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Final Report

Area-wide integrated pest management using the sterile insect technique to control the Queensland fruit fly

Olivia Reynolds
NSW Dept of Primary Industries

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Hort Innovation
Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

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Summary

Area Wide - Integrated Pest Management (AW-IPM) focuses on the preventive management of pest populations throughout a delimited geographic area. Isolation, i.e. geographic, a host crop surrounded by non-host species for several kilometres, or topographical (e.g. in a valley) is key in AW-IPM programs, to prevent re-infestation of an area by the target pest from surrounding areas. The sterile insect technique (SIT) is most effective under an AW-IPM scenario. The SIT involves the mass-rearing and release of irradiated, i.e. sterile male insects, that when released in the environment, mate with a fertile female thereby reducing the wild population. Sterile males are released in such large numbers that they 'overflood' the wild male population thereby reducing the chance of a fertile female encountering a fertile male. However, the processes involved in SIT programs, including domestication, mass-rearing, handling and irradiation, impact fly quality and performance. This degradation in quality includes the gut microbial community, adult fly emergence and flight.

In Australia the SIT has been used to manage the Queensland fruit fly, *Bactrocera tryoni* Froggatt, in pest-free areas for nearly two decades using a bisex strain, i.e. sterile males and females, but has not been used effectively to suppress wild endemic populations.

In our study, we established a pilot AW-IPM program which operated in a region in south-eastern Queensland, near Stanthorpe, and involved several growers and their properties collectively known as "Traprock" which are unique in that they are geographically isolated from urban centres and from each other. With the exception of the orchards, the country is largely grazing land and eucalyptus trees and is unsuitable fruit fly habitat. The target species was the native *B. tryoni*. The AW-IPM program targeted all habitats of the pest population throughout the year, not just crops at times when they are susceptible. A phased approach to *B. tryoni* management was used and included a pre-intervention phase, population reduction phase, release phase and a maintenance phase. The AW-IPM SIT program was established across two orchards, Warroo and Traprock, i.e. AW-IPM SIT treated orchards. Sterile *B. tryoni* were acclimated under local conditions at each orchard, whether undergoing pupal or adult release. Two control orchards, Top Lawson and Pikes Creek Bottom, followed the general principles of an AW-IPM program however i) suppressed *B. tryoni* populations using a management plan that did not incorporate sterile flies, i.e. AW-IPM without SIT and ii) utilized cover sprays as required in place of sterile flies. Using control orchards that were identical to AW-IPM SIT orchards with the exception of incorporating sterile flies was not possible as this program occurred in commercial crops, and it was recognised that either sterile flies or cover sprays were required on top of the other practices to achieve adequate control.

To differentiate between a sterile and a fertile mated female *B. tryoni*, one of the paramount methods to determine the reproductive potential of a population targeted by SIT, we aimed to develop a diagnostic to differentiate between sterile and wild male mated females. To increase the effectiveness and efficiency of SIT programs, we investigated the impacts of *B. tryoni* larval gut microbiota, the mass-rearing, handling and irradiation processes, with a view to better understand and improve fly quality and performance.

Our results show significantly reduced wild adult *B. tryoni* pressure (demonstrated by significantly lower trap captures) in SIT treated orchards compared to control orchards. Although control orchards were unable to reach the same levels of wild fly suppression as sterile fly treated orchards, fruit infestation did not differ between the control and SIT-treated orchards in the final year of the program. Therefore, this project shows that following the principles of an AW-IPM approach, with or without SIT, can be used to suppress *B. tryoni* populations and minimise infestation. There were no market access issues with fruit sent either domestically (including Adelaide which required a Plant Health Assurance

Certificate which stipulated ICA-55 and that the fruit had been irradiated) or to unregulated international markets throughout the duration of the 3-year program. However, it cannot be assumed that the approaches used in this study will enable market access in regulated international markets or elsewhere. The release of a bisex *B. tryoni* strain caused no market access issues in stone fruit across the three-year program. Our studies support a recommendation for the use of the current *B. tryoni* bisex strain in AW-IPM SIT programs, subject to economic viability.

To determine if a sterile male had mated with a wild fertile female, an attempt was made to develop a mating assay using a proteomic approach coupled with the design of target specific primers and using this in a PCR assay. However, the assay lacked sensitivity and therefore further development was not pursued.

This study demonstrates that an older fly colony, irradiation and transportation negatively affect the quality of sterile flies; considerable effort needs to be taken to minimize such negative impacts. These include renewing the colony every year with wild collected flies, irradiating at the lowest dose to induce sterility while balancing this with the risk of fertile F1 offspring and fly fitness and performance, and provision of adequate cushioning and insulation to reduce vibration and temperature extremes during transport. A previous recommendation to lower the dose at which *B. tryoni* is irradiated from 70-75Gy to 60-65Gy has significantly increased flight from 78.7% to 85%, therefore increasing the performance of sterile flies. Our findings suggest the gut bacterial communities of *B. tryoni* larvae are affected by the processes of domestication and mass-rearing with higher diversity present in wild larvae. Our study shows that bacterial probiotics fed to mass-reared larvae have the potential to positively influence a range of *B. tryoni* quality traits.

Five peer-reviewed journal articles, seven magazine articles, five oral presentations across four conferences, six posters across four events, one brochure and numerous other media based outputs were produced as part of this study.

The AW-IPM SIT is the first in Australia to show suppression of *B. tryoni* in an endemic region using this approach. Findings of this study show that the AW-IPM program is a model system for managing *B. tryoni* utilising the sterile insect technique to suppress populations in endemic areas. Similarly, an AW-IPM approach that does not incorporate sterile flies is equally effective at managing infestation of fruit. A cost:benefit analyses of this program would be valuable to understand if these scenarios are economically viable. Understanding the microbiome of larval *B. tryoni* could lead to improved diets and increased fly performance in AWIPM programs that incorporate SIT. Similarly, understanding the impact of the mass-rearing and domestication process, and mitigating negative impacts will further enhance the performance of flies, and increase the effectiveness of SIT programs.

Keywords

Bactrocera tryoni; Tephritidae; biosecurity; Area Wide - Integrated Pest Management; AW-IPM; Sterile Insect Technique; SIT; diet; microbiota; *Asaia;* probiotic

Acronyms

AW-IPM	Area Wide – Integrated Pest Management
bp	Base pairs
EMAI	Elizabeth Macarthur Agricultural Institute
FFPF	Fruit Fly Production Facility
IDW	Inverse distance weighted
MS/MS	Tandem Mass Spectrometry
MRS	De Man, Rogosa and Sharpe
PBS	Phosphate-buffered saline
rRNA	Ribosomal ribonucleic acid
SIT	Sterile Insect Technique
TSA	Trypticase soy agar

Introduction

Area Wide - Integrated Pest Management (AW-IPM) involves "control measures applied against a given plant pest over a geographically defined area that includes all known or potential hosts with the objective of preventing pest build-up while minimizing damage to commercial host. Control actions are conducted whenever and wherever the target pest exists regardless of host seasonality" (Enkerlin 2007). This technique has a strong emphasis on treating all habitats of the pest population preventing migrants re-establishing significant infestations which are damaging to crops (Enkerlin 2007). In contrast, conventional control focuses narrowly on protecting the crop from direct attack by pests. AW-IPM programs allow stakeholders to pool resources to employ technologies and expertise that are too expensive for individual producers. These may include trapping networks, mass-rearing facilities, adult rearing out facilities, release strategies, information technologies and highly trained specialists. AW-IPM also enables and enhances communication between growers, and enhances the learning experience of individual growers.

The sterile insect technique (SIT) is a target specific form of birth control imposed on a pest population that may be applied in the AW-IPM of insect pests of agricultural, medical and veterinary importance. There are several AW-IPM programs that have successfully used sterile insects including screwworm, moth and fruit fly (Mumford 2005). There are at least 20 AW-IPM programs worldwide that incorporate the SIT to prevent, contain, eradicate or suppress fruit flies (Enkerlin 2005). The SIT is environmentally benign and can be a cost-effective component of an AW-IPM program for the control of fruit flies of major economic importance, such as the cosmopolitan Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the Australian native, Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Fig. 1). The SIT for *B. tryoni* in Australia has been used to eradicate incursions in fruit fly free areas (e.g. Fruit Fly Exclusion Zone and South Australia); however its use has not been reported to suppress populations in areas which are endemic to *B. tryoni*. Like most pest control techniques, the SIT is not a stand-alone technique and, in most situations, requires pre-release population suppression to be effective and economically viable. Ongoing use of other techniques, including bait sprays, together with SIT is also common. Further, the processes involved in SIT programs (mass-rearing, domestication, handling, and irradiation) impact fly quality and performance traits.

Recently, severe restrictions were placed on a key pesticide used to manage *B. tryoni*; dimethoate and products containing fenthion may no longer be used or supplied in Australia. Although permits for alpha-cypermethrin and clothianidin have been granted to manage *B. tryoni* (Reynolds et al. 2017), there are increasing restrictions being placed on insecticides due largely to environmental and public health concerns. Therefore, there is a need to find alternate sustainable, 'softer' in-field control options for *B. tryoni*.

In Australia, *B. tryoni* is the most significant insect pest of biosecurity concern affecting Australia's $9 billion-plus per annum horticultural industry, impacting domestic and international market access (Hort Innovation 2016). As a result, the supply of fresh fruit must meet the requirements of importing domestic (http://www.interstatequarantine.org.au/; accessed 13 September 2017) and international (https://micor.agriculture.gov.au/Plants/Pages/default.aspx; accessed 13 September 2017) markets. In areas where fruit flies are endemic, rigorous field control must ensue to ensure the production of high quality produce. *Bactrocera tryoni* is a polyphagous, mobile and multivoltine species, therefore making it an ideal candidate for an AWI-IPM suppression program. AW-IPM aims to suppress the population through space and time as opposed to a conventional approach where each grower protects their own crop independently; targeting the pest population only when the crop is susceptible.

Figure 1. Sterile male Queensland fruit fly (*Bactrocera tryoni*); note orange coloured dye on top of head (i.e. ptilinum).

The main objective of this study was to establish an effective pilot AW-IPM SIT program to suppress *B. tryoni* in an endemic area that will inform the development of similar future campaigns. Specifically, this was considered successful when the target *B. tryoni* population was reduced to a level below 0.01 wild female flies/trap/day and wild males to below 0.05 flies/trap/day. We also aimed to develop a diagnostic assay to determine if a sterile or fertile male had mated with a female. The effect of irradiation, transportation conditions and the age of the fly colony on performance traits of *B. tryoni* was investigated with a view to develop protocols to improve sterile fly quality. The microbial community of individual larval midguts of *B. tryoni* were characterised and potential probiotic candidates were identified. By administering these bacterial candidates in the larval diet, we aimed to establish impacts on performance traits of domesticated, sterile *B. tryoni* with a view to enhancing the quality of mass-reared flies.

Findings of this study show that the AW-IPM program is a model system for managing *B. tryoni* utilising the SIT to suppress endemic populations in orchards which can be quarantined, or contained in some way, preventing re-incursion of the pest. Additionally, findings showed that an AW-IPM approach that does not incorporate sterile flies is very effective at reducing fruit infestation. The release of a bisex *B. tryoni* strain caused no market access issues in stone fruits in the three-year program and can be utilised without concern of the impact of stings on market access. The dose for irradiated fruit flies has been lowered from 70-75Gy to 60-65Gy which we have shown positively influences flight, directly improving sterile fly performance, although did not impact emergence. It is recommended that *B. tryoni* irradiated at 60-65Gy be released in AW-IPM SIT programs for endemic areas, and should also be considered for pest free areas. It is worthwhile investigating a further reduced irradiation dose at the mass-rearing scale. The mating assay lacked sensitivity and therefore was considered unfeasible to continue pursuing, however an assay based on the annotated Y-chromosome of fertile *B. tryoni* males may be worthwhile. The increased diversity and abundance of bacteria in wild larvae suggest that we can manipulate the microbiome of domesticated larval *B. tryoni* potentially leading to improved diets and increased fly performance in programs that incorporate SIT.

Methodology

AW-IPM SIT

Study area

The Southern Downs region in Queensland has a strong horticultural industry with 4,210 hectares of production by 277 horticultural producers with a value of nearly $300 million at the wholesale level (Tancred & McGrath 2013). There are 1,446 hectares of orchard crops grown with the main being apples, followed by stone fruit, with several small areas of pears, persimmons, figs and olives (Tancred & McGrath 2013).

The commercial orchards in the region that participated in this study included Warroo (28°36’07.32”S,151°26’21.59”E; 60Ha), Traprock (28°49’10.56” S, 151°31’23.44”E; 35 Ha), Top Lawson (28°40'24.69"S 151°31'46.47"E; 15 Ha) and Pikes Creek Bottom orchards (28°39' 56.43"S, 151°34' 51.38"E; 5 Ha) comprising a total of 115Ha of stone fruit (Fig. 2). Initially, there were an additional two orchards (totalling approximately 20Ha), however, due to financial and other constraints, the decision was made that they would not participate after the first year of the program. These two orchards are geographically isolated from the four trial orchards and continued to manage *B. tryoni* utilising controls including cover sprays as required, bait sprays, MAT and Fruit Fly Attractant Gel baited-Bio Traps (Bio Trap Australia Pty Ltd, Ocean Grove, Victoria 3226 Australia). They also committed to removing and/or managing alternate hosts up to 2km from their orchards, thereby they were not negatively impacting the AW-IPM program. As the tree crops are temperate, the orchards are situated in the higher altitude areas of the Region, centred on the Granite Belt.

These orchards are geographically isolated from urban centres, and aside from the orchards the surrounding area is largely grazing land. The medium chill stone fruit across the four orchards is valued at $5-6million/annum (Rowan Berecry, John Pratt and Andrew Finlay pers. comm. 2014) and is harvested from late October through to late February depending upon the variety. Warroo only grows plums (Queen Garnet) while Traprock, Top Lawson and Pikes Creek Bottom orchards all have a mix of plums, peaches and nectarines.

Traditionally the orchards (except Warroo which was newly planted in 2012) have followed a conventional program to control fruit flies which relied principally upon insecticide (largely fenthion prior to withdrawal of its use in Australia) with sporadic use of male annihilation technique (MAT) and other controls. Sanitation involved collection of discarded fruit and feeding it to stock (Top Lawson & Bottom Orchard) or destruction of fruit through other means.

Figure 2. Orchard location map

AW-IPM SIT Program

An Area Wide - Integrated Pest Management (AW-IPM) program incorporating the Sterile Insect Technique (SIT) was established across two orchards, Warroo and Traprock. Top Lawson and Pikes Creek Bottom orchards were designated as control orchards and followed a management plan based on the principles of AW-IPM, however they used cover sprays as required, rather than sterile flies to manage *B. tryoni*. The program that all orchards followed involved several phases (after Hendrichs et al 2005): pre-intervention phase, population reduction phase, and maintenance phase, with SIT treated orchards also incorporating a release phase.

Pre-intervention phase

The pre-intervention phase involved the collection of baseline data on the distribution, dispersal and population dynamics of the target species, the development of basic human and physical infrastructure (including emergence centres), and establishment of whether sterile female fruit flies cause issues with market access. Historic trapping data, including GPS coordinates of trap sites, trap catches and fruit maturation data were collected.

i) Sterile female stings

An assessment of releasing a bisex strain of *B. tryoni* was undertaken prior to the release of sterile flies as part of the AW-IPM SIT study to determine the impact on market access of fresh stone fruit.
Sterile male and female *B. tryoni*, i.e. bisex strain (See *Insects* below), were released weekly as pupae as per Reynolds & Orchard (2015) (also see *Release protocol* below) at an average rate of 3900 pupae/Ha/week in Warroo Orchard from

August 2013 to May 2014. In February 2014, plums were harvested from Warroo Orchard and taken to Pikes Creek orchard packing shed for inspection and packaging for market as per usual farm procedure. A total of 1600 plums were randomly sampled from three consecutive days. One plum per 10kg packed carton was sampled each day.

ii) Mapping and spatial analyses

The entire production area and identified wild and non-commercial hosts were mapped to allow for the strategic release of sterile flies, targeted control and placement of monitoring traps.
The boundaries of all the orchards in the program were mapped. Core areas and buffer (edge area which is included in the treatment zone) were identified.

Static maps

Static maps were generated for each orchard reflecting the location of *B. tryoni* traps and sterile release sites, orchard infrastructure, tree variety, alternative host and water sources (Appendix A). These reference layers were displayed over a series of basemaps including; slope, aspect and elevation surfaces derived from space shuttle radar digital elevation models and high resolution ADS40 aerial photography. Heatmaps were also generated to highlight where sterile and wild males and females were concentrated each season (Appendix B). Heatmaps were calculated using an inverse distance weighted (IDW) interpolation technique (http://pro.arcgis.com/en/pro-app/tool-reference/spatial-analyst/idw.htm; accessed 17 July 2017). IDW interpolation was used to determine cell values using a linearly weighted combination of weekly trap counts where weight is a function of the inverse distance of the interpolated cell from the *B. tryoni* trap. The resultant interpolated surfaces were displayed as stretched colour range to facilitate the identification of *B. tryoni* hotspots and over wintering sites.

Spatial Data Animation

Temporal analysis using the ESRI suite was used to identify the most appropriate location and timing of *B. tryoni* management options. Field data was recorded in Smartsheets by individual orchardists in preparation for extraction and conversion to Filegeodatabase for consumption by ArcMap http://desktop.arcgis.com/en/arcmap/latest/manage-data/main/what-is-geodata.htm; accessed 17 July 2017). Activities occurring within a defined time period such as *B. tryoni* trapping, sterile release and orchard management, critical tree stage (fruit shuck and harvest), notable weather events (such as hail, cold snaps and snowfall), were visualised using the ESRI time aware functionality. Analysis of time aware data was undertaken in conjunction with static datasets including high resolution aerial photography and digital elevation models. Seasons were interactively inspected using the time-slider-bar to view the temporal progression of *B. tryoni* activity through the season and to qualitatively assess the impact of management intervention options (Click on: http://www.dpi.nsw.gov.au/biosecurity/insect-pests/qff). Further, animation of the season's management activities and events was invaluable for gaining an understanding of timing of resource use and fly movement in the area.

Establishing core area and buffer zone

A core area (commercial orchard production) of control was established. In addition, a buffer zone to manage flies around the edge to intercept any immigrating insects and deal with progeny of any gravid female that enter the area was established. The buffer zone was considered the extensive native and other vegetation (essentially grazing land) that surrounds each orchard. Several small commercial (managed) orchards were identified in the region, in addition to two residential properties near Warroo that have fruit trees and/or vegetable gardens and were sources of *B. tryoni* infestation. These residential properties were managed in the same manner as the orchards, with bait sprays, MAT and sterile fly release.

i) Quarantine measures and procedures

These were established to intercept any fruit fly that is transported passively (i.e. in fruit) as this is a potential source of *B. tryoni* outbreaks. We conducted regular grower education activities with the growers engaged in the project (including engaging in the first year the two orchards that were not part of the three year AW-IPM program) to ensure the growers understood the program objective, their role and to inform the growers about fruit fly monitoring and management. This included regular face to face meetings, practical on-farm demonstrations of management activities, talks and demonstrations with pickers and an annual planning and evaluation meeting. Through engagement with the growers we established a 'flyer' highlighting the program objectives and detailing the lifecycle of *B. tryoni.* This flier, made available to all orchard workers throughout the program, communicated what practical measures farm staff could do to assist the program.

ii) Trapping

Male and female *B. tryoni* attractant based traps were used. To ensure accuracy of analysing and interpreting temporal and spatial monitoring data, standardised sampling procedures were used across all sites.

A trapping array of 15 to 21 (2014/2015), 21 (2015/2016; 2016/2017) 1 L Lynfield traps baited with the male attractant cue-lure (International Pheromone Systems, Cheshire, UK) and malathion (Meats *et al*. 2002) spaced at 400 m intervals (in vegetation) was established at Warroo, and the adjacent buffer zone which included two residential properties. Similarly, traps were space in the same array at Traprock, 14 to 15 (2014/2015) and 13 (2015/2016; 2016/2017). The control orchards, Top Lawson had six (2014/2015; 2015/2016) and four (2016/2017) traps, and Pikes Creek Bottom Orchard six (2014/2015), five to six (2015/2016) and two (2016/2017) traps. Lures were changed every three months.

A smaller array of McPhail traps were established at each orchard and buffer zone, baited with orange ammonia (19/6/2014 to 24/9/2015), and then Bio trap Fruit Fly Attractant Gel (24/9/2015 to 31/3/2017), which are mainly attractive to immature female *B. tryoni*, but also catch mature females and males. Traps comprised, Warroo five to six (2014/2015), six (2015/2016; 2016/2017), Traprock three to four (2014/2015), four (2015/2016; 2016/2017), Top Lawson four to five (2014/2015), five (2015/2016) and four (2016/2017) and Pikes Creek Bottom Orchard two to three (2014/2015), three (2015/2016) and two (2016/2017) traps. Orange ammonia lures were made fresh and changed weekly, while gel-based lures were changed every three months.

Traps varied slightly from year to year, typically due to grower practicalities, such as ease of checking, and removal or planting of orchard trees. All traps were mounted at 1.5 – 2.0 m above the ground. Traps in the SIT treated orchards were typically cleared weekly from September – April and fortnightly from May-August, although on different days at each orchard. Traps in the control orchards were typically cleared weekly from September – December and at irregular intervals from January – August.

