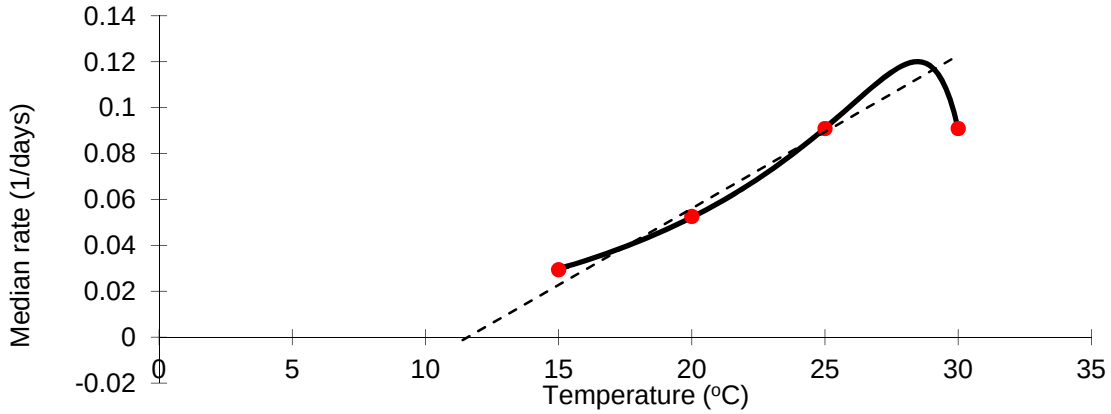

Figure 4: Influence of temperature on the egg to pupa development time of *Island fly*. The dotted line represents linear regression and the solid line represents non-linear regression.

The thermal constant for egg to eclosion stage was 500 degree-days and the lower threshold temperature was 10.4°C, while the lethal high temperature was estimated to be above 31°C (Figure 5). The quickest eclosion was noted at optimum temperature of 28.6°C at an intrinsic rate of increase of 0.05 and took 21.2 days to complete the egg to adult cycle. A positive and strong linear relationship was noted between development rates and temperature, with a R^2 value of 0.9471.

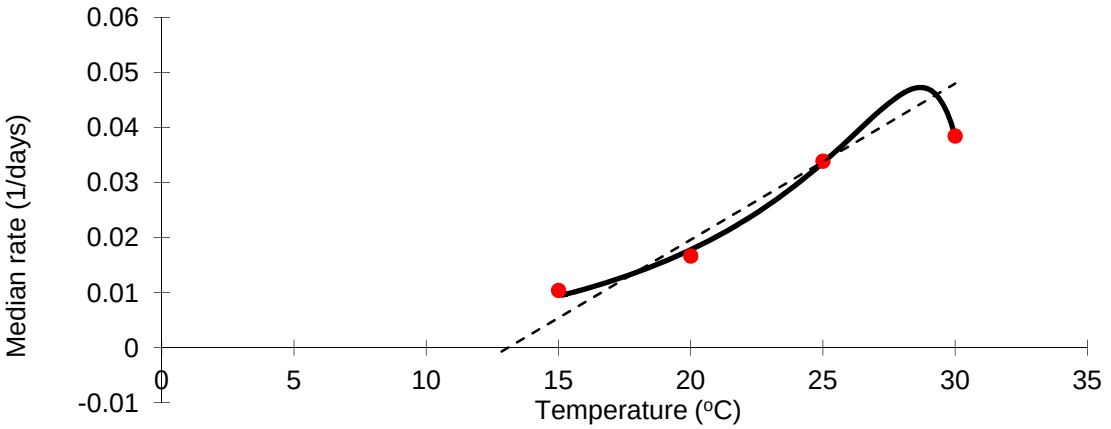

Figure 5: Influence of temperature on the egg to adult development time of *Island fly*. The dotted line represents linear regression and solid line represents non-linear regression.

Survival Rates

The 15°C temperature range replications had not completed the full experiment for life cycle to adult eclosion; and only two adult's eclosed due to the prolonged development period at low temperatures due to low metabolic activities. A more complete dataset would have enabled better analysis, but was beyond the time

limitations of this study. At 35°C no eclosions were noted because development did not proceed to pupation and the hatching rate percentage was also the lowest of all the temperature ranges (Table 4). The greatest hatching rate for eggs was 53.33% at 30°C, while best eclosion was 59.27% at 25°C. The temperature range of 25 to 30°C showed the best performance in terms of hatching and eclosion percentages, with the rates declining at lower or higher temperatures outside this range.

The calculated values for the temperature summation models are limited by the lower and upper development thresholds, below and above these temperatures development not occur (Honek & Kocourek 1990).

Table 4: Survival rates (%) of *Island fly* for hatching and eclosion stages at five constant temperatures.

Temperature (°C)	Hatching survival (%)	Eclosion survival (%)
15	42.5	3.47[1]
20	41.18	42.51
25	47.14	59.27
30	53.33	53.98
35	38.33	0

Different rates of survival were obtained for different life stages of *Island fly* at 5 constant temperature ranges.

[1] Data for 15°C was in progressive stages and incomplete when this experiment was concluded.

9.2.3 Host Status Verification (Navel Oranges)

An inspection of pin hole damaged Navel oranges in the first trial 7 days after exposure to adult Island fly were infested with Island fly larvae and more than 100 adult flies emerged from the 50 fruit (Table 5:).

In the second stage using undamaged oranges none of the fruit were infested with Island fly larvae regardless of whether they were kept separate or touching other fruit. On inspection some eggs were laid under the calyx of oranges (Figure 6) and although some had hatched, no larvae were able to penetrate the rind and successfully infest the fruit. No eggs were detected in the navel of the fruit or where the fruit were touching. No larvae were detected in either damaged or undamaged fruit in the field trial and no adult flies emerged. No eggs were detected under the calyx or in the navel.

Table 5: Host status verification assessment of Navel oranges for Island Fly. .

Location	Damaged/ Undamaged	Total number of fruit. (5 cages)	No of Adult flies (♀/♂) per cage	Eggs Detected	Larvae Present	Adults Emerged
Laboratory	Damaged	50	10/10	YES	YES	YES
Laboratory	Undamaged - individual	50	10/10	YES[1]	NO	NO
Laboratory	Undamaged -Touching	50	10/10	NO	NO	NO
Field	Damaged	25	10/10	NO	NO	NO
Field	Damaged	25	10/10	NO	NO	NO

[1] Eggs were found under the calyx of some fruit, some hatched but larvae did not successfully infest fruit.

Figure 6: Island fly eggs laid under the calyx of undamaged navel oranges in host status verification trials. Although some eggs hatched no larvae infested the fruit.

9.2.4 Fallen fruit and Island fly population density

The seasonal pattern of fluctuation in populations of Island fly in Riverland citrus orchards was similar regardless of population density. In 2011 when there was at least a 10-20 fold difference in the number of fallen fruit there was a significantly greater number of Island flies caught in traps placed in trees in orchards with a high density of fallen fruit than in low density orchards between October and January (except 7/11/2011 sample) (Figure 7). There was a strong correlation between the density of fruit on the orchard floor and numbers of Island flies caught in traps (Figure 8). In 2012 when number of fallen and discarded fruit in all orchards was considered low there was no significant difference in trap count among the eight orchards regardless of population and fruit densities in 2011 for samples taken on 6th December 2012.