Historical trapping data was reviewed to determine and define spatial and temporal fluctuations in density and structure of the target population. Historical trapping data was obtained from 2007 for the control orchards (Top Lawson and Pikes Creek Bottom), from 2013 for the SIT treated orchard Traprock, and from 2012 for the SIT treated orchard Warroo. Top Lawson typically monitored one or two cue-lure baited male trap (Bugs for Bugs, Qld) from 2007 and a single protein baited Cera Trap (Barmac, Lidcombe, NSW, Australia) from 2012 (all positioned within the orchard) at least once per week during the fruiting season (i.e. October - December) through to implementation of the AW-IPM program. Pikes Creek Bottom followed the same trap monitoring routine but only had a single cue-lure baited male trap. Traprock monitored twenty cue-lure baited traps (Bugs for Bugs) from 2013 (one in orchard; 19 in surrounding native vegetation) at least once per week during the fruiting season (October – early January) through to the commencement of the AW-IPM program. Warroo monitored fifteen cue-lure baited traps (Bugs for Bugs) from 2012 (10 in orchard; four in surrounding backyard

fruit trees/native vegetation), weekly except from June – August when traps were monitored fortnightly through to the commencement of the AW-IPM program. The historical data was referred to when interpreting the field data collected during the suppression and sterile insect release phases, however a statistical comparison of historical data with the data obtained in the AW-IPM program was not feasible due to several factors including several orchards being used for various research prior (including sterile insect releases), irregularity of trap monitoring, position of traps, the limited number of traps utilized, the differing types of traps/baits utilized, and infrequency/non-recording of lure changes.

Population reduction phase

i) Orchard treatments

In the SIT treatment orchards, Warroo and Traprock, in addition to sterile flies, several control tactics including bait sprays (Bugs for Bugs, Mundubbera Queensland 4626 Australia; Fig. 3) and male annihilation technique (MAT; Bugs for Bugs; Fig. 4) were implemented. These management plans were modified year to year, as required (Fig. 5) in consultation with Olivia Reynolds (Project leader) and Dan Papacek (Bugs for Bugs). The SIT treatment orchards used bait sprays at a rate of 420g/L of protein (14L/1000L), with gum (1kg/1000L) (Bugs for Bugs) and insecticide Hymal (4.35L/1000L). Cover sprays were also available as required.

Figure 3. Bait spraying using a quad-bike.

Figure 4. A male annihilation technique (MAT; Bugs for Bugs, Mundubbera Queensland 4626 Australia) device used in the AW-IPM program.

The control orchards, Top Lawson and Pikes Creek Bottom, developed their management plan in consultation with Olivia Reynolds, Dan Papacek and crop and trap consultants. The control orchards used several control techniques including a combination of bait sprays, Fruit Fly Attractant Gel baited-Bio Traps, MATs, orchard and non-commercial host sanitation and cover sprays. Bait sprays were utilised once every fortnight from the end July until end October in a ring around the orchard in the vegetation bordering the orchard, and in several blocks of early season fruit. Bio Traps were positioned in a single concentric ring, spaced approximately 15m apart, around Top Lawson (170 traps) and Pikes Creek Bottom (100 traps) orchards and the lures replaced as per label instructions. Three concentric rings of MATs were placed in the native vegetation surrounding each orchard, with the rings spaced 200m, 400m and 600m from the orchard boundary and replaced every 4 months. MATs were spaced approximately 200m apart and replaced every 3-4 months. Cover sprays of alpha-cypermethrin and/or clothianidin were used as required.

These programs were modified as required, at least every year, based upon the continual evaluation and assessment of the effectiveness of *B. tryoni* suppression and infestation levels. This also applies where relevant to any other unmanaged properties/residences harbouring host plants within the buffer of the targeted AW-IPM campaign. It should be noted that no two orchards or farms in an AW-IPM program (whether receiving sterile flies or not) are likely to manage their orchard and fruit fly pest in exactly the same way due to a range of factors including the pest complex, fruiting periods of susceptible host crops, among other factors. For example, in orchard bait spray frequency is higher earlier in Traprock orchard because their fruit varieties mature earlier than Warroo each year.

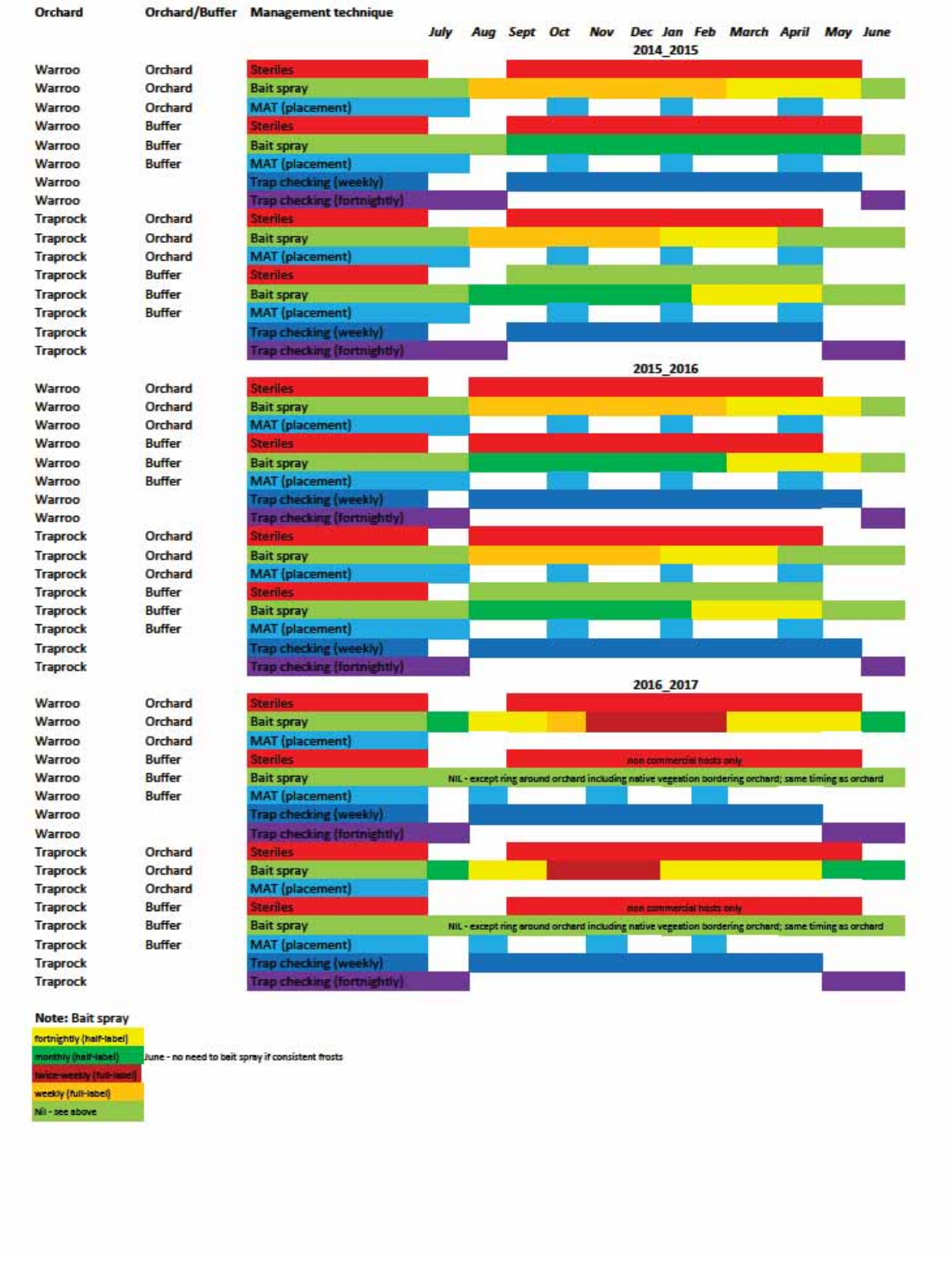

Figure 5. Management Plan for AW-IPM SIT treated orchards across the three year program.

ii) Fruit monitoring (in orchard)

Fruit monitoring also occurred in SIT treatment orchards on a routine basis to determine levels of infestation (if any) and effectiveness of the sterile insects. Individual fruit were inspected for 'stings' and eggs and larvae (see *Packing shed sampling* for method followed if fruit fly were detected). A trained independent consultant was employed during the first and second season but towards the end of the second and the third season on-farm workers were trained by Terry Osborne (NSWDPI). Fruit on the tree were inspected for fruit fly stings. For the SIT treated orchards, blocks were sampled weekly once the fruit started to colour, taking into account identified hotspots and known susceptibility of fruit until harvest. Typically, Traprock orchard followed a program of inspecting 20 random trees per block, and five fruit selected randomly per tree, while for Warroo which had a majority one plum variety, five trees were randomly selected per block and five random fruit per tree. Fruit monitoring in control orchards, Top Lawson and Pikes Creek Bottom was done on a weekly basis by an agronomist. Nectarines were monitored from bud burst through until harvest, while peaches, plums and apricots were monitored from small fruit size through until harvest. Typically, up to 10 random trees per block and 10 random fruit per tree were selected for fruit inspection. If suspected infestation was found, these were cut under a good light source to check if any larvae or eggs were present. Detected numbers were so low, i.e. dominated by zero detections that the data was not able to be analysed, however the raw results for the AW-IPM SIT orchards are reported for each season (see after figure 14).

iii) Orchard and packing sanitation

Growers followed their own standard orchard and packing quality assurance/hygiene practices as these are an important part of *B. tryoni* management. There was little late hanging fruit left in the orchards after harvest. Where some may have been missed, this was managed through careful monitoring (control and SIT treatment orchards), summer pruning immediately after picking (SIT treatment orchards) and/or dimethoate sprays (control orchards only; one block sprayed in 2015). During picking, only fruit that was to be packed was placed in packing bins (Fig. 6). During picking, only fruit that was to be packed, or was for juicing (e.g. fruit damaged by sunburn, hail damage, thrips or Helicoverpa spp.) was placed in packing bins (Fig. 6). Fruit suspected to have fruit fly or brown rot damage was collected and appropriately disposed (typically within plastic bags and placed in the sun for 3-5 days). Any fruit suspected to have fruit fly damage was first taken to a supervisor/grower (trained on fruit fly sting/damage by Terry Osborne) for confirmation. This was very rare, and this data is not reported. Fruit that had bird or flying fox damage was thrown onto the ground in the middle of the row and exposed to intense heat (Warroo harvest during February when temperatures are high), crushed underfoot, or macerated using a variety of techniques including a modified harvester. Waste fruit from packing sheds was removed regularly and disposed well away from packing facilities by feeding to stock, or deep burial. Sampling fruit for *B. tryoni* was done separately as detailed above under Fruit monitoring.

Figure 6. Harvest at Traprock orchard. Picked damaged fruit is thrown into the centre of the orchard rows, and macerated using for example, a modified harvester.

iv) Non-commercial and wild host treatments

Surveys of non-commercial and wild hosts were conducted. Residential properties which harboured non-commercial hosts were managed as per treatment orchards. An action plan was developed for any wild plant found to be a host of *B. tryoni*. Wild and unwanted hosts were instructed to be managed (typically sprayed, removed or destroyed) from around sheds, houses and up to 2km from the orchard boundaries.

Release phase

This involved the sequential release of sterile insects over the target area to reduce the target population to a level below 0.01 wild female flies/trap/day and below 0.05 wild male flies/trap/day. The aim was to preclude migration of mated fertile females into the core area. There is some overlap of this phase with the reduction phase.
Initially, the area to be targeted with sterile *B. tryoni* was determined. Trapping and release sites were mapped for each orchard and year (Appendix A). These were modified each year depending upon a range of practical or logistical factors, including the annual area under orchard, accessibility, farming practices and trap captures.

v) Sterile insect releases

Insects
Bactrocera tryoni were obtained as dyed irradiated pupae from the Fruit Fly Production Facility at the Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia, under standard rearing conditions of 26 ± 1°C, 65 ± 10% RH and a light : dark period of 14:10, with a simulated dawn and dusk as the lights ramped up and down at the beginning and end of the light phase. Larvae were reared on a standard lucerne chaff diet (Dominiak et al. 2008), and pupae were dyed (0.8 g dye

per 100 g pupae) with one of each of several fluorescent pigments; Fiesta FEX 1 Arc chrome or Astral Pink (Swada, 30-32 Kilkenny Court, Dandenong South, Victoria, Australia) as described by Reynolds, Dominiak, and Orchard (2010). Weekly consignments of dyed *B. tryoni* were sent as late-stage pupae under hypoxic conditions in plastic bags in 1L cardboard boxes to the Australian Nuclear Science and Technology Organisation (ANSTO) facility, Lucas Heights, where they were irradiated under hypoxia at 60-65 Gy of gamma irradiation from a cobalt-60 source at a dose rate of approximately 6 Gy min^{-1}.

Timing and transport

Pupae were packaged in cardboard boxes on a Monday, irradiated at the Australian Nuclear Science and Technology Organisation (ANSTO), in Lucas Heights, NSW on a Tuesday and transported in polystyrene boxes by air and road the same day, to arrive at Traprock on a Thursday, and Warroo on a Friday. Upon receipt, the plastic bags containing the pupae were opened and distributed between the release containers. Temperature during transit ranged from 17.7°C – 25.8°C and relative humidity ranged from 47% – 83%.

Release protocol

This program released a bisex strain of *B. tryoni*, i.e. sterile males and females (the females do not contribute to the program). As this program was protecting commercial orchards, we were very conservative with the number of sterile males released. As we did not have a central rearing out facility to rear out the flies and assess quality control variables (pupal weight, emergence and flight post-transport), we allowed the flies to acclimate at each orchard site. Using the number of pupae sent to the orchards each week (based on the mean weight of pupae for each year) and the mean number of fliers pre-transport, we calculated the mean released sterile male *B. tryoni*. On average, the effective sterile male fliers for 2014/2015 were 3722 males per Ha/week, 5,917 males per Ha/week (2015/2016; this year more pupae were sent as the FFPF had excess) and 3279 males per Ha/week (2016/2017). Any impact of environmental conditions at the orchard sites, on emergence and release were not factored into these values. These means include the increased targeted releases of sterile *B. tryoni* that occurred in hot spots as required and indicated by trapping.

The *B. tryoni* bisex strain were either released as pupae or adults following the methods detailed in Reynolds, Dominiak, and Orchard (2010) and Reynolds and Orchard (2015) respectively. In years 1 and 2, the flies were released as pupae, with the exception of Warroo which trialled the release of adults in year 2 (approximately 10% of the total flies released). In year 3, both orchards performed adult release. Briefly, for adult releases, the pupae were placed in well ventilated boxes (up to 25,000 pupae per box), acclimated under local conditions and reared to adult under a roofed farm shed (Reynolds et al 2015), and released when the majority were typically aged 2-3 days (Fig. 7). For pupal release, the pupae were placed in the field in suitable rearing-out containers (up to 80,000 pupae/box), acclimated under local conditions and allowed to emerge over time (Fig. 8). A full diet (yeast hydrolysate, sugar & water) to promote maturity and longevity was provided at the point of eclosion for both pupal release and adult release as recommended by Reynolds et al. (2014).

Figure 7. Adult release boxes, containing sterile Queensland fruit fly pupae. Emerged adults are fed and watered, and when 2-3 days, these boxes are taken to the release sites, the lids opened and the flies allowed to leave the boxes.

Figure 8. Pupal release station. Sterile Queensland fruit fly pupae are placed in the polystyrene boxes and permitted to leave the boxes (see openings in boxes) as they emerge. Water (blue bottle) and food (placed on top of the boxes) are provided for the emerged flies.

Release sites were selected so that they were not closer than 150m from a trap to minimise released sterile flies directly entering traps. Release sites were selected based on the current practice of 400m gridded spacing's and trapping data. Different dye coloured flies were allocated to each property, with the same colour for each property used for the duration of the trial to aid identification of release/recapture sites. Flies were released weekly in Warroo and Traprock orchards, Stanthorpe, Queensland, from August-April/May 2014 – March 2017, with the exception of 2016/17, when releases commenced in September. Winter and/or very low numbers of flies trapped permitted the cessation of releases from May/June – July/August; a cost saving. Across the three year trial, depending on each consignment, individual pupal weight ranged from 8.90–11.20 mg (mean 9.87mg, SE ± 0.05mg), which is within the acceptable range for Fruit Fly Production Facility *B. tryoni* pupae. The control orchards Top Lawson and Pikes Creek Bottom did not receive sterile flies.

Climate data (temperature, relative humidity (RH) and rainfall) was recorded at each site using a Davis Vantage Pro 2 weather station. From 29/3/2016 the data was taken from Stanthorpe BOM, station No. 41095, with the exception of Warroo rainfall which was recorded from BOM station 4137, while Warroo temperature and RH was taken from BOM station 41100.

Maintenance phase

This involved the management of the *B. tryoni* population through the ongoing implementation of control and release activities after year 2. Monitoring the success of the program broadly followed the model and recommendations of Vreyson (2005). This largely involved monitoring in various forms including sampling the male portion of the population (which provides crucial feedback on sterile to wild male ratios and on the dispersal characteristics of released sterile males) and in orchard sampling. Trapped females (as they are the target of the SIT program and provide crucial information on the rate of sterility induced in the population) were also sampled for the development of the mating diagnostic (see below). Sampling included:

vi) Trap monitoring

Traps were checked as detailed under trapping above. Captured flies were collected into vials, labelled with the collection date, property & trap number and sent to the Orange Agricultural Institute (OAI), Orange, NSW, Australia or EMAI, where the presence or absence of dye observed in the ptilinal fissure (Norris 1957; Steiner, 1965) was recorded for each fruit fly.

vii) Fruit monitoring

See above.

Review

After each season, modification to the control program occurred based on evaluation and feedback, and included assessment of trap records and fruit sampling surveys. An external national AW-IPM expert, Mr Dan Papacek (Bugs for Bugs) was engaged throughout the project and provided an evaluation of the programme and constructive advice and guidance on management.

Packing shed sampling

In the third season, approximately 600 - 2000 individual whole fruit per day, were randomly selected and inspected for stings (Fig. 9) caused by fruit fly across both SIT treatment orchards and the control orchard, Top Lawson. The fruit was

chosen from field bins that were tipped onto the sorting machine, before the first packing line inspection point. Each randomly selected fruit was inspected for fruit fly stings, or damage. Starting at the stem of the fruit, the top of the fruit was checked, rotating it slowly until 100% of the skin area was checked for stings. This was done under well-lit conditions. Any suspect fruit was examined with a 10x headband magnifier. If white gloss was apparent on the fruit, the fruit was wiped with paper towel prior to inspection. The number of stings on each fruit was recorded. Any stings found in individual fruit that were suspected of harbouring eggs and/or larvae were dissected and washed into a water bath (with a black filter paper base). If any eggs or larvae were found, their number was recorded. Eggs were maintained on moistened black paper in a petri dish labelled with the fruit number, for 48h after which they were inspected to determine egg hatch and the number of hatched eggs recorded. If any larvae were found in the fruit, the fruit was set up over moistened vermiculite for the larvae to develop into adults for identification.

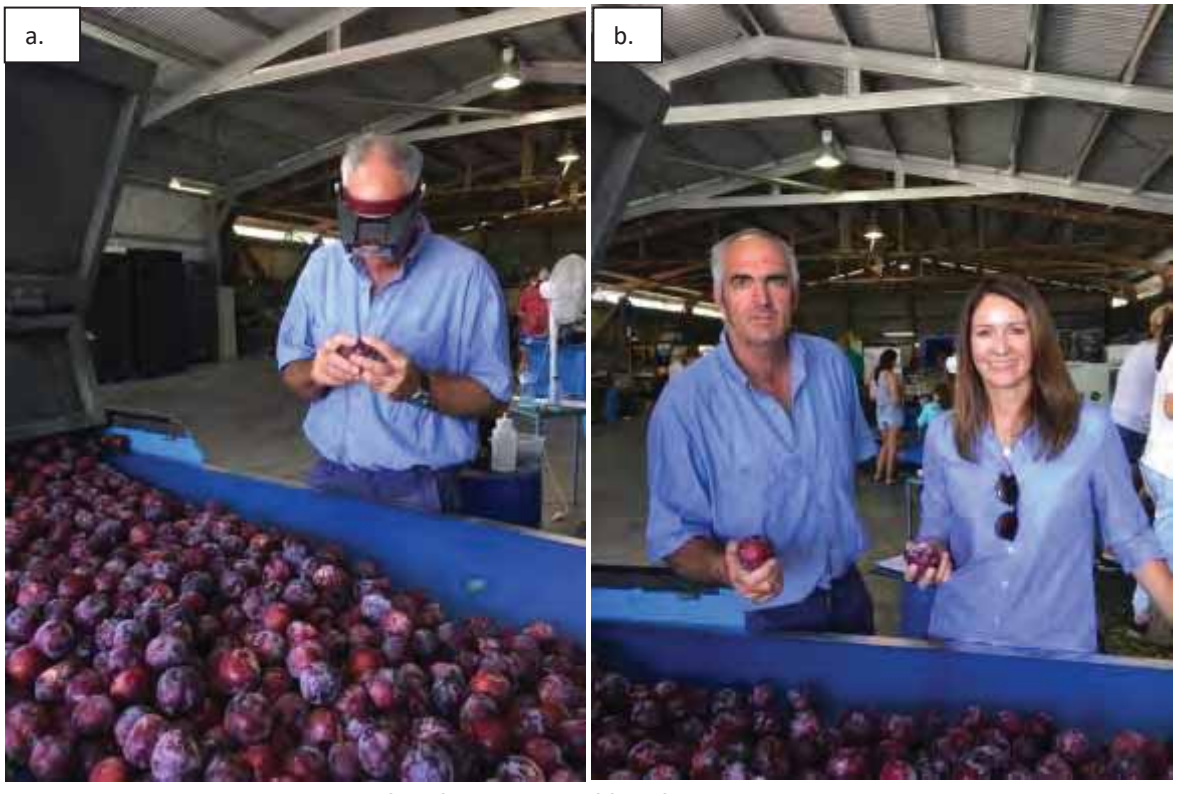

Figure 9. Terry Osborne inspecting fruit for evidence of fruit fly stings or larval damage in the Pikes Creek packing shed (a); Terry Osborne and Olivia Reynolds in the Pikes Creek packing shed, inspecting plums from Warroo orchard (b).