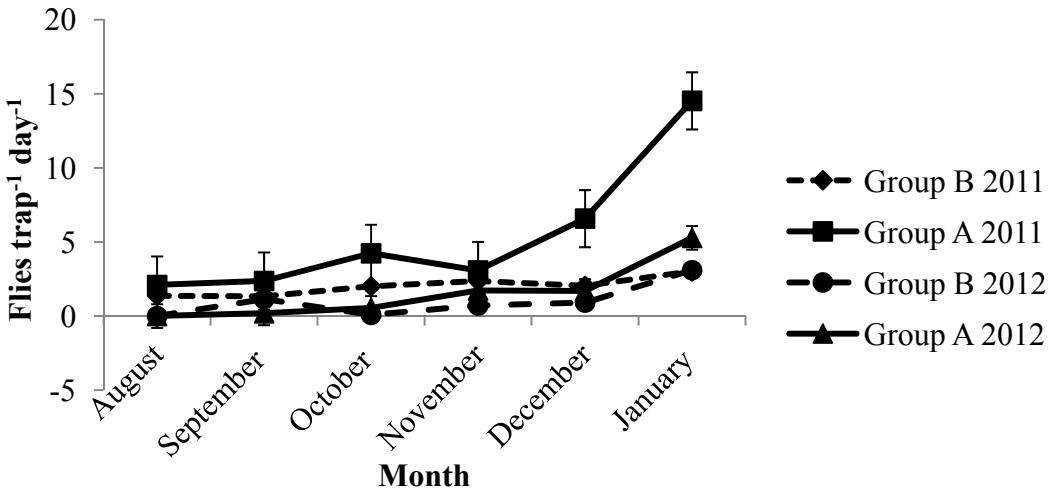

Figure 7: Number of Island fly adults caught in McPhail traps in eight citrus orchards in the Waikerie region. Group A: >50 fallen and discarded fruit per tree in 2011, <5 fallen and discarded fruit per tree in 2012 and Group B: <5 fallen and discarded fruit per tree 2011 and 2012.

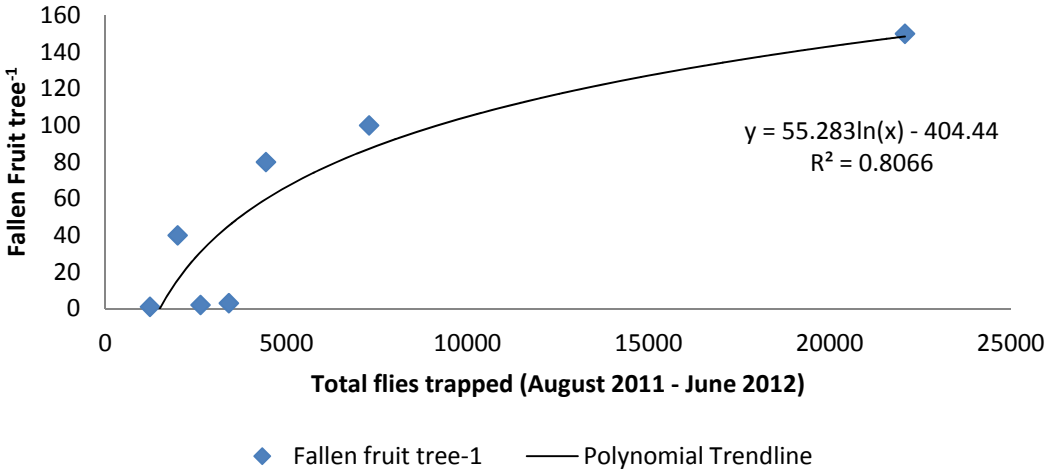

Figure 8: Relationship between number of fallen and discarded fruit on an orchard floor and the number of Island flies caught in McPhail traps from August 2011 to June 2012.

9.2.5 Packing Shed Surveillance

Island fly adults were regularly caught in the McPhail traps placed in the orchards surrounding the packing shed and those placed in the ornamental trees adjacent to the packing sheds. When fruit is delivered to the packing shed it is stored in a breezeway adjoining the packing area until processed, while this is usually same day, in some cases the fruit is left in this area overnight, as was the case in CT 07045 no flies were caught in the traps placed in this area.

10. Discussion

10.1 Fullers Rose Weevil

The results of the large scale field trials and orchard surveys conducted to assess the efficacy of the FRW Best Management recommendations indicated that while generally very effective some fine tuning was required. In most cases where there were after-harvest detections of FRW eggs on oranges, the detection could be traced back to a management failure, either insufficient trunk band applications, extended intervals between applications, occasional weeds bridging to the canopy or high FRW densities in adjoining orchards. There were however some instances where the management appeared to have met all recommendations. One major concern is the movement of FRW adults across narrow farm tracks from highly infested untreated orchards to adjacent 'export' orchards; to address this we recommend that 2 buffer rows between orchards be treated as per the best-practice management recommendations but fruit from these rows not be harvested for export.

Of some concern was the detection of an adult FRW on a picking bin on receival at the packing shed when no adults or eggs had been detected in the orchard. It is possible that this adult may have been knocked onto the picking bin whilst being moved from the orchard. However it came to be there, it presents a risk of laying eggs under the calyx of fruit in the bin. In two orchards FRW eggs were detected by calyx sampling despite no adults being detected during shaking. This suggests that the shaking sampling rate should not be reduced and may need to be increased.

Research conducted in California, USA assessing a range of trunk banding options has found that Brigade® WSB (100 ml L^{-1} bifenthrin, FMC Corporation, USA), which contains Kaolin clay, appears to have greater persistence on the trunk than other treatments trialled including lambda-cyhalothrin (Morse J. pers. comm. 2012). While difficult to make direct comparisons with the persistence of the chemicals trialled in Australia due to different application concentrations and climatic variations, these Californian data have relevance for Australia. An obvious advantage of the presence of Kaolin clay in the formulation is that it clearly marks the area the spray contacts, making it easy to assess coverage achieved on the trunk and also giving a guide to any drift into the canopy that may be occurring (Figure 9). Although Brigade® WSB is not available in Australia and bifenthrin is not registered for trunk banding in Australian citrus, Kaolin clay is inexpensive and can easily be added to spray tanks used for banding. The addition of Kaolin or similar clay products is recommended for trunk banding so that coverage can be monitored.

Brigade WSB
trunk spray 0.5 lb
ai/ acre 1 day
after treatment

Figure 9: Navel orange tree in USA sprayed with Brigade® WSB clearly showing the coverage achieved by the spray unit. Photo courtesy of J Morse (University of California, Riverside).

The results of trials and surveys conducted as part of CT11002 have demonstrated that when followed carefully, the updated Best Management Guidelines (Appendix 4) provide citrus growers who are targeting FRW export markets with a sound and reliable, but not perfect, field management tool that will minimise the number of FRW eggs produced under the calyces of fruit. In most cases this will enable growers and packing houses to export fruit with confidence that it will meet required phytosanitary standards. However, further research is required with post harvest treatments to kill and remove any egg masses that may still be present at low incidence and thus provide the industry with a full systems approach to the management of FRW. Also, further development of vital stains will enable a means of assessing the viability of any egg masses detected on fruit, which may then allow for negotiation of acceptance of fruit with minimal egg mass detections if they can be demonstrated to be non-viable.

10.2 Island Fly

The research conducted as part of CT11002 has increased the knowledge base on the biology of Island fly and the development of a reliable rearing method will allow future research to areas of the biology and control options not previously possible. The constant rate development data and day degree model will provide a basis for the development of management programs of Island fly if required in the future.

The host status verification experiments support the physiological data gathered in CT07045 and the long held understanding by Australian entomologists that Island fly, at least in the case of Navel oranges, can only infest damaged fruit, and even when given no choice cannot infest sound fruit. Even where eggs were laid under orange

calyxes and hatched the fruit remained uninfested, suggesting that the larvae are unable to penetrate the rind. The failure to infest damaged fruit on the trees in the field trial is of interest, but needs to be repeated to determine if this result was an artefact of the environmental conditions. Repeating these trials for other varieties of citrus would also strengthen the data set for negotiation with trading partners if Island fly were to become a quarantine issue.

The fallen fruit experiments show that the population densities of Island fly in an orchard are directly related to the density of fallen and discarded fruit on the orchard floor. The Group A orchards had significantly higher population densities than group B orchards in 2011 when fruit densities were high but similar populations when fallen fruit densities were similar. This suggests that the simple hygiene practice of minimising the number of fallen fruit on the orchard floor will assist in the suppression of Island fly populations in orchards; this practice is used in some countries to assist with the management of Mediterranean fruit fly. The seasonal variation in population density was similar in 2011 and 2012 (Above average rainfall) compared with that of 2007 - 2009 (Below average rainfall) reported in CT07045. The differences in population densities between under canopy sprinkler and drip irrigated orchards observed in CT07045, were not evident in 2011 or 2012, which suggests that this effect related to irrigation system is more pronounced in dry years than wetter seasons, highlighting the importance of humidity or possible free water to Island fly populations.

The packing shed surveillance program conducted in 2012 was targeted to a packing shed from which fruit with Island fly larvae were detected in markets. As was the case with the results from CT07045 from a similar surveillance program, while Island fly were well established in orchards around the packing shed no flies were trapped within the shed or storage area. This result suggests that the fruit are being infested in the field rather than in the packing process. Two experiments were established as part of this project to attempt to infest harvested fruit sitting in picking bins in the orchard for up to three days, without success. The harvested fruit experiments need to be repeated along with the field host status trials to elucidate where infested fruit is getting into the system, as at this point fallen fruit appears to be the only source.