Mating Diagnostic

A proteome analysis of sperm and seminal fluid was conducted on sterile and fertile files. To isolate the sperm and seminal fluid male flies were dissected. A small amount of wax was placed on a glass mounting block and a fly positioned on its back and allowed to set. Three drops of sterile PBS were placed onto the fly, and using fine forceps, the abdomen was gently removed revealing the internal organs. The testicles were then collected and placed on a slide with a drop of sterile PBS and covered with a cover slip. Each slide contained ten testicles from individual flies. Using the handle end of the forceps, the cover slide was tapped several times to disrupt the testicle so that the sperm could escape. The cover slip was then removed and using a pipette, the PBS/sperm mixture was washed into a 1.5 mL tube, leaving the outer casing of the testicle behind. To obtain an understanding of the proteome, a tryptic digest of each proteome was subjected to nanoflow liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS or shotgun proteomics) with each

sample analysed in triplicate to determine if there were significant changes in protein abundance between the fertile and sterile samples. A list of 83 proteins was found exclusively in tested sterile files; of these eleven proteins had valid tandem mass spectrometry (MS/MS) spectra. From the eleven proteins, five of the more prevalent profiles (regions) were selected for primer design. Across the five regions 34 primers were designed; these primers were tested in different combinations of pairs against the DNA extracted from the sperm and seminal fluid from sterile and fertile male *B. tryoni*. The sperm and seminal fluid were centrifuged at 2000 rpm 5 minutes. The supernatant was removed and 100 µl water was added before centrifuging again at 2000 rpm for 5 minutes. The supernatant was then removed and 50 µl water added. The DMA was then extracted using the Qiagen Dneasy Blood and Tissue Kit (cat #69504), using the "Purification of total DNA from animal sperm" methodology provided. These assays were run under different optimization conditions.

Domestication, Irradiation & Transportation

In this study, we simultaneously assessed the effect of irradiation, transportation and age of fly colony on quality of mass-reared *B. tryoni*. Flies were reared at the Fruit Fly Production Facility, Menangle, NSW, during the 2013/2014 production season (mid August 2013 – mid April 2014) and the key quality parameters were measured before and after pupae transportation following the international IAEA standards (FAO/IAEA/USDA 2014).

Fruit fly larval diet was made using 19kg lucerne chaff (as bulking agent), 5.6kg torula yeast, 11.3 kg white sugar, 307g Sodium benzoate, 250g methyl paraben and 588g citric acid. All the diet ingredients were mixed together with 100L warm water for 30 minutes in a power-operated stainless steel mixer and 4.5kg of the diet mix was placed on each diet tray (total of 28 trays) and each tray was then placed in one larval growth tower with a capacity of holding a total of 28 trays. After the diet had cooled down to the standard growth room temperature (26±1^{o}C), 3.5ml of *B. tryoni* eggs collected from large adult fly cages at the FFPF were placed on each diet tray. Eggs and larvae were left to develop in the tower in a room set at 26±1^{o}C and 80±5% RH. Four samples of 100g pupae (~ 10,000 pupae) from the first larval hopping day (i.e. first day larvae 'hopped' from the tray to pupate) recovered from this tower were then used to undertake the various quality control (QC) tests. The four samples were grouped into, i) fertile pupae that would not undergo transportation, ii) fertile pupae that would undergo transportation, iii) sterile (irradiated) pupae that would not undergo transportation, iv) sterile (irradiated) pupae that would undergo transportation. This process was repeated each week for 28 weeks.

Each week, all sterile pupae were enclosed in tightly sealed plastic bags to achieve anoxic (i.e. low level of oxygen) conditions and transported in an air-conditioned vehicle to ANSTO to be irradiated as described above, except that pupae were irradiated at 70-75 Gy. After irradiation, the sterile pupae together with the fertile pupae (both still under hypoxic conditions) to undergo transportation were placed in a tightly fitted one litre cardboard box and expressed posted using Australia Post to Orange Agricultural Institute (OAI), which is 266km from the FFPF by road, usually arriving the following day (after 24h). A data logger recording temperature was included during transport. The fertile and sterile pupae that did not undergo transportation were kept at the FFPF.

As soon as the test pupae were received by each laboratory (FFPF and OAI), various quality control tests were undertaken on each group of pupae. The key quality control tests undertaken included pupal weight, adult emergence (%), flight ability of adults (% fliers) and rate of fliers (calculated as (% fliers / % emergence)*100). All quality control measurement procedures and the test room conditions followed that of the FAO/IAEA/USDA international standards (FAO/IAEA/ USDA, 2014).

Reduced Irradiation Dose

Our study compared the quality control parameters of emergence, flight and sterility of flies irradiated at either 70-75 Gy or 60-65Gy. Late-stage FFPF pupae were irradiated at ANSTO as described above. Pupae from the 21 November 2012 – 15 May 2014 were irradiated at a dose of 70-75Gy, while pupae from 9 September 2014 – 23 August 2016 were irradiated at the current recommended dose of 60-65 Gy. Quality control data of sterile (i.e. irradiated) and fertile (i.e. unirradiated) adult *B. tryoni* was obtained from the FFPF during these periods, held under standard rearing conditions of 26±2°C and 60% ± 10% relative humidity (RH) and emergence and flight ability analysed following the detailed international standards of FAO/IAEA/USDA (2014).

Microbial Symbionts

i) Larval Microbiome Characterization

In this study, we used a novel near full-length (>1300 bp) 16S rRNA gene amplicon next-generation sequencing (NGS) on the Illumina platform to characterize midgut bacterial communities of individual larval *B. tryoni.* Larvae were collected from two NSW (Buxton and Tumut) field populations developing in peaches and three domesticated populations (Gosford Primary Industries Institute, Ourimbah; Fruit Fly Production Facility, Menangle; and Macquarie University, North Ryde) reared on a standard carrot diet. Confirmation that the wild larvae were *B. tryoni* was attained by sequencing of the COI gene and rearing out larvae from infested fruit collected from the same tree. A total of 58 larvae (35 wild and 23 domesticated) were surface sterilised and dissected as described in Deutscher et al., 2017. The midgut was collected and DNA was extracted. 16S rRNA gene libraries were constructed and sequenced on an Illumina MiSeq following the method by Burke and Darling (2016).

To isolate microbiota candidates, individual *B. tryoni* midguts were ground and spread onto a range of agar plates. Dominant isolates were Gram stained and examined under the microscope. Yeast-like isolates were identified by sequencing of the ITS1, 5.8S rRNA gene, and ITS2 regions (Appendix C), while bacterial isolates were identified through 16S rRNA gene sequencing.

ii) Larval Probiotics

Individual and a blend of live bacteria from the genera Asaia, Enterobacter, Lactobacillus, and Leuconostoc were isolated from wild larvae and provided as probiotic supplements to mass-reared larvae. To isolate the bacteria from larval *B. tryoni*, midguts were dissected and then suspended in 200uL of sterile 1 x phosphate-buffered saline (PBS), homogenised by hand with a sterile plastic mortar, then 50 uL of the homogenate was plated on to 2 x trypticase soy agar (TSA) and 2 x De Man, Rogosa and Sharpe (MRS) agar plates. Plates were incubated in the dark at 25 °C for 3 days. Individual colonies were sub-cultured, and identified to genus level using morphology (gram stain, colony morphology, cell morphology), and molecular methods (16S rRNA locus, phylogenetics). Bacteria were administered in a standard larval carrot diet at a rate of 1 x 10^8 cells per 20 grams of diet for individual treatments, and 2.5 x 10^7 cells of each bacteria (*Asaia, Enterobacter, Lactobacillus, Leuconostoc*) in the blend treatment. The control had 1 x PBS added to the diet at a rate of 1 mL per 20 grams of diet. Eggs were placed on top of the diet and eclosed larvae permitted to feed until pupation (Fig. 10). Pupae were sifted from the diet and placed under moistened vermiculite (water 1: vermiculite 3) until adult emergence. Fitness and performance traits were tested including larval development time, pupal weight and adult activity. Twelve replicates were selected for larval development time and pupal weight. One hundred and fifty pupae per replicate were counted in the larval development assay (1800 per treatment), and 10 pupae per replicate were individually weighed 7 days after pupation for pupal weight (120 per treatment). For the adult activity assay a locomotor activity monitor was used (Trikinetics, USA). Four replicates were selected per bacterial treatment, each replicate representing a single fly placed in a single tube. The experiment was run for 3 days. Measurements were recorded every 5 mins over each 24 hour period.

Figure 10. Larval Queensland fruit fly feeding on a carrot based diet supplemented with bacteria.

Data Analyses

AWIPM SIT

Trapping

To determine male and female wild fly pressure, and because there were a large number of zero trap catches, data were fitted with GLM, where the errors were assumed to follow a Binomial distribution. A logarithmic link function was used and all parameters were estimated using a REML estimation. The parameter estimates of the linear predictor were compared at a 5% probability level of student *t*-distribution. We were not able to utilise 'true' control orchards, i.e. AW-IPM orchards that were identical to AW-IPM SIT orchards with the exception of incorporating sterile flies, as this program occurred across commercial orchards and it was recognised that either sterile flies or cover sprays are required in addition to other management practices to achieve adequate *B. tryoni* control.

Packing shed fruit sampling

Because there are so many zeros in the data the statistical analysis was run on replicate averages of the number of *B. tryoni* stings and larvae. A conventional analysis of variance and standard error of difference were calculated for site comparisons as these data met the assumption of homogeneity of variance.

Fruits with fruit fly stings were analysed separately using a generalized linear model with errors assumed to have a Bernoulli distribution. A 95% confidence interval was calculated on the linear model for site comparisons. The estimates were then compared using least significant difference (LSD) at the 5% level.

Domestication, Irradiation & Transportation

Statistical analyses were done using IBM SPSS Statistic 24 for Windows. Apart from transportation, irradiation (sterilization) of *B. tryoni* pupae does significantly affect fly quality. Since each test pupae groups involve irradiated and non-irradiated pupae/ flies, the data was first subjected to univariate analysis (a general linear model, GML) to determine the main effect of each of these factors on the various fly quality parameters. This analysis further helped determine if there was any interaction between these factors, as this then helped on how the data was used for subsequent comparative analysis (i.e., if there was significant interaction between the factors, the transportation data was not separated from the sterility data of one pupal group). For each univariate analysis transportation and pupae sterility were the independent factors while the tested QC parameter was the dependent variable.

Data for comparing the effect of transportation and sterilization were first subjected to parametric tests. Prior to conducting any parametric test all data were checked for normality and homogeneity of variance was tested using Levene's test. Data comparing three or more pupal groups were subject to one-way analysis of variance. When a significant difference (α set at 0.05) was detected by one-way analysis of variance (ANOVA), Tukey's HSD test or Games-Howell test (when variance was heterogeneous) was used for *post hoc*, pair-wise comparisons of the means. An independent sample *t*-test was used when comparing mean of two pupal groups.

A linear regression analysis was used to determine the impact of temperature during transport on pupal weight, emergence and flight.

Reduced Irradiation Dose

Data (emergence and flight ability) were analysed separately using a generalized linear mixed model where fly condition (Fertile, Sterile), irradiation dose and their interaction were assumed to have fixed effects whereas batches and replicates within batches were assumed to have random effects. A logit link function was used to relate the response variable to the causal factors. A least significant difference (LSD) test at the 5% significance level was used to compare treatment means. As the application of different doses was not concurrent, i.e. across different time periods, any significant effects on emergence and flight, could also be caused by other factors.

Microbial Symbionts

i) Larval Microbiome Characterization

Sequences (scaffolds) >1300 bp were analysed using QIIME. OTUs were picked using open-reference picking method at 99% similarity against the Greengenes 16S rRNA reference database. OTU taxonomic assignment was also checked against RDP 16S rRNA, NCBI RefSeq_genomic and NCBI nr/nt databases. The Mann-Whitney U test was used to compare the median number of OTUs between domesticated and wild populations and alpha diversity was assessed using QIIME. Sequence variation (proxy for species or strain variation) within the dominant OTU was assessed by clustering sequences from this OTU at 100% similarity, as described above.

ii) Larval Probiotics

A Quasi-Poisson analysis was applied to the larval development time data, and ANOVA was applied to the pupal weight data and locomotor data (Ultra violet (UV) beam crosses); for the larval development time and pupal weight analyses df= 55, and for the locomotor analysis df = 15. Data sets were analysed with the R 3.3.3 statistical package.

Outputs

1. Empirical data demonstrates that an AW-IPM program incorporating the SIT is effective for the suppression of endemic *B. tryoni* in stone fruit under similar conditions and pest pressure of this study.
2. Research has demonstrated that the release of a bisex strain of sterile *B. tryoni* does not cause problems for the sale of fresh stone fruit to either domestic, or unregulated international markets. However, it cannot be assumed that the approaches used in this study will enable market access in regulated international markets or elsewhere.
3. Recommendations on the establishment of an AW-IPM SIT programs in Australia and implications for its wider use (See Recommendations below).
4. Area Wide Management Plans were produced for each season.
5. Extensive spatial maps were produced to illustrate i) trap deployment, release sites (Appendix A) and ii) hotspots (heatmaps) (Appendix B) to aid in targeting control methods and observe trends in the data.
6. Mp4 clips were produced to inform management of the program (http://www.dpi.nsw.gov.au/biosecurity/insect-pests/qff).
7. Selection of potential probiotic candidates for *B. tryoni* larval mass-rearing.
8. A list of 83 proteins were found exclusively in sampled sterile files; of these eleven proteins had valid Tandem Mass Spectrometry (MSMS) spectra. Despite sampling, there were no proteins identified exclusively in fertile males
9. Two primers pairs; one pair that amplifies only sterile sperm/seminal samples (CG10527-500F/CG10527-1200R) and a second set (GF20908-1500F/GF20908-2300R) that only amplifies fertile sperm/seminal samples.
10. Magazine and other articles (Appendix D)
    a. Reynolds OL & Osborne T. 2017. Pilot program in Stanthorpe suppresses fruit fly. Australian Tree Crop. December 2016 /January 2017
    b. Reynolds OL & Osborne, T. 2015. Area Wide Management Program: effective at suppressing Queensland fruit fly populations after first year. Australian Stonefruit Grower. Issue No. 4/15, November 2015.
    c. Deutscher AT. 'NSW Department of Primary Industries (NSW DPI) and University of Western Sydney (UWS) Researchers Investigating Differences in Gut Bacteria between Wild and Artificial Diet Fed Larval and Adult Queensland Fruit Flies [*Bactrocera tryoni* (Froggatt)],' TAAO Newsletter, April 2015.
    d. Deutscher AT. Graham Centre's Innovator Newsletter Summer 2015–16 Edition (see pp. 10-11).
    e. Berecry R. 2015. Bush Telegraph. Warwick, Qld, Australia.
    f. Reynolds O. 2014. Sterile Insect Technique as part of an Area Wide-Integrated Pest Management campaign for Queensland fruit fly control. Australian Stonefruit Grower. Issue No 1/14, February 2014.
    g. Goodrich B. 2014. High-antioxidant plum developed and grown in Queensland. Growcom Fruit And Vegetable News. April 2014.
11. Radio

    Reynolds O. 2015. Trial using sterile fruit fly opens new markets for growers. http://www.abc.net.au/news/rural/2015-12-16/fruit-fly-trial-gets-results/7033720. ABC Rural News. December 2015.

12. Brochure (Appendix E)

A brochure was written at the request of David Moore, HIA, for distribution at the 2015 'Fruit Fly Roadshows' and to interested stakeholders: Reynolds, O.L & Papacek, D. 2015. Manage Queensland fruit fly. It's your responsibility… and it's as easy as ABC. Brochure. Horticulture Innovation Australia, Sydney, Australia.

13. Flyer (Appendix F)

In consultation with the growers, a flyer titled 'Help us control Queensland fruit fly (Qfly)' was written for distribution to all the farm workers and people in the region of the Trap Rock growers. The aim of this district flyer was to inform people living and working in the area about the AW-IPM SIT trial which has commenced and to equip them with the knowledge to support the program.

14. eBooks

Shuttleworth L. et al. under review. Area Wide Integrated Pest Management incorporating the Sterile Insect Technique: gut microbiota impacts on tephritid fitness and performance. Proceedings of the International Symposium on the Biological Control of Arthropods – Extended Abstract, Langkawi, Malaysia, 11-15 September 2017.

15. Oral Presentations (presenter is underlined; Appendix G)

a. Shuttleworth L. Deutscher, A.T. et al. Presented by OL Reynolds. 2017. Gut Microbiota to Improve the Quality of Mass-Reared Queensland fruit fly under the Sterile Insect Technique. Fourth IAEA/FAO Research Coordination Meeting of Coordinated Research Projects on 'Use of Symbiotic Bacteria to Reduce Mass-Rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application', Vienna, Austria, 17-20 May 2017.
b. Deutscher AT. et al. 2017. Diet Influences the Gut Microbiome of Queensland Fruit Fly Larvae: Understanding Gut Microbiota to Improve the Quality of Mass-Reared Flies for the Sterile Insect Technique. AusME 2017. 13-15 February, 2017 Peter Doherty Institute for Infection and Immunity, Melbourne, Australia.
c. Deutscher AT. et al. 2015. A Novel Molecular Sequencing Technique to Determine the Effect of Mass-rearing on the Queensland Fruit Fly Larval Gut Microbiome. Third IAEA/FAO Research Coordination Meeting of Coordinated Research Projects on 'Use of Symbiotic Bacteria to Reduce Mass-Rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application', Antigua, Guatemala, August 2015.
d. Reynolds OL, Liedo P & Barnes, B. 2016. Sterile Insect Technique programs for fruit flies across Australia, Mexico and South Africa: a detailed comparative. 3rd International Symposium of the Tephritid Workers of Europe, Africa and the Middle East, Stellenbosch, South Africa, 11-14 April 2016.
e. Deutscher AT. et al. Presented by OL Reynolds. 2016. Characterisation of larval tephritid gut microbiota: towards understanding sterile fly fitness and performance. Australian Entomological Society 47th Annual General Meeting and Scientific Conference, Melbourne, Victoria, 27-30 November 2016.

16. Posters (Appendix H)

a. Shuttleworth L, Collins D, Osborne T & Reynolds OL. 2017. The effects of bacterial probiotics fed to larvae of Queensland fruit fly (*Bactrocera tryoni*). Do they improve fitness and performance under the Sterile Insect Technique? Area Wide Management conference. Vienna, Austria, May 2017.
b. Deutscher AT, Burke CM, Darling AE, Riegler M, Reynolds OL* &. Chapman TA* *joint last author. 2016. Near full-length 16S rRNA gene NGS revealed *Asaia* as a common larval midgut bacterium of *Bactrocera tryoni*.

The Microbiome: Exploring the role of microorganisms in ecosystem processes and health @ Theo Murphy Australian Frontiers of Science, Adelaide, Australia, 29 November – 1 December 2016.

c. Reynolds OL, Osborne T, Balagawi S & Worsley P. 2016. Effective Area-Wide Management of the Queensland fruit fly. SITplus Fruit Fly Mass Rearing Facility Opening, November 2016.

d. Deutscher AT, Burke CM, Darling AE, Chapman TA, Reynolds OL and Riegler M. 2016. Gut Bacterial Diversity of Wild and Domesticated *Bactrocera tryoni* Larvae. SITplus Fruit Fly Mass Rearing Facility Opening, November 2016.

e. Balagawi S, Osborne T, Bloomfield C, Dominiak B & Reynolds, OL. 2016. Impact of irradiation, transportation and fly generation on key quality parameters of Queensland fruit fly. SITplus Fruit Fly Mass Rearing Facility Opening, November 2016.

f. Balagawi S, Osborne T, Liang W, Aiken D, Edwards D. 2016. Queensland fruit fly mass production for the Sterile Insect Technique: where it starts and finishes. SITplus Fruit Fly Mass Rearing Facility Opening, November 2016.

17. Television

A Landline segment titled 'The Colour Purple' was broadcast on the ABC during February 2015. Globally, Warroo Orchard is the only commercial producer of Queen Garnet 'QG' plums. Five times higher in anthocyanin (an anti-oxidant) than ordinary plums, these antioxidant QG plums are receiving a great deal of interest as a health product with studies reporting reductions in obesity in laboratory rats, which could translate to humans. This segment highlighted that Warroo is collaborating with NSWDPI releasing sterile Queensland fruit fly, to manage their crop using a sustainable, target-specific approach.