While not reported at any length or detail in this report one of the most interesting developments was the importance of certain gut bacteria to Island fly management, both in enabling the establishment of a self-sustaining long term culture, but also because some of the bacteria were shown to be pathogenic to Island fly and may provide a management option for Island fly and a range of other Tephritid fruit flies. Additionally, some of the identified bacteria are highly attractive to Island fly and Queensland fruit fly. Further research into the volatile metabolites produced by the bacteria could lead to the development of improved lures for monitoring and lure-and-kill technologies particularly for species like Queensland fruit fly where current lures are only weakly attractive.

11. Technology Transfer

Fullers Rose weevil

The information gathered in CT07045and CT11002 has been developed into a Best Practice Management Guide (Appendix 4, page 38) which has been circulated widely throughout the citrus industry. The most recent update to the guide includes the recommendations made in this report.

Additionally, the Best Management protocols have been presented as posters at the 2011 and 2012 National Citrus Conferences.

The findings and recommendations have also been presented at a number of grower days in SA, NSW and Victoria.

The protocols and spray equipment have also been discussed and demonstrated on a one on one basis with growers and Citrus exporters.

There have also been a number of articles published on the FRW protocols in Industry and regional press.

The Best Management practice protocols were taken up by at least 50 major growers in 2012 and are being adopted by many more in 2013.

Island Fly

As the current research on Island fly is less advanced than that of FRW in relation to management practice the circulation of information has been communicated primarily within the research community.

Results of some of the early research into the bacteria have been presented at an International Atomic Energy Agency Co-operative Research project meeting that is focused on improvement of the management of Tephritid fruit flies using Sterile Insect Technology.

The research has also been shared with Australian research teams working on Citrus pests at DPI NSW and DAFF Queensland.

Some information was presented in poster form at the 2012 National Citrus Conference in Leeton.

12. Recommendations

Fullers Rose Weevil

We recommend that growers wishing to export fruit to FRW-sensitive markets adopt the protocols in the Best Practice Guide including the updates added as a result of the findings for CT11002.

1. That Kaolin be added to spray tanks when applying trunk bands to provide a means of assessing coverage of the trunk achieved by the growers spray equipment, and assessing spray drift. (Based on finding of US research the kaolin may improve the longevity of the insecticides in the trunk band treatment.)
2. That where export orchards are in close proximity to or adjoining orchards not being treated for FRW two buffer rows be established using the Best Management Guide protocols to reduce the risk of movement of adults from untreated orchards to export orchards.

Island Fly

Recommendations for Island fly are primarily based around further research itemised below. However, the research results indicate that orchard hygiene plays a large role in Island fly population density, and hence reducing fallen and discarded fruit in the orchard is likely to minimise populations and the risk of infestation. It is also recommended that the message to pickers to **not** pickup fallen fruit be reinforced as this would appear to be the most likely pathway for Island fly to be in harvested fruit.

We recommend that further research be undertaken to:

1. Evaluate other FRW trunk band insecticidal active options that may be suitable alternatives to the current APVMA approved options.
2. Evaluate other formulation options which may improve the longevity of the FRW trunk band treatments in the field, such as Kaolin.
3. Assess post-harvest options for removal of FRW egg masses from under the fruit calyx.
4. Develop control protocols for Island fly.
5. Assess the potential of new fruit fly lures derived from bacterial volatiles.
6. Assess the potential of pathogenic bacteria as a management option for fruit flies, including Island fly.

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Appendix 1: Dirioxa pornia Adult Diet.

Adult Fly Diet Food Supplement mixture for *Island fly* rearing used for Culture maintenance and in the Constant Rate of Development and Host Status experiments.

Part 1

Sl. No.	Ingredient	Quantity	Unit	Source	Sl. No.	Ingredient	Quantity	Unit	Source
1	Water	610.00	ml	Reverse Osmosis	**2**	Vinegar	2.50	ml	Goodman Fielder, Aust.
3	Agar	6.00	g	Amyl Media Pty Ltd., Aust.	**4**	Dextrose	50.00	g	Ace Chemical Co., Aust.
5	Fructose	25.00	g	Lotus Foods Pty Ltd., Aust	**6**	Torula Yeast	40.00	g	Lotus Foods Pty Ltd., Aust
7	Potato starch	20.00	g	Lotus Foods Pty Ltd., Aust	**8**	Soy flour	25.00	g	S F Health Foods Pty Ltd., Aust
9	Bran	10.00	g	Lotus Organic, Aust					

Part 2

Sl. No.	Ingredient	Quantity	Unit	Source	Sl. No.	Ingredient	Quantity	Unit	Source
1	Distilled water	31.00	ml	Distillation process	**2**	Wesson Salt Mix	0.25	g	M P Biomedicals, LLC, USA
3	VanDerZandt Vitamin Mixture	0.25	g	M P Biomedical, LLC, USA	**4**	Sorbic Acid	1.00	g	Ace Chemical Co., Aust.

Part 1 was measured (±1%) and mixed thoroughly, autoclaved at 121^{o}C for 20 minutes and cooled down, and then Part 2 was added and mixed thoroughly to complete the diet formulations, which were poured into smaller containers to settle down and set. The diet was stored at 5^{o}C.

This diet was further supplemented with enterobacteria, grown on yeast extract agar, isolated from the gut of adult *Island fly* and selected for probiotic properties.

Appendix 2: *Dirioxa pornia* larval diet.

Diet mixture for *Island fly* larvae used at SARDI Laboratories in 2012 for culture maintenance and Constant Temperature Development (Modified from Baker *et al*, 2011).

Sl. No	Ingredient	Quantity	Unit	Source
1	Orange juice	400	mL	Golden Circle, Aust.
2	Torula Yeast	10	g	Lotus Foods Pty Ltd
3	Sorbic Acid	0.2	g	Ace Chemical Co., Aust.
4	Methyl P	0.2	g	Ace Chemical Co., Aust.
5	Apple cider vinegar	10	mL	Goodman Fielder, Aus.
6	Penicillin-Streptomycin Solution	4	%	Sigma-Aldrich, USA

Appendix 3: Bacteria isolation and culture

The flies were killed in the laboratory by freezing them at -20 ˚C for five minutes. All of the following steps in this section were carried out in a laminar air flow cabinet to prevent contamination. Petri dishes were prepared with Trypticase Soy Agar (TSA) and Yeast Extract Agar (YEA) in order to ensure that most of the bacteria species that could be cultured were isolated. The dead flies were surface sterilised by immersing them in 70% ethanol for one minute and then washing them in sterile distilled water (Thaochan *et al*, 2010). Each fly was pinned from the thorax with a sterile pin onto a sterile wax under a microscope.

The cuticle on the abdomen was removed using a thin sterile scalpel to reveal the gut, which was picked up by sterile tweezers and placed on both types of agar on Petri plates ensuring that the gut contents were spread on the plates. Overall, 60 flies were dissected for this experiment including 50 gut isolates, and for comparison purposes, three ovary isolates, three crushed heads, two whole flies crushed and cultured, and two non-bacteria-fed flies from the insectory at the Waite Campus – University of Adelaide.

The plates were incubated at 35 ˚C for 24-48 hours (Murphy *et al*, 1994). Afterwards, bacterial isolates were sub-cultured by taking a part of each individual bacterial colony using a sterile loop and spreading it onto the new Petri plates (TSA and YEA) and incubating again at 35 ˚C for 24-48 hours. Each bacterial growth was isolated and sub-cultured twice to ensure purity for the next step which involved running a PCR method to amplify the bacterial cells.

The four gram negative, rod-shaped Enterobacteriaceae bacteria species (provisionally named A, B, C and D) used for various experiments were those extracted from the gut of *Island fly* in 2011 as part of CT07045 and maintained on 2.3% Yeast Extract Agar (YEA). The bacteria were sub-cultured and purified using sterile techniques and the process of isolation of individual colonies used was according to Thaochen *et. al* (2010). The bacterial isolates were then incubated at 30±2º C for 24 - 48 hours and then refrigerated at or below 4^{o}C until required. To make dense bacterial cultures for use as supplementary diets for experimental *Island fly* adults, the YEA media was streaked with the inoculated metallic loop in a dense crisscross/ spiral manner so that the resulting culture would spread throughout the surface of the YEA media.

Appendix 4: Field management of Fuller's Rose Weevil in Citrus

The Problem

Fuller's Rose Weevil (FRW; Asynonychus cervinus) is a high-priority quarantine issue for some key export markets for Australian citrus. FRW lays eggs on citrus fruit and although the pest does not cause significant damage to trees or fruit, the presence of eggs, larvae or adults in shipments can result in the rejection of those shipments by sensitive markets. Groves supplying fruit for those markets require field management

of FRW to prevent eggs being laid on fruit. Any field management approach needs to avoid or minimise disruption to established citrus IPM programs.