18. Media Release (Appendix I)

Media release titled 'Innovative birth control program to protect Fruit Crops'. Released wc 24 March 2014.

Outcomes

There are several outcomes as a result of this work and include,

AW-IPM SIT

1. The current sterile *B. tryoni* bisex strain (which includes females) was released in stone fruit orchards with nil risk of market access issues due to 'stung' fruit, where no larvae develop. However, it cannot be assumed that the approaches used in this study will enable market access in regulated international markets or elsewhere.
2. The lowest male and female wild fly pressure was experienced by the SIT treated orchards
3. AW-IPM, with or without SIT, is an effective method to suppress *B. tryoni* in endemic regions of Australia under similar conditions and pest pressure as Traprock
4. the number of wild female Queensland fruit flies trapped over consecutive seasons in SIT treatment orchards have been reduced to less than 0.01 flies/trap/day (FTD); an extremely low rate of fly trapping. It is expected that if the growers continue with the management plans that they will be able to maintain low fruit fly pressure
5. The number of wild males trapped per day over consecutive seasons in SIT treatment orchards have been reduced to less than 0.05 flies/trap/day; a very low rate of trapping
6. Reduction in fruit fly populations to low levels using AW-IPM, demonstrated by no market access issues with fruit sent either domestically or internationally throughout the duration of the 3-year program
7. The market destinations for the harvested fruit throughout the duration of this trial are shown in Table 1.
8. Reduction in pesticide use for *B. tryoni* across SIT treatment orchards from up to four sprays per season over the entire orchard down to one (Traprock; selected blocks across all seasons) to nil (Warroo) sprays.
9. Reduced secondary pest outbreaks, likely associated with reduced pesticide usage for *B. tryoni* and associated control costs in SIT treated orchards; a similar reduction was also observed in the control orchards
10. Protecting the environment and the health of farm workers due to decreased pesticide usage
11. Increased communication amongst the growers who participated in the trial; "…there are many ideas and strategies that evolved from working with the like-minded individuals in our group along with specific input from entomologists - confident that this has put us in good stead for not only confronting the QFF issue" John Pratt, pers. comm. 6 September 2017
12. Research and development support of the stone fruit industry has been strengthened through engagement
13. Identification of a new host for *B. tryoni*. Osage orange*, Maclura pomifera*, was identified as a host of Queensland fruit fly. This has management implications for this pest. Removal of the fruit from this tree or of the tree itself, in horticultural production areas should be considered to prevent fly populations breeding up in these hosts and/or this tree acting as a successive host for this pest.
14. There are further empirical outcomes that will be completed outside the scope of this project, which will include ongoing trap monitoring (maintenance phase). It is expected that this work will result in at least two publications that will be submitted to journals in the next 18 months.

Mating Diagnostic

There are no outcomes from this component of the work. Given the lack of sensitivity with the assay, further work was not justified.

Domestication, Irradiation & Transportation

This study demonstrates that an older fly colony, irradiation and transportation negatively affect the quality of sterile flies; considerable effort needs to be taken to minimize such negative impacts

Reduced Irradiation Dose

A reduced irradiation dose led to increased flight ability of *B. tryoni* (60-65 Gy; 85%) compared to flies that were irradiated at the higher dose (70-75Gy; 78.7%). However, while irradiated flies had a significantly decreased emergence rate compared with fertile flies, irradiation dose did not influence emergence.

Microbial symbionts

15. Near full-length 16S rRNA gene sequences of gut bacteria were obtained for 56 *B. tryoni* larvae; this is the first study to assess the bacterial communities of individual *B. tryoni* larval midguts.

16. *B. tryoni* gut bacterial diversity was low, and significantly lower in domesticated larvae.

17. Bacteria commonly associated with fruit (belonging to the families Acetobacteriaceae, Enterobacteriaceae and Leuconostocaceae) were detected in wild larvae, but were largely absent from domesticated larvae. However, acetic acid bacteria of the genus *Asaia* (Acetobacteriaceae) were detected in larvae of both wild and domesticated populations (55 out of 56 larval gut samples).

18. Larvae from the same single peach shared a similar gut bacterial profile, whereas larvae from different peaches collected from the same tree had different gut bacterial profiles. Wild flies from different locations had different *Asaia* strains.

19. Microbial symbiont characterization of the *B. tryoni* gut, together with the published literature has informed our selection of four bacterial candidates to test as probiotics which include members of the genus *Asaia*, *Enterobacter*, *Leuconostoc*, and *Lactobacillus*

20. Increased the NSWDPI collection of bacteria and yeasts cultured from the *B. tryoni* larval gut, which can be used in future studies aimed at improving larval diets, or identifying new lures etc. The collection is stored at EMAI.

21. The probiotic treatments were found to have various effects on fitness and performance of mass-reared *B. tryoni* larvae and adults. *Enterobacter* and *Asaia* both reduced larval development time, while *Lactobacillus*, *Leuconostoc* and the blend delayed this. The pupae resulting from larvae fed *Asaia*, *Enterobacter*, and *Leuconostoc* were all significantly lighter than the control, while *Lactobacillus* and the blend were not significantly lighter or heavier than the control. Larvae fed *Enterobacter* and *Asaia* may improve the ability for male flies to find mates after release in to the field. The application of probiotics that reduce development times such as *Enterobacter* and *Asaia* can reduce rearing costs.

Table 1. Market destinations of fresh fruit sold from the SIT-treated and control orchards for the duration of the three-year Area Wide - Integrated Pest Management pilot program.

Orchard	Season	Domestic Markets	Unregulated International Markets
Warroo (SIT)	2014/15	Supermarket (Woolworths) in Queensland, New South Wales and Victoria and Brisbane markets	Singapore, Hong Kong, Malaysia, Indonesia and the United Kingdom
Traprock (SIT)	2014/15	Supermarkets (Woolworths, Coles, Aldi, IGA and Costco), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	nil
Pikes Creek Top Lawson (control)	2014/15	Supermarkets (Woolworths, Coles, Aldi, IGA and Costco), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	Malaysia
Pikes Creek Bottom (control)	2014/15	Supermarkets (Woolworths, Coles, Aldi, IGA and Costco), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	Singapore
Warroo (SIT)	2015/16	Supermarket (Woolworths) and markets in Brisbane and Sydney and markets in Adelaide	Singapore, Hong Kong, Malaysia, Indonesia and the United Kingdom (UK)
Traprock (SIT)	2015/16	Supermarkets (Woolworths, Coles, Aldi, IGA and Costco), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	nil
Pikes Creek Top Lawson (control)	2015/16	Supermarkets (Woolworths, Coles, Aldi, IGA and Costco), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	Hong Kong
Pikes Creek Bottom (control)	2015/16	Supermarkets (Woolworths, Coles, Aldi, IGA and Costco), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	nil
Warroo (SIT)	2016/17	Supermarket (Woolworths) and markets in Brisbane, Sydney, Melbourne and Adelaide	nil
Traprock (SIT)	2016/17	Supermarkets (Woolworths, Coles, Aldi, IGA and Costco), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	nil
Pikes Creek Top Lawson (control)	2016/17	Supermarkets (Woolworths, Coles, Aldi and IGA), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	nil
Pikes Creek Bottom (control)	2016/17	Supermarkets (Woolworths, Coles, Aldi and IGA), Harris Farm Markets, Greengrocers and Brisbane & Sydney markets	Singapore

Note: All fruit sold to Adelaide, a fruit fly free state, was accompanied by a Plant Health Assurance Certificate which stipulated ICA-55 and indicated the fruit had been irradiated. For domestic access, all orchards follow the Freshcare (https://www.freshcare.com.au/) program for Food Safety and Quality Assurance and Pikes Creek and Traprock orchards are approved Coles Suppliers. Pikes Creek also has full HACCP accreditation. However, it cannot be assumed that the approaches used in this study will enable market access in regulated international markets or elsewhere.

Evaluation and discussion

AWIPM SIT

Overall, the pilot program demonstrated suppression of Queensland fruit fly, *Bactrocera tryoni* (Froggatt) populations in endemic areas under an Area Wide-Integrated Pest Management (AW-IPM) Sterile Insect Technique (SIT) program. The SIT treatment and control orchards experienced extremely low wild fly pressure and detections of larvae each year and there was not a single detection of infested fruit sent to either domestic or international markets over the three-year program from any orchards. This project has shown that following the principles of an AW-IPM approach with or without SIT can achieve a very good level of *B. tryoni* control.

Prior to the implementation of the AW-IPM SIT program, we assessed the impact of releasing a bisex strain of *B. tryoni*, which includes sterile females that are capable of 'stinging' fruit, i.e. laying eggs in fruit, on the subsequent market access of fruit. A low percentage of fruit fly stings were detected throughout the duration of the 3-day trial, sampled from packed fruit, with 1.36%, 2.57% and 2.4% of fruit recording stings on each day respectively. Our results suggests that the detected fruit fly stings were aborted stings or merely excavations with no evidence of eggs, larval development or feeding, suggesting stings (aborted or otherwise) were likely caused by either sterile or wild females that were unmated, or had mated with sterile males. There was no issue with the sale of stone fruit for the entire season that was due to fruit fly (Rowan Berecry pers. comm. 18 April 2014). This assessment allowed us to confidently pursue an AW-IPM SIT program across two commercial stone fruit orchards with no concern about the impact of sterile female 'stings' on the market access of stone fruit.

Although trap catches were fluctuating prior to the AW-IPM SIT program, since implementation we have kept wild male and female *B. tryoni* trap catches consistently low across the three years of the program (Figs. 11 & 12), with SIT treated orchards experiencing significantly less pressure than control orchards (Tables 2 & 3). The mean wild male (Fig. 11) and female (Fig. 12) *B. tryoni* flies per trap per day are shown from 2007 to 2017 for the control orchards and for the SIT treated orchards, from 2012 for Warroo and from 2013 for Traprock. Prior to implementation of the AW-IPM program in 2014 / 2015, trapping data was mined from growers who often used few male or female attractant baited traps, checking them irregularly mainly during the fruiting season. This variability made it problematic to statistically compare between the two periods.

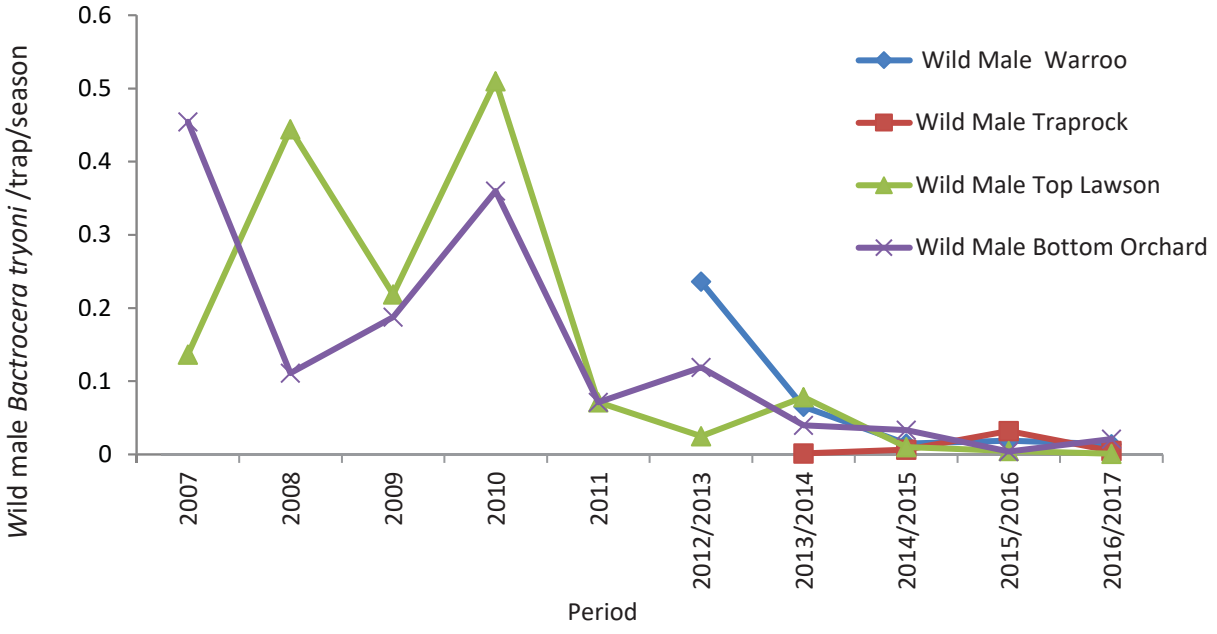

Figure 11. The wild male *B. tryoni* caught per trap per season across all trap types at each location. Note the AW-IPM program was implemented in 2014/2015. Calendar years show when data was only recorded during the growing season for each orchard. This is also true for 2012/2013 with the exception of Warroo, which trapped for the entire fiscal year.

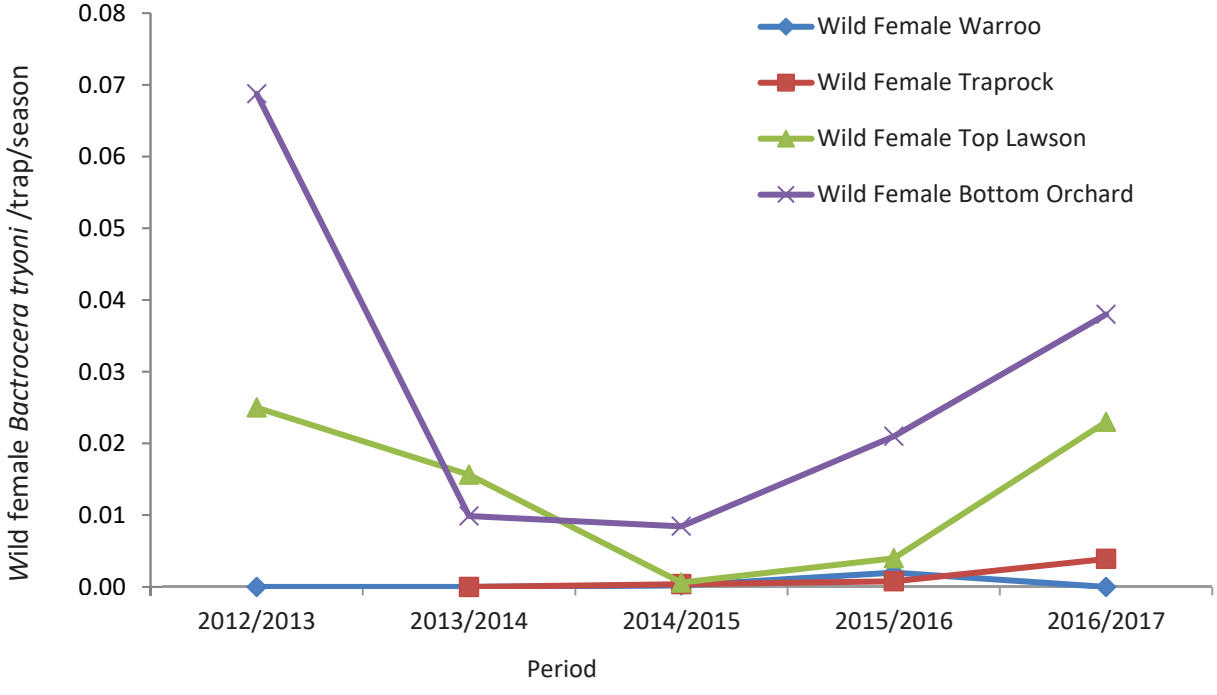

Figure 12. The wild female *B. tryoni* caught per trap per season across all trap types at each location. Note the AW-IPM program was implemented in 2014/2015. Calendar years show when data was only recorded during the growing season for each orchard. This is also true for 2012/2013 with the exception of Warroo, which trapped for the entire fiscal year.

The lowest male and female wild fly pressure was experienced by the SIT treated orchards (Tables 2 & 3). The SIT treatment orchard Warroo had the lowest proportion of traps with non-zero wild male fly catches, followed by Traprock,

then the control orchards, Top Lawson and Pikes Creek Bottom orchards respectively (Null deviance: 3503.3, df =5579; Residual deviance: 3473.6 , df=5576; p > 0.001; Table 2). As expected, the same trend was reflected in the mean number of wild male flies trapped per day during the program (Fig. 11).

Table 2. The mean (%) of all traps that captured wild males across sterile *B. tryoni* treated and control orchards throughout the AW-IPM program (June 2014 – March 2017).

Orchard (treatment)	Mean (%) traps that captured wild males
Pikes Creek Bottom (control)	8.5a
Top Lawson (control)	3.8b
Traprock (SIT)	2.6c
Warroo (SIT)	1.7d

Note: Within a column, means followed by a different letter are significantly different from one another (P<0.05). Mean (%) are back transformed logit means.

Similarly, the SIT treatment orchard Traprock had the lowest proportion of traps with non-zero wild female fly catches, followed by Warroo, then the control orchards, Top Lawson and Pikes Creek Bottom orchards respectively (Null deviance: 488.90, df =1863, Residual deviance: 468.09, df=1860, p = 1.59585e-06; Table 3). The same trend was reflected in the mean number of wild female flies trapped per day during the program (Fig. 12).

Table 3. The mean (%) of all traps that captured wild females across sterile *B. tryoni* treated and control orchards throughout the AW-IPM program (June 2014 – March 2017).

Orchard (treatment)	Mean (%) traps that captured wild females
Pikes Creek Bottom (control)	16.9a
Top Lawson (control)	10.3b
Traprock (SIT)	8.2c
Warroo (SIT)	8.8d

Note: Within a column, means followed by a different letter are significantly different from one another (P<0.05). Mean (%) are back transformed logit means.

We were not able to achieve male sterile:wild ratios of 100:1, or even 50:1 or 30:1 (Figs. 13 & 14) which are the targets of most tephritid AW-IPM SIT programs. Overflooding ratios must account for any propensity of the target population to increase, since the SIT interacts with a pest population at the point of reproduction (Lance & McInnis 2006). Although there are no published studies for *B. tryoni* to determine the required overflooding ratios, the often high numbers of sterile flies required in SIT programs due to a range of factors such as reduced survival, migration, mortality due to predation (Hendrichs et al 2006) may be reduced by enhancing performance traits (e.g. Barry et al 2003; Reynolds et al 2014; Khan et al in press). It is not clear why this is the case, however overall we did achieve higher sterile fly to wild fly ratios in Warroo compared to Traprock. Warroo is a continuous orchard of one variety on relatively flat ground. Traprock comprises blocks of different stone fruit varieties often separated by grass strips several metres wide over hilly terrain. Similarly, a study released the same batches of sterile flies concurrently over four periods in both NSW and South Australia (SA), yet SA consistently showed a reduced recapture rate despite being corrected at each location for the number of fliers, i.e. flies that were released (flew) into the environment (Reynolds et al 2012). Those authors proposed that *B. tryoni* may have a higher post-release mortality rate prior to reaching maturity in SA. This difference could be due to a number of variables including reduced fly quality when released due to longer pupal transport periods, climatic conditions, food

availability, predation and variation in response to cue-lure baited traps (Reynolds et al 2012).

In the present study, higher numbers of sterile males were detected compared to sterile females, as is typically observed in most *B. tryoni* bisex strain releases. Cue-lure is a powerful attractant for many male Dacine fruit flies, including *B. tryoni* (Metcalf & Metcalf 1992; Metcalf 1990). Further, the protein-based attractants used in the present trial are non-selective, but typically attract more immature female fruit flies, than protein-fed mature females or males (Prokopy et al 1991) and are probably only detected by *B. tryoni* over relatively short distances (Clarke et al 2011) due to their low volatility (Morton & Bateman 1981). This would, at least in part, explain why female *B. tryoni* are detected at lower rates than males.

Pupal release was initially used, as no cover for adult release boxes could be identified to allow the flies to develop to 2-3 day adults, prior to adult release. When adequate cover (space in a roofed open-air shed at each orchard) was available, the growers moved to adult release in 2016-2017. This meant that we could release sterile flies from further within the orchards (Appendix A). This is likely to have provided a more even distribution of flies within the orchards. Pupal release stations were in fixed positions, sometimes on the edges of the orchard blocks and governed by several factors including practicalities for growers, including allowance for tractors and other farm machinery and away from spraying (bait and cover) operations.

Bactrocera tryoni successful release and survival is influenced by several factors including temperature, humidity, food availability, predation (Reynolds et al 2012), irradiation, pupal dye and transport of pupae (Campbell et al. 2009). These factors, among others, are important considerations for sterile fly release. Although this trial was not designed to assess the interaction of climate and successful release a study by Reynolds et al. 2010 showed the complex relationship between external and internal temperature and relative humidity of pupal release systems at different pupal loadings. Simply, they showed that temperature above 35°C and below 10°C lead to reduced emergence, while for successful flight, the temperature should not fall below approximately 6°C while the maximum temperature should exceed 16°C. So, during days or periods when temperatures were not optimal in the present study, we expected lower numbers of sterile flies in the environment. This would likely be a similar scenario for adult release as the flies were acclimated, as they are under pupal release. The high numbers of pupae that we used in the present trial, would assist in minimizing the impact of a decreased number of released flies, however extreme temperatures would also impact wild *B. tryoni* in the environment.