The Pest

- FRW pupate in the soil, then adults emerge and begin feeding on leaves of weeds and citrus.
- Peak adult emergence occurs from midsummer to autumn.
- Adult FRW are flightless -to reach citrus fruit they must crawl up the tree trunk or enter the canopy by way of tall weeds, sprinkler risers or tree foliage that is touching the ground.
- Peak egg laying occurs from late summer to autumn.
- After hatching, FRW larvae drop to the ground and burrow into the soil where they feed on tree roots.

Grove risk assessment

Younger groves typically have lower risk of FRW infestation. Before any grove can supply fruit for export to FRW sensitive markets, it must be officially inspected for compliance with market requirements regarding FRW status and management. Growers may determine their own grove's FRW status before deciding on involvement in the export programs. To do this, randomly select at least ten trees per block for inspection. At each tree:

- Look for typical FRW feeding damage on leaves in lower parts of the canopy
- Look for egg masses under the calyx of five fruit per tree
- Sharply beat some lower foliage over a white sheet or tray and check for adult FRW

All groves intending to export to FRW sensitive markets should maintain the skirting and weed management program outlined below. Groves with obvious signs of FRW infestation should implement the full program (ie. including trunk banding) to reduce FRW populations to low levels in the longer-term.

Objective of FRW management
The objective of FRW management is to prevent eggs from being laid on fruit. This has two aspects:

- Immediate: Prevent FRW from accessing fruit by preventing their entry into the tree canopy.
- Longer-term: Suppress FRW populations to reduce the overall risk of egg laying on fruit.

Current best-bet management approach

1. **Maintain good weed control to prevent weeds acting as a bridge into the canopy**
   - Even single blades of grass have been observed to allow FRW access into a tree canopy.
   - Groves should be inspected frequently enough to detect and combat weed regrowth before weeds contact the tree foliage.

2. **Skirt trees to ensure that low foliage does not touch the ground or weeds**
   - Trees should be skirted high enough to prevent foliage or fruit touching the ground at any time.
   - Skirt height must take into account the future sagging of branches as a result of fruit growth.
   - Skirts should be at least 50cm high to allow for easy trunk treatment and inspection for weeds.
   - Skirting and weed control should be maintained from December until harvest.

3. **Spray a band of insecticide onto tree trunks to repel or kill FRW that try to climb the trees**
   - Karate®, Trojan®, and Matador® are registered in lemon and orange.
   - The band should be at least 20cm wide and fully encircle the trunk.
   - Commence trunk applications in December
   - Reapply every six weeks (refer to product labels).
   - For mandarins and grapefruit, several carbaryl products are registered.
   - Create buffer rows of treated trees around export blocks
   - Mix kaolin with sprays 1) Potentially improves chemical persistence
     2) Coverage can easily be checked
     3) Any drift can be detected

Critical comments from the insecticide labels:
- Firstly ensure that the trees are skirted and that all weeds under the trees are removed. Apply 250ml spray solution to the tree trunk at about 300mm from the ground in a 100mm band. Trees must be treated in the early stages of the adult weevils emerging from the ground.
- Skirt trees to 0.5 m above ground. Apply spray to the lower trunk in December and again every 6 weeks until harvest.

4. **Maintain a good level of grove hygiene and cleanliness**
   Light prunings, tumbleweeds, polystyrene boxes etc should be kept out of the grove as they are easily blown under trees where they create bridges between the ground and foliage.

5. **Monitor the treated trees regularly to ensure that:**
   - Weed control is effective
   - Tree skirts are well clear of the ground, weeds and cover crop
   - Insecticide bands are reapplied regularly as per the label instructions

The risk of fruit being infested with FRW eggs will be much higher if any of these aspects of management are compromised, even for a short period.

6. **Sanitation and exclusion**
   - FRW adults have limited in their capacity to disperse unaided.
   - Dispersal between orchard blocks is largely reliant on human intervention.
   - Dispersal can occur either amongst soil with new plantings, or on clothing, machinery and equipment moving into established orchards from infested blocks.
   - Simple quarantine and cleaning methods can be used to help prevent FRW from entering non-infested orchards.