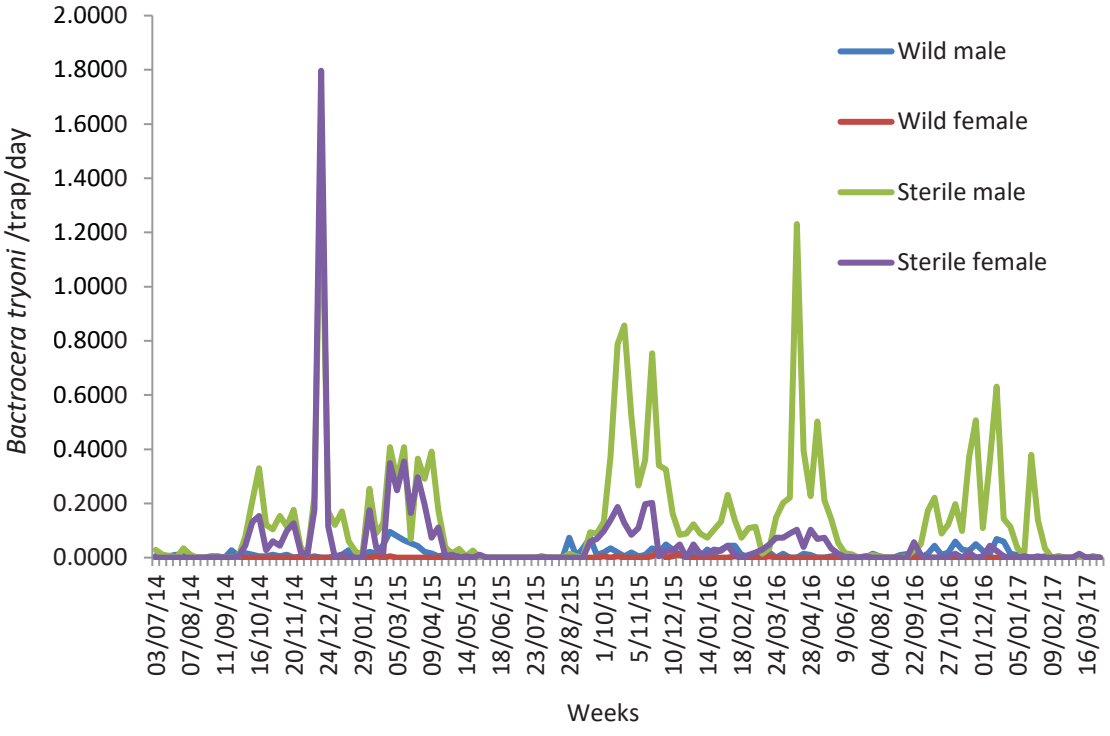

Figure 13. The sterile and wild, male and female *Bactrocera tryoni* flies/trap/day for Warroo Orchard over the entire three year Area Wide - Integrated Pest Management program incorporating the Sterile Insect Technique.

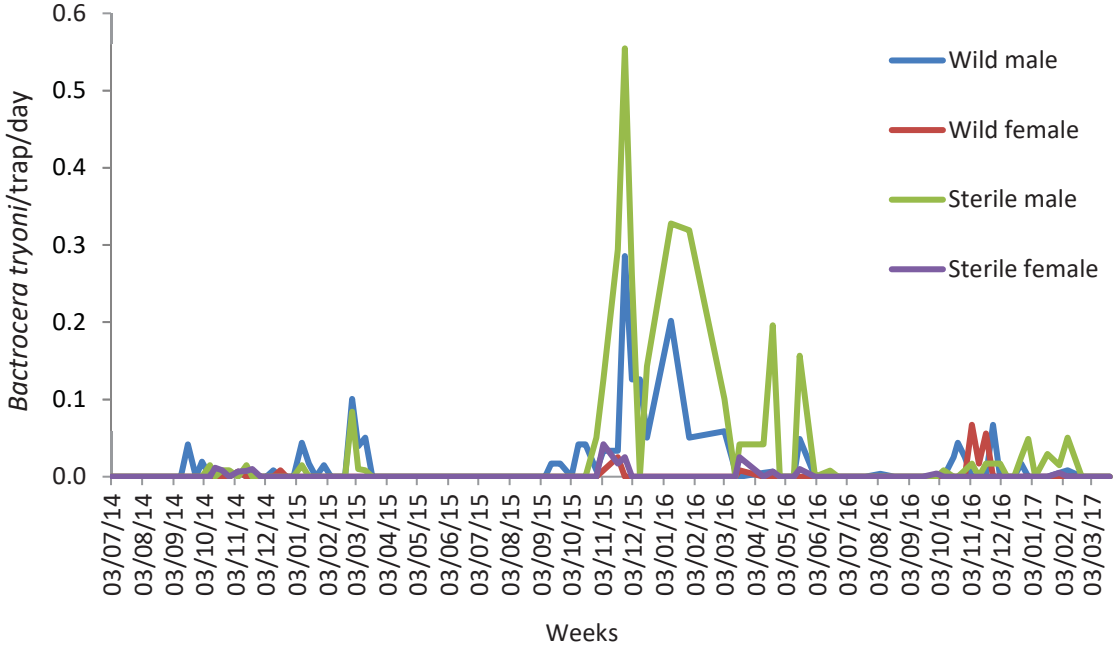

Figure 14. The sterile and wild, male and female *Bactrocera tryoni* flies/trap/day for Traprock orchard over the entire three year Area Wide-Integrated Pest Management program incorporating the Sterile Insect Technique.

Regular fruit monitoring by a trained individual which followed a sampling protocol (rare detections by pickers were not included here), detected very low levels of sting/egg/larvae infested fruit across the SIT treated orchards i.e. Warroo 25/0/26 (2014/2015), 32/0/53 (2015/2016), 3/0/0 (2016/2017) and Traprock 0/0/0 (2014/2015), 15/0/6 (2015/2016) and 22/0/0 (2016/2017. Typically, Traprock orchard has very little, if any *B. tryoni* pressure until late October, early November (http://www.dpi.nsw.gov.au/biosecurity/insect-pests/qff) and so their early season fruit has a reduced risk of being stung. As Warroo only harvests their entire commercial plum crop in February, they face the peak time for *B. tryoni* populations; however our program has kept pressure extremely low. Maintaining low pest pressure reduces the risk of fruit infestation, therefore reducing the risk of lost fruit, and therefore profitability and market access.

However, it should be noted that no management scenario is fail safe. On 20 November 2015 a block of Crimson Lady yellow peaches from Traprock orchard (SIT treated) were harvested and packed. Larvae were detected once the fruit was in the packing house and in cold store. Although there was an extremely low level of infestation (estimated at less than 1%; John Pratt pers. comm. 2015), the entire picking to that point was discarded as the grower, after consultation with his buyer, decided the risk of *B. tryoni* detection was too high to send to market. The same block had earlier in October been sprayed with spinetoram for western flower thrips (20g/100L) which would have also impacted sterile and wild *B. tryoni* present in the crop. In the lead to harvest this block was not monitored weekly for eggs/larvae as per protocol which should have detected this low level incidence of *B. tryoni*. Although a loss for the grower, this perhaps demonstrates the importance of regular weekly fruit checking. Subsequently, the grower applied two consecutive cover sprays (Samurai; 40g/100L) to control *B. tryoni* on his remaining Crimson Lady yellow peaches (Blocks 2 & 4) (22/11/15 and 29/11/15) in the lead up to harvest. In Top Lawson (control orchard) in November 2015, a single block of the last plums of the season, which contained a mix of maturities, had an estimated 50% fruit fly infestation in the mature fruit (Graham Finlay pers. comm. 2016). This block had received two sprays of Samurai. Once the infestation was detected alpha-cypermethrin was sprayed. Further, young trial trees (not for commercial harvest) that had not been cover sprayed were also found to have up to 50% infestation. It should be noted that the 2015-2016 season was a particularly favourable season for *B. tryoni* in the region, with warm, humid weather; ideal conditions for this fly to proliferate.

Significantly, pesticide usage for *B. tryoni* was down across all SIT treated orchards from previous conventional managed years, from 3-4 cover sprays to nil – 1. The use of a cover spray was always available to a grower if they feel a need. Notably, Warroo did not spray any pesticide for any pest throughout the entire 3 year program, with the exception of the first year of the program where only some blocks were sprayed. Despite few detections of *B. tryoni* by regular fruit monitoring (excepting the above), Traprock did elect to spray some later season fruit blocks during each season, although greatly reduced over previous conventional treated years. This is a confidence item for the growers as they adapt to a new program of control, but still well below rates they sprayed prior to the programs implementation. Conversely, the control orchards, Top Lawson and Pikes Creek Bottom, used cover sprays as a key component of their management program, and used these as required at the lowest label rate. Pesticide Sprays for secondary pests reduced for all treated SIT and control orchards over time, likely due to a combination of factors, including an increased awareness of what pests were present in the orchards and a reduction or change in the rate and type of pesticides used.

We identified that non-pest species including *Dacus newmani*, *Dirioxa pornia*, *B. jarvisii* and *B. aequalis* were also trapped in the region, although at very low numbers. This highlights the importance of local crop consultants, or managers of AW-IPM programs understanding the fruit fly complex in the target region/crop, to reduce the risk of misidentification.

During harvest of the final year of the three year AW-IPM program, importantly, our results show that under an SIT treated or control program, stone fruit infestation levels are extremely low (Table 5). Indeed, although wild fly pressure was lower in SIT treated orchards (Table 2 & 3), larval infestation levels (no eggs were detected) were no different to the

control orchard that received no sterile flies (Table 5; F=0.656, df=2, 3, p=0.589). There is no published evidence showing a correlation between *B. tryoni* trap catches and larval infestation of fruit. This suggests that an AW-IPM program, with or without SIT, can be successfully implemented to suppress endemic *B. tryoni* populations in areas with similar conditions as Traprock, however an economic analyses is required to assess the cost effectiveness of these scenarios. Selecting whether to include SIT as one of the tools will depend upon several factors including whether an area or region has minimal fruit fly incursions (through geographic isolation or quarantine), can realise greater access to markets (e.g., demonstration of area freedom or international demand for low-pesticide or pesticide-free fruit), and is cost-effective.

Traprock Orchard (SIT treated) and Top Lawson orchard (control) did not differ in the mean number of ovipositor excavations, or 'stings' (Note: record of a sting does not necessarily mean there is an infestation, i.e. eggs/larvae and there is no way of differentiating a sting between a wild and a sterile female, unless there is a presence of sterile/fertile eggs and/or larvae) per fruit (F=3.98, df=2,3, p=0.143; Table 5). However, Warroo Orchard had a greater number of stings per fruit than Top Lawson orchard (control). This is not surprising given the higher number of sterile flies recorded in Warroo (Fig. 13) than Traprock (Fig. 14), despite similar sterile release rates.

Table 5. Fruit fly stings and larvae recorded from stone fruit sampled at Pikes Creek Packing shed from sterile release and control orchards during the 2016/2017 stone fruit season.

Orchards	Total fruit sampled	Total Stings	Total Larvae	Mean no. fruit fly stings /fruit	Mean no. fruit fly larvae/fruit
Top Lawson (Control)	4674	128	10	0.027a	0.002a
Traprock (SIT)	6807	442	99	0.065ab	0.015a
Warroo (SIT)	8315	902	21	0.109b	0.003a

Within each column, values followed by the same letter are not significantly different from one another ($p > 0.05$).

The proportion of fruit with 'stings' (but not necessarily infestation) was highest for Warroo, followed by Traprock and then the control orchard ($X2=311.3$, df=2, $p<0.001$; Table 6). These stings are typically not visible except to a trained eye and when not infested (i.e. contain no eggs and/or larvae) did not cause any discernible damage to the fruit. Regardless, these 'stings' caused no market access issues across the three year AW-IPM program. Indeed, during the 2017 packing of SIT treated Warroo Orchard plums it was observed that "To date we have packed 170,000kg of Queen Garnet and have not seen any damage to fruit that could be attributed to sting marks caused by sterile Queensland fruit fly" (Andy Finlay pers. comm., Chair Summer Fruit Australia, 7 February 2017). It should be recognised that fruit is not perfect, and may experience a range of imperfections, both minor, and more severe such as sunburn, hail damage, lepidopteran larval feeding and thrips damage. As the control orchard (i.e. no sterile flies released) also recorded stings, but not necessarily eggs, or larval feeding, suggests that abortive stings by wild females are likely to contribute up to 2% of fruit with fruit fly stings in the SIT treated orchards.

Table 6. Mean (%) fruit with *Bactrocera tryoni* stings and 95% confidence interval (CI)

Orchards	Mean (%) fruit with stings	Mean (%) lower CI	Mean (%) upper CI
Top Lawson (Control)	1.93 a	1.57	2.36
Traprock (SIT)	2.95 b	2.58	3.38
Warroo (SIT)	7.87 c	7.31	8.46

Within each column, values followed by the same letter are not significantly different from one another ($p > 0.05$). Means and confidence intervals (CI) are back transformed from logit means and confidence intervals

Although anecdotal, numerous beneficial insects have been reported in the orchards since the program was implemented. However, a significant thrips problem in Traprock and the control orchards during 2015/16 and 2016/17, and associated insecticide application is likely to have impacted sterile male populations and impacted the number of beneficial and sterile flies in the orchards.

The maps generated for each orchard were provided to growers and consultants, to assist with fruit monitoring and other activities, such as trap checking runs. The mp4 clips allowed seasons to be interactively inspected using the time-slider-bar to view the temporal progression of *B. tryoni* activity through the season and to qualitatively assess the impact of management intervention options (http://www.dpi.nsw.gov.au/biosecurity/insect-pests/qff). Animation of the season's management activities and events was invaluable for gaining an understanding of timing of resource use and fly movement in the area. It appears that the flies utilise the surrounding native and non-native vegetation as a resource (food, shelter, etc.), particularly in the pre- and later post- harvest periods. An increased focus on managing *B. tryoni* in non-commercial hosts year round is warranted, and is in line with the principles and practices of AW-IPM programs. Further, the flies appear to be utilising the native vegetation to overwinter, with movement into the orchards around the time of pruning/thinning, which generates a distinct aroma the flies are likely attracted. It is also likely that the flies roost in the native vegetation, at least when the stone fruit has no leaves. After particularly high humidity, or rain, we see an increase in trap catches, presumably as a result of increased fly movement. This suggests the flies are nearby, and not travelling extensive distance, although this does not preclude that this is also a possibility.

Three wild fruiting plants were identified in the region, Osage orange, *Maclura pomifera*, tree pear, *Opuntia tomentosa* and bumble fruit, *Capparis mitchelli*. Samples of all three fruit were collected and placed over vermiculite to determine their host status. *Maclura pomifera* was identified as a new host of *B. tryoni* (Appendix J). This has management implications as it is considered a weed, and may act as a successive host for *B. tryoni*. No larvae were recorded, or *B. tryoni* adults were reared from *O. tomentosa* or *C. mitchelli*.

During the three year AW-IPM program, all orchards sent fruit to domestic and unregulated international markets, except Traprock which only supplied domestic markets (Table 1). In 2014/15 season, Pikes Creek (Top Lawson and Bottom) and Traprock orchards sold their fruit domestically to supermarkets (Woolworths, Coles, Aldi, IGA and Costco), Harris Farms, Greengrocers and Brisbane and Sydney markets. In addition, Top Lawson sent some fruit to Malaysia and Bottom orchard to Singapore. Approximately 70% of the fruit from Warroo was marketed domestically, as either pre-packs or loose through Woolworths in Queensland, New South Wales and Victoria, with a small percentage that went through the Brisbane Market. The remaining 30% was sent to Singapore, Hong Kong, Malaysia, Indonesia and the United Kingdom (UK). Every fruit sent to the UK was individually inspected. Similarly, the majority of fruit was sold domestically in 2015/16 and 2016/17, although Warroo and Pikes Creek orchards sent some fruit to international markets. In 2015/16, approximately 94% of the fruit from Warroo was marketed domestically as fresh fruit and approximately 6% as juice. The fresh fruit was marketed through Woolworths and markets in Brisbane and Sydney with approximately 1.8% going to

markets in Adelaide and 0.8% to overseas markets. In 2016/17 approximately 36% of the fruit from Warroo was marketed domestically as fresh fruit through Woolworths and markets in Sydney, Brisbane, Melbourne and Adelaide. Approximately 63% of the fruit produced was sold for processing as juice, due largely to *Helicoverpa* damage, and the inclusion of fruit from pollinator trees. In 2015/2016 Top Lawson sent fruit to Hong Kong and in 2016/2017, Bottom orchard sent fruit to Singapore. No fruit from any SIT treated orchard has ever been, or required, post-harvest treatment, with the exception of Warroo orchard which sent fruit to Adelaide (*B. tryoni* free state) accompanied with a Plant Health Assurance Certificate stipulating ICA-55 and indicating it had been irradiated at Steritech, Brisbane (7 tonnes; 2015/2016 and 14.3 tonnes; 2016/2017). However, it cannot be assumed that the approaches used in this study will enable market access in regulated international markets or elsewhere.

The social engagement program included regular face to face meetings with the growers, talks with pickers and packers, handing out the *B. tryoni* management brochure (Appendix E), and the production of a flyer produced in association with the growers, targeted at the workers which enter the property each year (Appendix F).

A cost:benefit analyses of the AW-IPM study is being conducted separately. Labour stands as one of the most significant cost factors. Reducing requirements of labour, for example by producing bait sprays with greater longevity therefore reducing frequency of application, and reducing handling of pupae and adults will assist in reducing costs.

From this study the following recommendations on the establishment of AW-IPM SIT programs for *endemic B. tryoni* in Australia and implications for its wider use include:

- An AWM program that includes SIT can be used when an area or region has minimal fruit fly incursions (through geographic isolation or quarantine), can realise greater access to markets (e.g., demonstration of area freedom or international demand for low-pesticide or pesticide-free fruit), and is shown to be cost-effective
- AW-IPM programs can achieve excellent suppression without the use of sterile flies and should also be considered across areas, or regions of Australia where *B. tryoni* is endemic
- Start with a pilot AW-IPM SIT program; there is little room for error in a full-scale program. Use the pilot to smooth out any challenges, both foreseen and unforeseen
- Pilot projects should now extend to an area that is adjacent to a peri-urban/urban area; recognize that no two regions/scenarios are the same, but important lessons are learned from pilot projects
- Establish clear core and buffer zones; manage these appropriately
- Surveys should be used to identify alternate hosts; these should be suitably managed
- Sanitation should be observed for both infested fruit fallen on the ground, and fruit left on the tree after harvest
- Monitoring both male and female populations should occur to measure success (e.g. suppression, eradication) and identify 'hotspots' which may require additional treatments
- Know your orchard!; conduct regular, weekly fruit monitoring within each orchard
- Know your pest!; understand the pest complex in each region, including other pest fruit fly species
- Conduct regular packing shed assessments to monitor infestation levels
- Damaged fruit (e.g. after a hail storm) may be more susceptible to *B. tryoni* attack, and should be carefully monitored and managed

- Engage grower 'champions' from the start which are involved in the project, and can promote the successes of the program
- Engage local crop consultants from the outset; educate on management of *B. tryoni* (where required)
- Social engagement programs should extend to include the yearly influx of farm workers (e.g. pickers, pruners etc); you can expect better levels of detection of any infestations pre- or post-harvest through doing this
- Develop regional/crop specific management plans 'cards' (i.e. simple at a glance) to provide to growers to manage *B. tryoni* (could extend to include pest complex)
- Future research should focus on techniques which reduce the cost of AW-IPM SIT programs; e.g. enhancing sterile male fitness and performance (e.g. understanding the role and function of microbial symbionts in the insect gut, development of probiotic diets), reducing the response of sterile males to male attract and kill systems (e.g. Male Annihilation Technique (MAT)), and the development of target-specific female baits that can be readily applied by growers and have good longevity
- Management tools and techniques that are compatible with SIT under an AW-IPM warrant further study and include, but are not limited to habitat management principles and practices, longer-life baits (protein, or other female attractants), augmentative release of parasitoids, and entomopathogenic fungi

Mating Diagnostic

A list of 83 proteins were found exclusively in tested sterile files, of these eleven proteins had valid MSMS spectra. There were no proteins identified exclusively in fertile males. From the eleven proteins, five of the more prevalent profiles were selected for primer design. The sequences were checked via blast in NCBI to determine matches. The primers were checked for in silico matches to ensure it was specific for the target protein. The testing of the primers was conducted on different sperm and seminal samples from sterile and fertile flies. All indications pointed towards a working assay, until a second subset of flies were analysed. During this analysis, the primer pair for the sterile marker amplified in the fertile flies. Possible reasons for this include a mix up of flies, mix up of DNA, the irradiation process is not affecting the same proteins each time, or the primers are not as specific as first thought. Any of these scenarios are possible and all are relatively easy to test. However, the main draw back with the assay was its lack of sensitivity. The assay was tested for 1, 5, 10, 20 and 60 flies. Amplified product was only observed with the 60 flies. It has been demonstrated that a sterile fly passes a small quantity of sperm across in the first mating and that this is reduced in subsequent matings to mainly seminal fluid. For this reason the sperm and the seminal fluid was targeted for protein analysis. However, if these specific target regions are in such low concentrations then detection of these proteins in a female fly using a whole body DNA extraction method is not likely to be viable. Future studies might consider the development of a mating assay based on the annotated Y-chromosome of a fertile *B. tryoni* male. A primer set that could detect the presence of a Y-chromosome in a female fly would be an indicator of mating. The next phase would be to determine if irradiation has an effect on this Y-chromosome and if this assay could then be adapted to a mating assay.

Irradiation and Transport

There was no significant interaction between transportation and sterility ($F_{1,108} = 0.111$, $p = 0.740$), and pupal weight was not affected by transportation ($F_{1,108} = 0.111$, $p = 0.740$) or pupae sterility ($F_{1,108} = 0.075$, $p = 0.785$). A separate one way analysis of variance showed that there was no significant difference ($F_{3,108} = 0.087$, $p = 0.967$) between weight of fertile or sterile pupae that were transported to Orange Agricultural Institute (OAI) for quality control testing as compared to those that were not transported and tested at the fruit fly production facility at EMAI (Fig. 15).

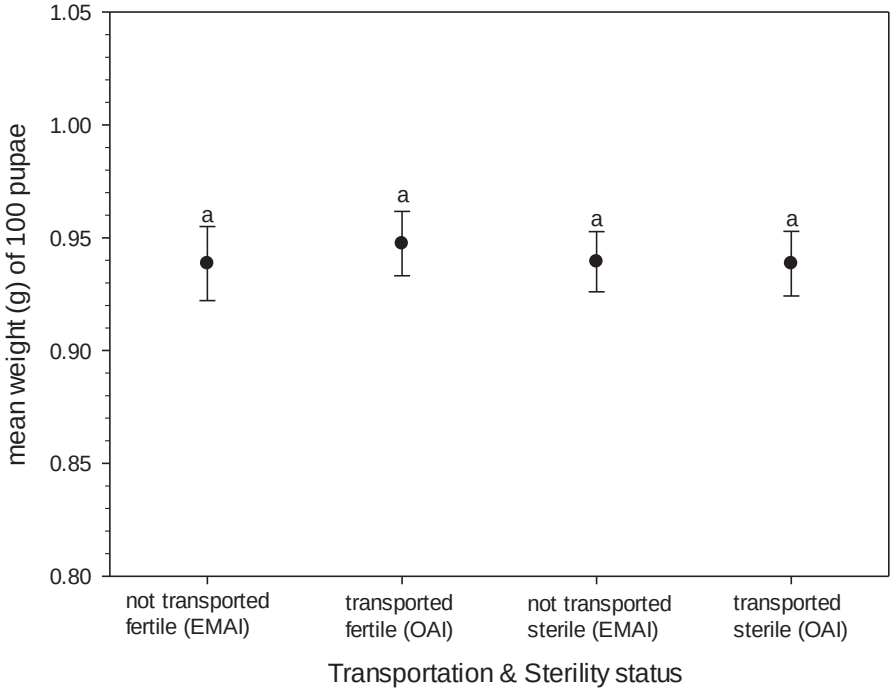

Figure 15. Effect of transportation of Queensland fruit fly pupae on weight of fertile and sterile pupae. Dots are mean weight (g) and bars are error bars. Bars with the same letter are not significantly different.

A univariate analysis with percent of adult *B. tryoni* emergence as the dependent variable and transportation and pupae sterility as independent factors showed that there was no significant interaction between transportation and sterility ($F_{1,108} = 0.066$, $p = 0.798$), but both transportation ($F_{1,108} = 15.435$, $p < 0.001$) and sterility ($F_{1,108} = 7.954$, $p = 0.006$) did have significant effect on adult *B. tryoni* emergence. An independent sample t-test showed that significantly higher percent of flies emerged from pupae that were not transported (81.6 ± 0.01%, mean ± SE) (i.e., test done at EMAI) compared to fly emergence from pupae that were transported (73.2 ± 0.03%, mean ± SE) to OAI ($t = 3.825$, df =110, $p < 0.001$). Similarly, a separate independent sample t-test showed that there was a significant difference in adult *B. tryoni* emergence between flies emerging from fertile and sterile pupae ($t = 2.662$, df =110, $p = 0.009$). A higher percent emergence of flies were observed from fertile pupae (80.0 ± 0.02%, mean ± SE) compared to sterile (irradiated) pupae (74.8 ± 0.01%, mean ± SE).

Similar results to that described for percent adult fly emergence were also observed for percent fliers (flight ability) and rate of fliers. There were no interactions between pupae transportation and fly sterility status, but both transportation and fly sterility status did significantly affect flight ability and rate of fliers (Table 2). Separate t-test showed that significantly higher percent of fliers were observed from flies that emerged from pupae that were not transported (65.46 ± 0.02%, mean ± SE) (i.e., test done at EMAI) compared to flies from pupae that were transported (43.98 ± 0.03%, mean ± SE) to OAI ($t = 6.278$, df =110, $p < 0.001$). Another independent sample t-test showed that there was a significant difference in percent fliers of *B. tryoni* between fertile and sterile flies ($t = 4.572$, df =110, $p < 0.001$). A higher percent of *B. tryoni* fliers were observed from fertile pupae (63.18 ± 0.03%, mean ± SE) compared to sterile (irradiated) flies (46.27 ± 0.02%, mean ± SE). When the data was grouped into sterility (fertile or sterile) and transportation status (not transported [EMAI] or transported [OAI]); a one-way analysis of variance showed that there was a significant difference

(F3,108 = 7.228, p < 0.001) between transportation and sterility status with non-transported and fertile pupae producing higher fliers than the transported sterile flies (Fig. 16). Again, flies from pupae that were transported to OAI provided lower rate of fliers as compared to those not transported (t = 6.278, df =110, p < 0.001), while irradiated (sterile) pupae also provided lower rate of fliers than the non-irradiated (fertile) flies (t = 4.612, df =110, p < 0.001).

Table 7. Summary of two-way analysis of variance on the effect of *Bactrocera tryoni* pupae transportation and fly irradiation (sterility) on flight ability (% fliers) and rate of fliers.

Quality Control Parameter	Source of Error	*df*	*F*	*p*
% fliers	transportation	1	50.005	< 0.001
	sterility	1	30.269	< 0.001
	transportation* sterility	1	1.273	0.262
Rate of fliers (%)	transportation	1	50.857	< 0.001
	sterility	1	31.242	< 0.001
	transportation* sterility	1	2.69	0.104

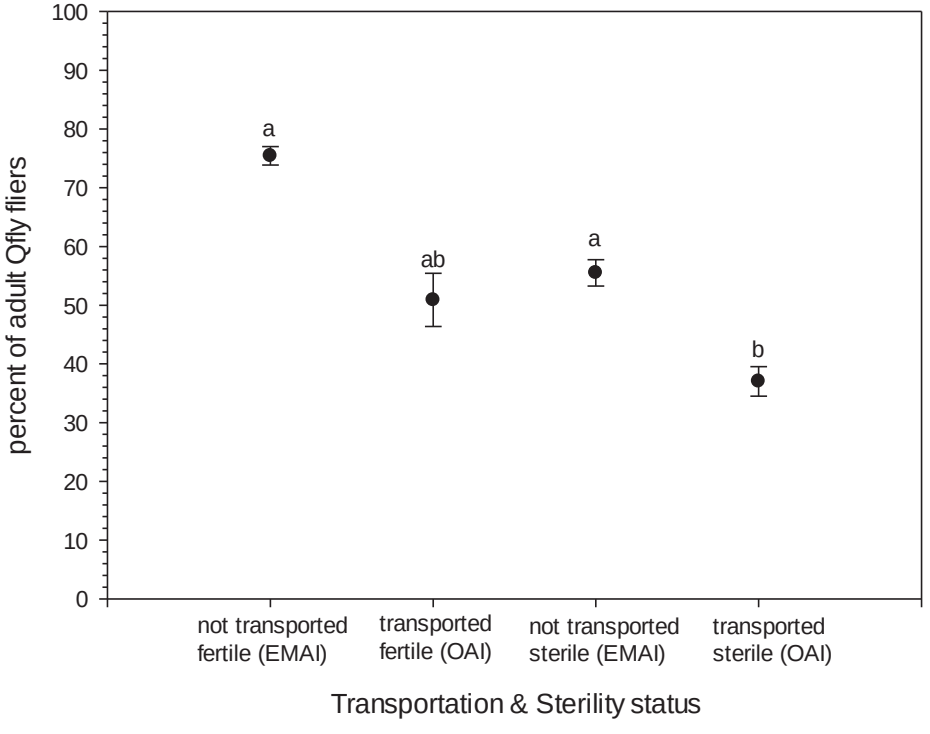

Figure 16. Effect of Queensland fruit fly pupae transportation on flight ability (% fliers) of flies emerging from fertile and sterile pupae. Dots are mean percent flight ability and bars are error bars. Bars with the same letter are not significantly different from each other.

There was a significant effect of temperature on flightability of transported sterile *B. tryoni* (Table 8). The percent of sterile *B. tryoni* fliers decreased as the temperature increased (Fig. 17). Temperature during transportation can explain 29.3% of this decrease in flightability of sterile *B. tryoni* while 78.7% of this decrease in flighability may be explained by

other factors.

Table 8. The impact of temperature during transport on sterile and fertile *Bactrocera tryoni* pupal weight, emergence and flight.

Relationship	Statistic			
	standard coefficient	**R^2**	**F**	**P**
temperature vs fertile pupal weight	0.002	< 0.001	< 0.001	0.994
temperature vs sterile pupal weight	-0.038	0.001	0.023	0.881
temperature vs fertile % emergence	0.164	0.027	0.442	0.516
temperature vs sterile % emergence	0.285	0.081	1.410	0.252
temperature vs fertile % fliers	-0.172	0.030	0.488	0.495
temperature vs sterile % fliers	-0.541	0.293	6.621	0.020*

* statistically significant

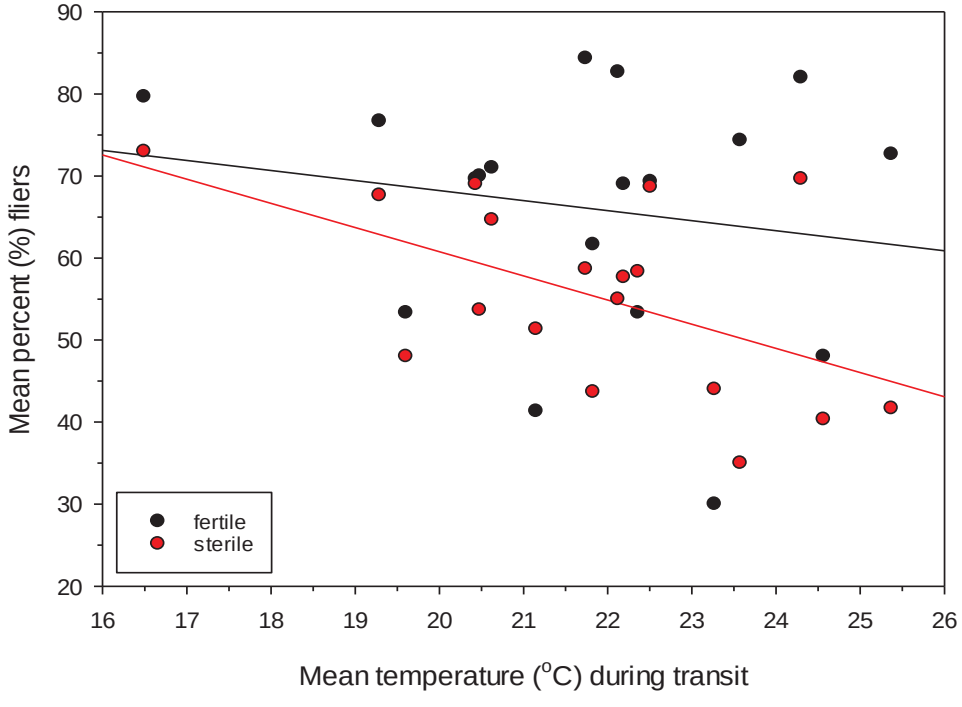

Figure 17. Mean (%) of *Bactrocera tryoni* capable of flight across a range of mean temperatures during transport.

The present study showed that transportation of *B. tryoni* pupae from mass production facilities to field release sites negatively affects the quality of flies. Transportation of pupae decreased percent fly emergence, percent flight ability and rate of fliers. This decrease in fly quality is further negatively compounded when pupae are irradiated to produce sterile flies. This is the first reported study to demonstrate that during transport, temperature contributes to nearly 30% of the

decrease in flightability of sterile *B. tryoni*. Campbell et al 2009 showed that vibration experienced during transport under a constant temperature did not impact fly quality, however suggested that trialling this under a range of temperatures warrants investigation. The authors did show that dye has a significant impact on fly quality. Similarly, a trial on Anastrepha obliqua in Mexico showed that sterile male performance can be achieved (without additional expense) by keeping flies under hypoxic conditions for less than 12h during transport, reducing the amount of fluorescent dye used to mark the flies and minimising the irradiation dose (Rull et al 2012). Hence, research is needed to further identify and improve the likely combination of several factors, including temperature, hypoxia, vibration and dye during the transportation process that contribute to poor *B. tryoni* quality.

Reduced Irradiation Dose

Our study showed that the reduced irradiation dose of 60-65Gy implemented by the FFPF in September 2014, increases flight in sterile Queensland fruit fly, *Bactrocera tryoni* (Froggatt), but not emergence. Further, sterility has not been impacted by this reduced dose.

Flight

There was strong evidence that irradiation at the higher dose significantly lowers the flight ability of *B. tryoni* ($F_{1, 109}=14.34$; $p<0.001$) and the reduced flight of sterile compared with fertile flies ($F_{1, 106}= 97.76$, $p<0.001$; Table 9). As the application of different doses was not concurrent, the significant effect might have also been caused by other factors. Sterile flies also had consistently lowered flight ability across the four year trial ($p<0.05$; Table 10).

Table 9. The mean (%) flight ability of sterile *Bactrocera tryoni* irradiated at different doses, and fertile flies.

	Mean (%) flight ability
Sterile (irradiated)	74.7a
Fertile	87.7b
60-65Gy irradiation	85.0b
70-75Gy irradiation	78.7a

Within each column, values followed by the same letter are not significantly different from one another ($p > 0.05$). Means are back transformed from logit means. Time period 1: 21 November 2012 – 15 May 2014; Time period 2: 9 September 2014 – 23 August 2016

Table 10. The mean (%) flight ability of sterile and fertile *Bactrocera tryoni* across two time periods.

	Time period (irradiation dose)	Mean (%) flight ability
Sterile (irradiated)	1 (70-75Gy)	64.9 a
Sterile (irradiated)	2 (60-65Gy)	82.5 b
Fertile	1	88.1 c
Fertile	2	87.2 c

Within each column, values followed by the same letter are not significantly different from one another ($p > 0.05$). Means are back transformed from logit means. Time period 1: 21 November 2012 – 15 May 2014; Time period 2: 9 September 2014 – 23 August 2016

Emergence

Irradiated flies had a significantly decreased emergence rate compared with fertile flies ($F_{1, 107}=24.74$; $p<0.001$; however irradiation dose did not influence emergence ($F_{1, 110}=3.88$; $p=0.053$; Table 11). Emergence of *B. tryoni* irradiated at the lower dose did not differ to the fertile flies across both time periods ($p>0.05$; Table 12).

Table 11. The mean (%) emergence of sterile and fertile *Bactrocera tryoni* when irradiated at different doses.

	Mean (%) emergence
Sterile (irradiated)	82.5 a
Fertile	85.6 b
60-65Gy	85.3 b
70-75Gy	83.0 b

Within each column, values followed by the same letter are not significantly different from one another ($p > 0.05$). Means are back transformed from logit means. Pupae irradiated at 70-75Gy were recorded during time period 1: 21 November 2012 – 15 May 2014; pupae irradiated at 60-65Gy were recorded during time period 2: 9 September 2014 – 23 August 2016 .

Table 12. The mean (%) emergence of sterile and fertile *Bactrocera tryoni* across two time periods.

	Time period (irradiation dose)	Mean (%) emergence
Sterile (irradiated)	1 (70-75Gy)	80.5 a
Sterile (irradiated)	2 (60-65Gy)	84.4 b
Fertile	1	85.1 b
Fertile	2	86.1 b

Within each column, values followed by the same letter are not significantly different from one another ($p > 0.05$). Means are back transformed from logit means. Time period 1: 21 November 2012 – 15 May 2014; Time period 2: 9 September 2014 – 23 August 2016

Our results provide support at the mass-rearing scale for the reduced dose of 60-65Gy, compared to the long-standing rate of 70-75Gy used to sterilize *B. tryoni*, and points to the possibility of a further reduced dose.

It is apparent that a higher irradiation dose and transportation both impact the emergence and flight of *B. tryoni*. A dose of 60-65Gy has been implemented at the FFPF for nearly 5 years with good quality control parameters/performance of *B. tryoni* while maintaining sterility, however a further reduced irradiation dose should be trialled at the mass rearing scale.

Microbial symbionts

This study is the first report of yeasts and yeast-like fungi in the midgut of wild-collected larval *B. tryoni* (Appendix C). There is only a single previous published report of yeasts in the alimentary canal of tephritid larvae (Darby and Kapp 1934). Our study identified yeasts and yeast-like fungi of the genera *Aureobasidium*, *Candida*, *Cryptococcus*, *Hanseniaspora*, *Pichia*, and *Starmerella*. The occurrence of these yeasts in fruits suggests that larvae consume these as part of their diet. Yeasts may play several roles including the provision of nutritional benefits, antagonistic activities against undesirable gut bacteria, and enhancement of bacterial probiotics. This work highlights that yeasts, and not solely bacteria, should be considered in future tephritid larval gut microbiota studies.

Overall, gut bacterial diversity was low for larval *B. tryoni*; however, wild larvae had greater bacterial diversity than domesticated larvae. This suggests that diet and the environment play a role in gut bacterial diversity. Further, high bacterial diversity between wild larvae feeding on peaches from the same tree, suggests that different bacteria may perform the same functional role/s. A single dominant bacterial genus *Asaia* (Acetobacteriaceae) was detected in all larvae (except one), suggesting this genus may have a fundamental role in *B. tryoni* larval development. In the mosquito, *Anopheles gambiae*, for example, the genus Asaia is important for larval development (Mitraka et al., 2013). Using microarray analysis, the authors showed that the larval genes, generally members of the CPR gene family of structural cuticular proteins, commonly affected are involved in cuticle formation. Other prevalent families found in *B. tryoni*

included Enterobacteriaceae and Leuconostocaceae. Understanding the gut microbiome and functional role of bacteria in *B. tryoni* will aid the development of probiotics, and therefore the quality of flies produced in SIT programs.

We selected four bacterial candidates from wild *B. tryoni* larvae. Our findings show that bacteria fed to larvae influence quality traits such as development time (Fig. 18) and pupal weight (Fig. 19). Shortened development time can reduce rearing costs (FAO/IAEA/USDA 2014). Higher pupal weight equates to heavier flies, and larger tephritids are generally more competitive than smaller ones (FAO/IAEA/USDA 2014). Therefore, flies produced from larvae fed *Lactobacillus* and the blend may be more competitive than those produced with *Asaia*, *Enterobacter*, and *Leuconostoc*, however this remains to be tested. There was no significant effect of feeding the bacterial treatments to the larvae on locomotor activity, i.e. there were no deleterious effects of the treatments on locomotor activity of mass-reared flies (Fig. 20). While these studies have implications for fertile *B. tryoni*, future studies need to test the effects of probiotic fed larvae on post-irradiated sterile males under SIT programs. Other performance traits also need to be measured including flight, survival and mating performance.

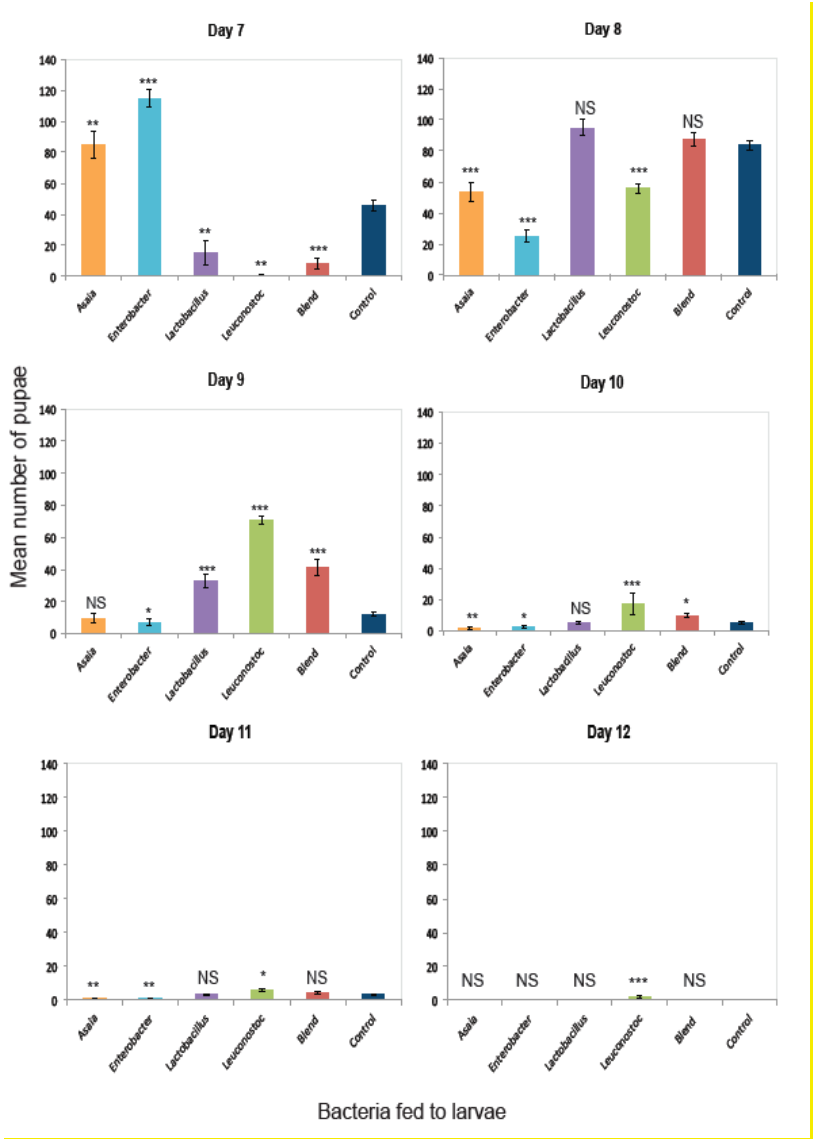

Figure 18. Larval development time in days (time from egg hatch to pupation) of Q-fly larvae fed various wild bacteria. Error bars indicate standard error of the mean. Asterixes '*' above columns indicate if the treatment is significantly different to the control within each day. * indicates p<0.05, ** indicates p<0.01 , *** indicates p<0.001. NS indicates not significantly different to the control within that day.

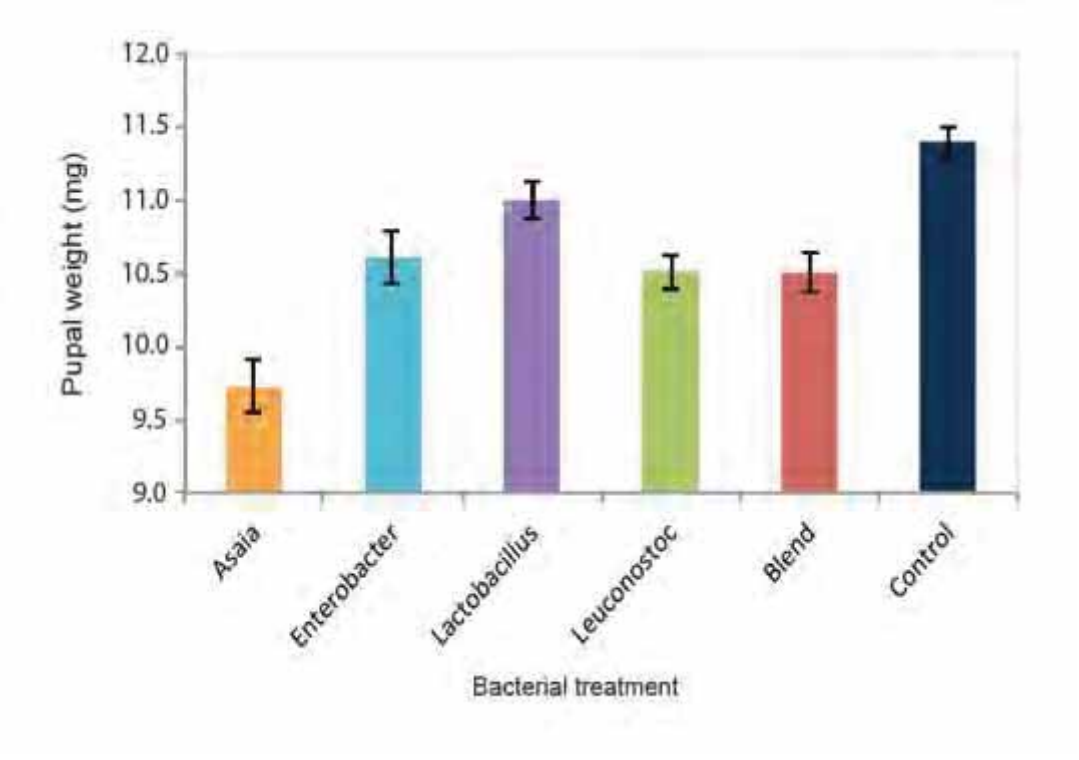

Figure 19. Pupal weight of mass-reared Q-fly larvae fed various wild bacteria. Error bars indicate standard error of the mean. Letters on the top of columns indicate the results of the ANOVA. If letters above two columns are different, they are significantly different to each other (df=55, $p<0.001$).

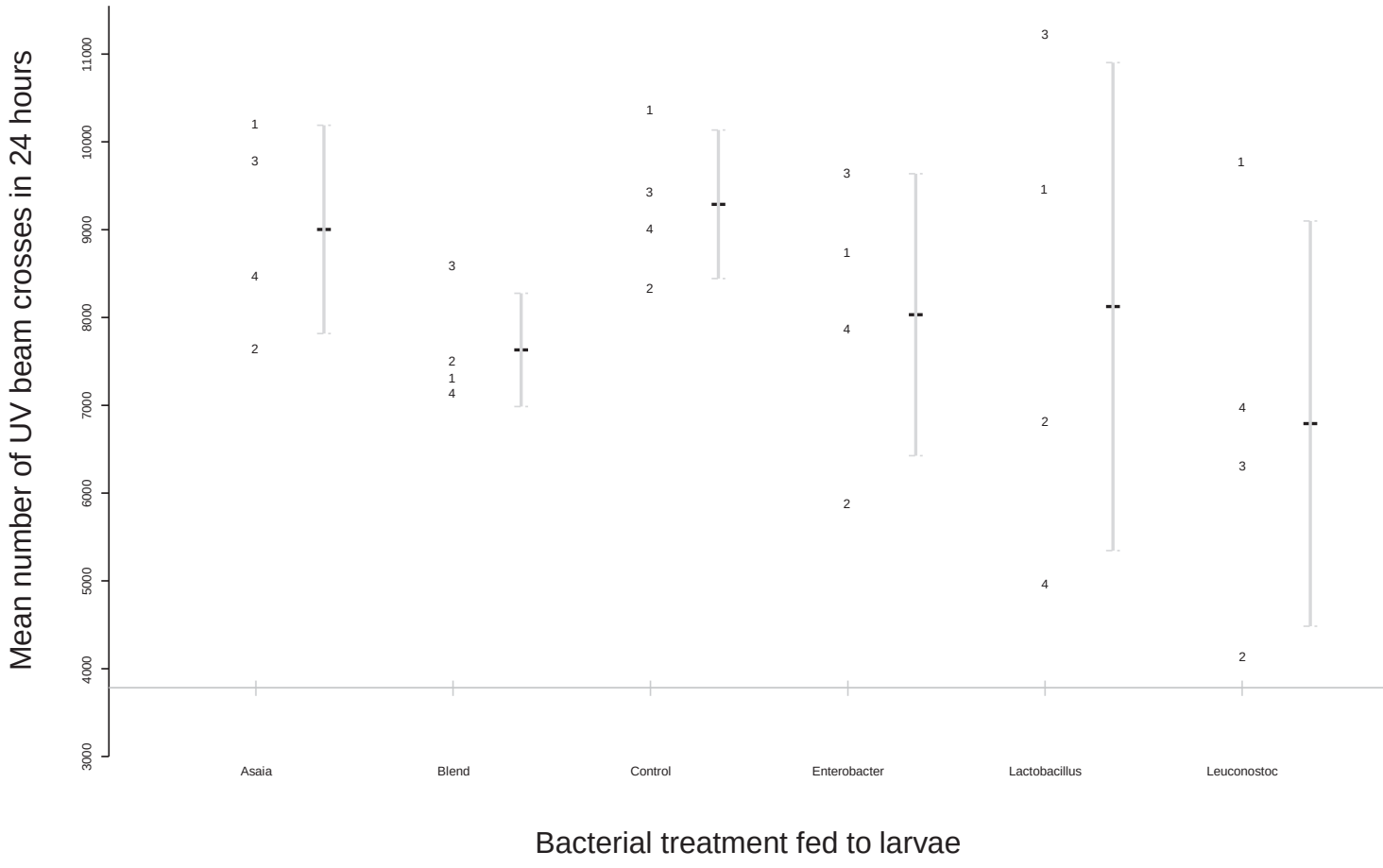

Figure 20. Total number of ultra-violet (UV) beam crosses by individual adult flies within single tubes in the locomotor activity monitor in a 24 hour period. Short black bars indicate mean of each treatment, vertical grey bars indicate standard error of the mean of each treatment. Numbers 1-4 indicate the total UV beam crosses over 24 hours of each replicate within each bacterial treatment.

Conclusions and Future Directions

When referring to AW-IPM SIT pilot studies Hendrichs et al., 2007 stated “They are a step of fundamental importance in progressing carefully towards the establishment of operational AW-IPM programmes”. The knowledge generated from this study should therefore be utilized to inform the development of future similar campaigns, and to increase the efficiency and effectiveness of the sterile insect technique under an AW-IPM scenario.

Recommendations

- The current sterile bisex *B. tryoni* strain can be released in commercial stone fruit orchards with little concern about the impact of stings caused by sterile females.
- Recommendations on the establishment of an AW-IPM SIT programs in Australia and implications for its wider use presented in the evaluation and discussion section should be adopted by industry.
- *Bactrocera tryoni* irradiated at 60-65Gy is recommended to be released in AW-IPM SIT programs for endemic areas, and should also be considered for pest free areas.
- The reduced irradiation dose of 60-65Gy currently used by the FFPF to irradiate *B. tryoni*, shows an increase in flight, and the same levels of sterility as flies irradiated at 70-75GY; therefore a further lowered irradiation dose should be trialed at the mass-rearing scale.
- Improve packaging and transport processes to minimize decreases in sterile *B. tryoni* performance
- Incorporate both yeast and bacteria in tephritid-microbial symbiont studies to increase fly performance and fitness as part of the SIT.
- Further develop larval (and adult) probiotics, to increase the fitness and performance of mass-reared and sterile *B. tryoni.*
- Test pathogens identified in tephritid-microbial studies and their potential in pest management.
- Develop a mating assay based on the annotated Y-chromosome of fertile *B. tryoni* males.

Scientific refereed publications

Journal articles

Deutscher AT, Burke CM, Darling AE, Riegler M, Reynolds OL# and Chapman TA#. In prep. Near Full-length 16S rRNA Gene Next-Generation Sequencing Revealed *Asaia* as a Common Midgut Bacterium of Wild and Domesticated Queensland Fruit Fly Larvae. To be submitted to *Microbiome*. # joint senior authors.

Shuttleworth L, Khan M, Collins, D and Reynolds OL. under review. Friend or foe? Wild larval gut bacteria fed to mass-reared larvae of Queensland fruit fly (*Bactrocera tryoni*) have varying effects on larval and adult fitness. Submitted to the Special Issue of *BMC Microbiology*.

Deutscher AT, Chapman TA, Riegler, M and Reynolds OL. under review. Symbiotic Associations of Mass-Reared Tephritid Fruit Flies: Implications for Sterile Insect Technique Programs. Submitted to the Special Issue of *BMC Microbiology*.

Deutscher AT, Reynolds OL and Chapman TA. 2016. Yeast: an Overlooked Component of *Bactrocera tryoni* (Diptera: Tephritidae) Larval Gut Microbiota. *Journal of Economic Entomology* 1–3 doi: 10.1093/jee/tow262.

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Intellectual property/commercialisation

No commercial IP generated.

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Acknowledgements

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Figure 21. Grower participants and project research leader from left to right: Andrew Finlay, Angus Ferrier, Nigel Pratt, Bim Goodrich, John Pratt, Olivia Reynolds, Rowan Berecry and Graham Finlay.

Appendices

Appendix A. Maps – orchard trap and sterile insect release sites

Appendix B. Static Heat Maps

Appendix C. Deutscher AT, Reynolds OL & Chapman TA. 2016. Yeast: an Overlooked Component of *Bactrocera tryoni* (Diptera: Tephritidae) Larval Gut Microbiota. *Journal of Economic Entomology* 1–3 doi: 10.1093/jee/tow262

Appendix D. Outputs – Magazines & other articles

Appendix E. Brochure - Reynolds O and Papacek D. 2015. Manage Queensland Fruit Fly. Its Your Responsibility. July 2015.

Appendix F. Queensland Fruit Fly Flyer

Appendix G. Oral Presentations

Appendix H. Posters

Appendix I. Media Release

Appendix J. Reynolds OL, Finlay A & Osborne T. 2015. Osage orange, *Maclura pomifera* (Rafinesque.) C.K. Schneid.: a new host record for *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) and *Delia platura* (Meigen) (Diptera: Anthomyiidae). *General and Applied Entomology*, 43, 19-23.

Appendix A.

Bottom Orchard

Queensland fruit fly 'Qfly'

Trap Sites

2015/2016

B5
B2
BF2
B3
BF3
BF1
B1
B6
B4b

Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Alternative hosts
- Orchard blocks

Meters
0 62.5 125

N

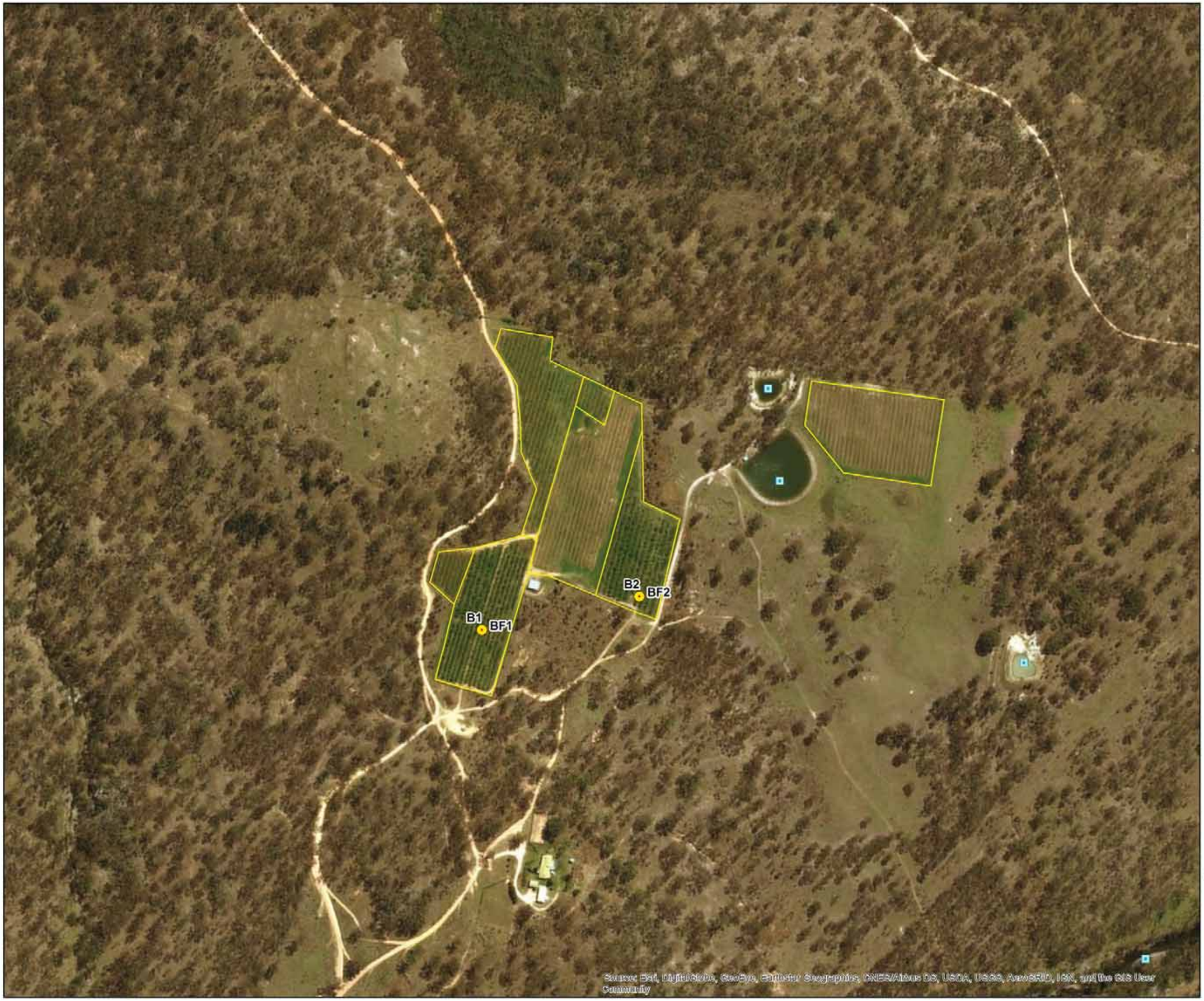
NSW GOVERNMENT Department of Primary Industries

Bottom Orchard

Queensland fruit fly 'Qfly'

Trap Sites

2016/2017



Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Alternative hosts
- Orchard blocks

Meters
0 62.5 125

N

NSW GOVERNMENT Department of Primary Industries

Toplawson Orchard

Queensland fruit fly 'Qfly'

Trap Sites

2015/2016

TL3
TLF3
TL5
TLF5
TLF4
TL4
TLF2
TL2
TL1b
TLF1
TL6

Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Alternative hosts
- Orchard blocks

Meters
0 50 100

N

NSW GOVERNMENT Department of Primary Industries

Toplawson Orchard

Queensland fruit fly 'Qfly'

Trap Sites

2016/2017

TL3
TLF3
TL4
TLF4
TL2
TLF2
TL1
TLF1

Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Alternative hosts
- Orchard blocks

Meters
0 50 100

N

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Queensland fruit fly 'Qfly'

Trap and Release Sites

2014/2015

T1b
T12b
TR8
T11b
TR7
TF1
T2b
TR9
T10b
TR6
T15b
TR10
TR2
T4b
TF3
TR5
T7b
T8b
TF4
T9b
TR1
TR4
T13b
T3b
TF2
T6b
TR3
T14b
T5b

Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Orchard blocks

Meters
0 175 350

N

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Queensland fruit fly 'Qfly'

Trap and Release Sites

2015/2016

T1b
TR11
TR15
TR8
T11b
TR7
TF1
T2b
TR9
T10b
TR6
T15b
TR10
TR12
TR2
TF3
T4b
TR5
T7b
TF4
T8b
T9b
TR1
TR4
TR14
T13b
TR13
TF2
T3b
T6b
TR3
T14b
T5b

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Orchard blocks

Meters
0 175 350

N

NSW Department of Primary Industries

Traprock Orchard

Queensland fruit fly 'Qfly'

Trap and Release Sites

2016/2017

T1b
No17
No16
No18
T11b
TF1
T2b
No15
T10b
No7
No14
T15b
No12
No5
No6
T4b
No13
T7b
TF4
T8b
No4
No3
No1
T9b
No2
No8
T13b
TF2
T3b
No9
No11
T6b
No10
T14b

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Orchard blocks

Meters
0 175 350

N

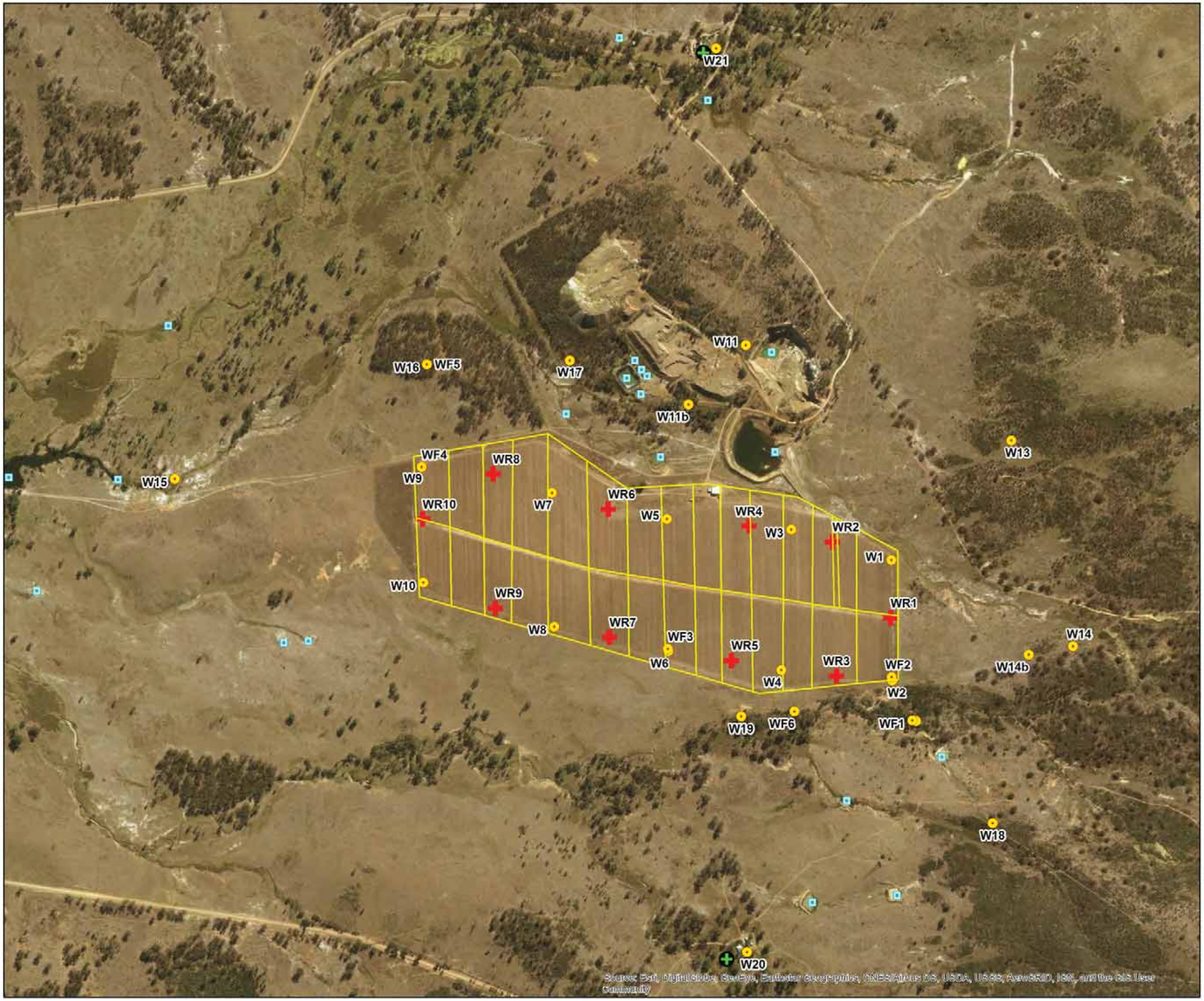
NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Queensland fruit fly 'Qfly'

Trap and Release Sites

2014/2015



Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Alternative hosts
- Orchard blocks

Meters

0 225 450

N

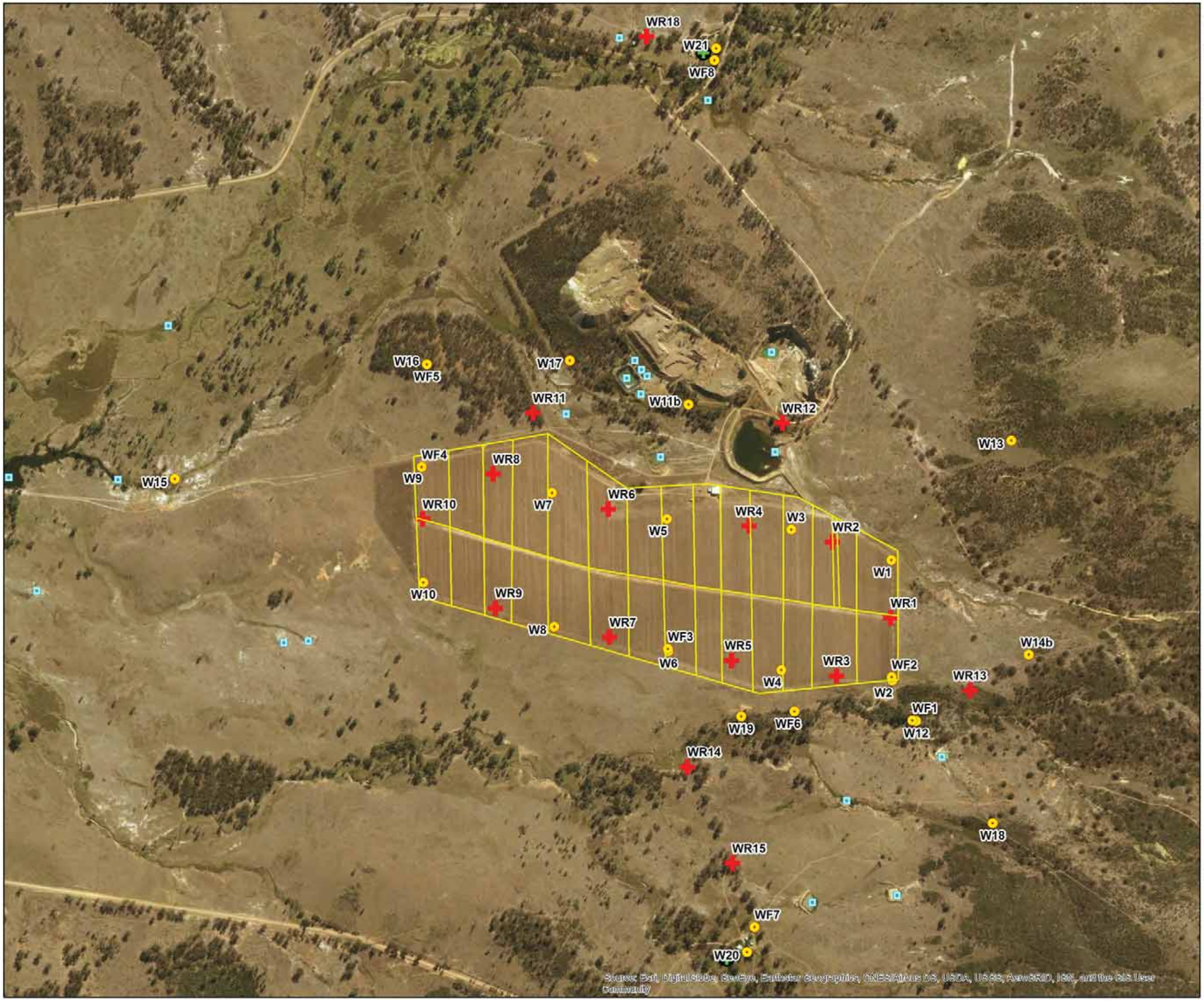
NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Queensland fruit fly 'Qfly'

Trap and Release Sites

2015/2016



Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Alternative hosts
- Orchard blocks

Meters

0 225 450

N

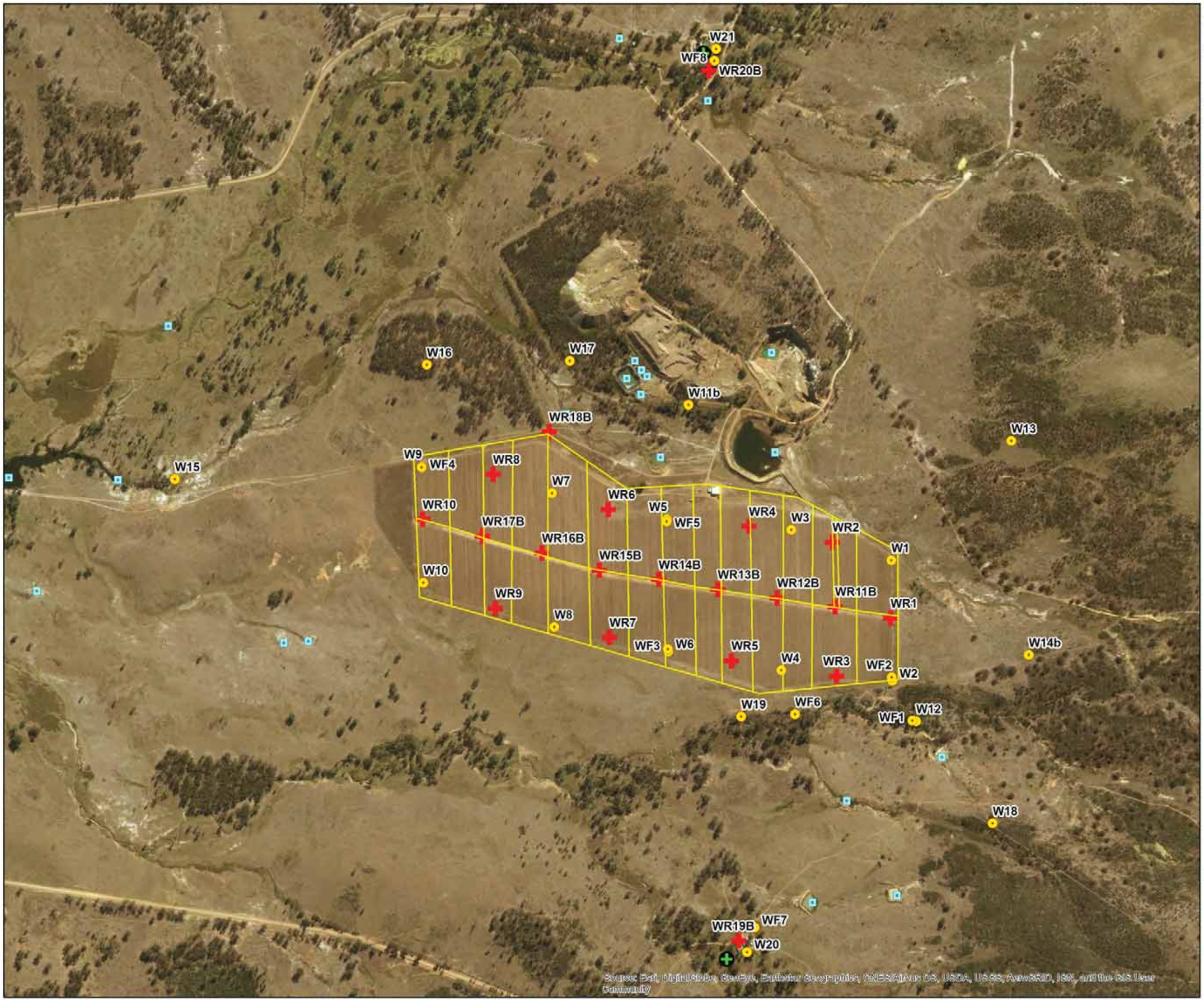
NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Queensland fruit fly 'Qfly'

Trap and Release Sites

2016/2017



Legend

- Qfly Traps
- Adult Qfly Release Sites
- Water Source
- Alternative hosts
- Orchard blocks

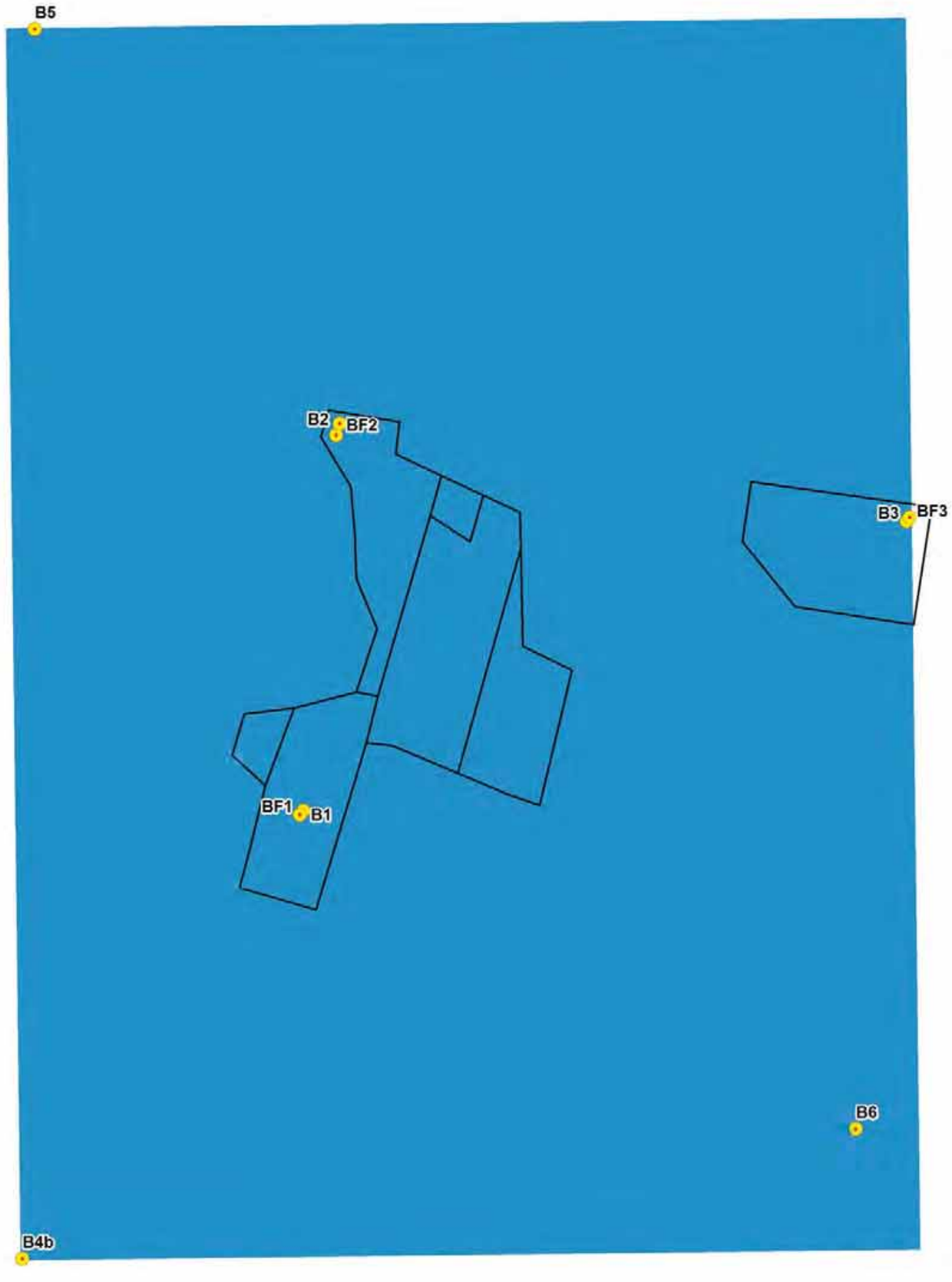
Meters

0 225 450

N

NSW GOVERNMENT Department of Primary Industries

Appendix B.



Bottom Orchard

Wild Female Queensland fruit fly 'Qfly'

2015/2016

Legend

Qfly Trapping density

High

Low

Qfly Traps

Water Source

Alternative hosts

Orchard blocks

N

Meters

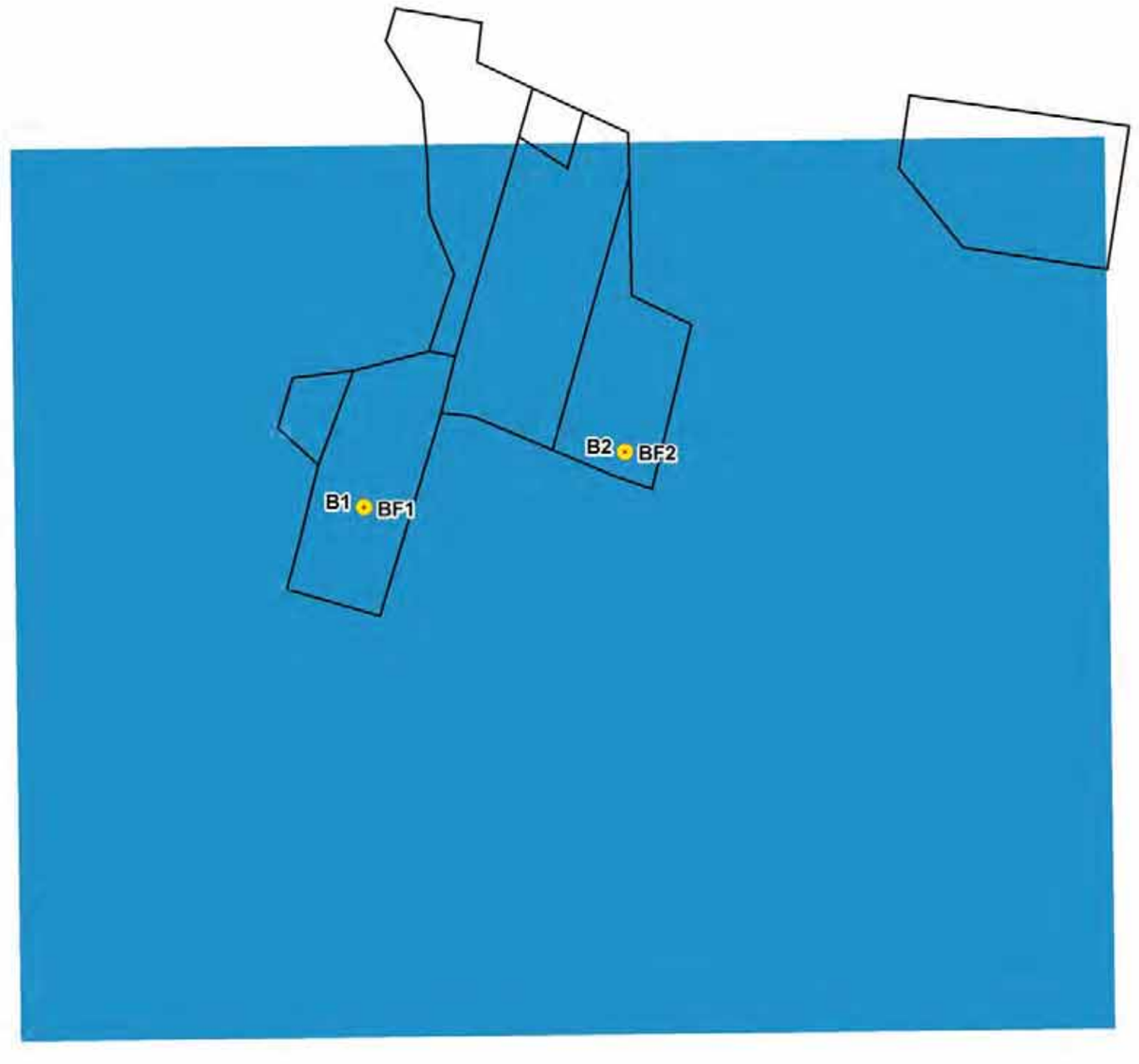
0 100 200

NSW GOVERNMENT Department of Primary Industries

Bottom Orchard

Wild Female Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Qfly Traps

Water Source

Alternative hosts

Orchard blocks

N

Meters

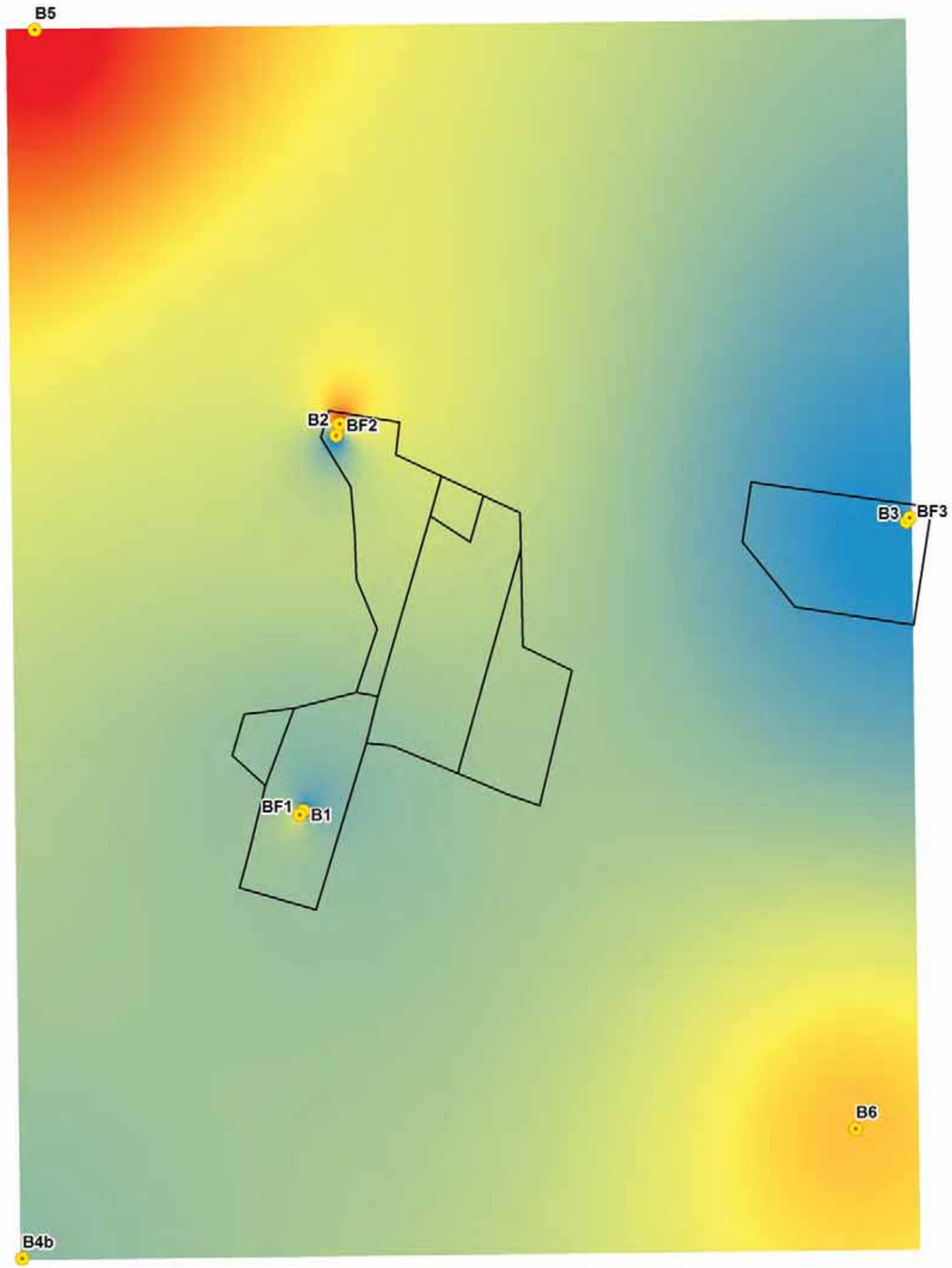
0 100 200

NSW GOVERNMENT Department of Primary Industries

Bottom Orchard

Wild Male Queensland fruit fly 'Qfly'

2015/2016



Legend

Qfly Trapping density

High

Low

Qfly Traps

Water Source

Alternative hosts

Orchard blocks

N

Meters

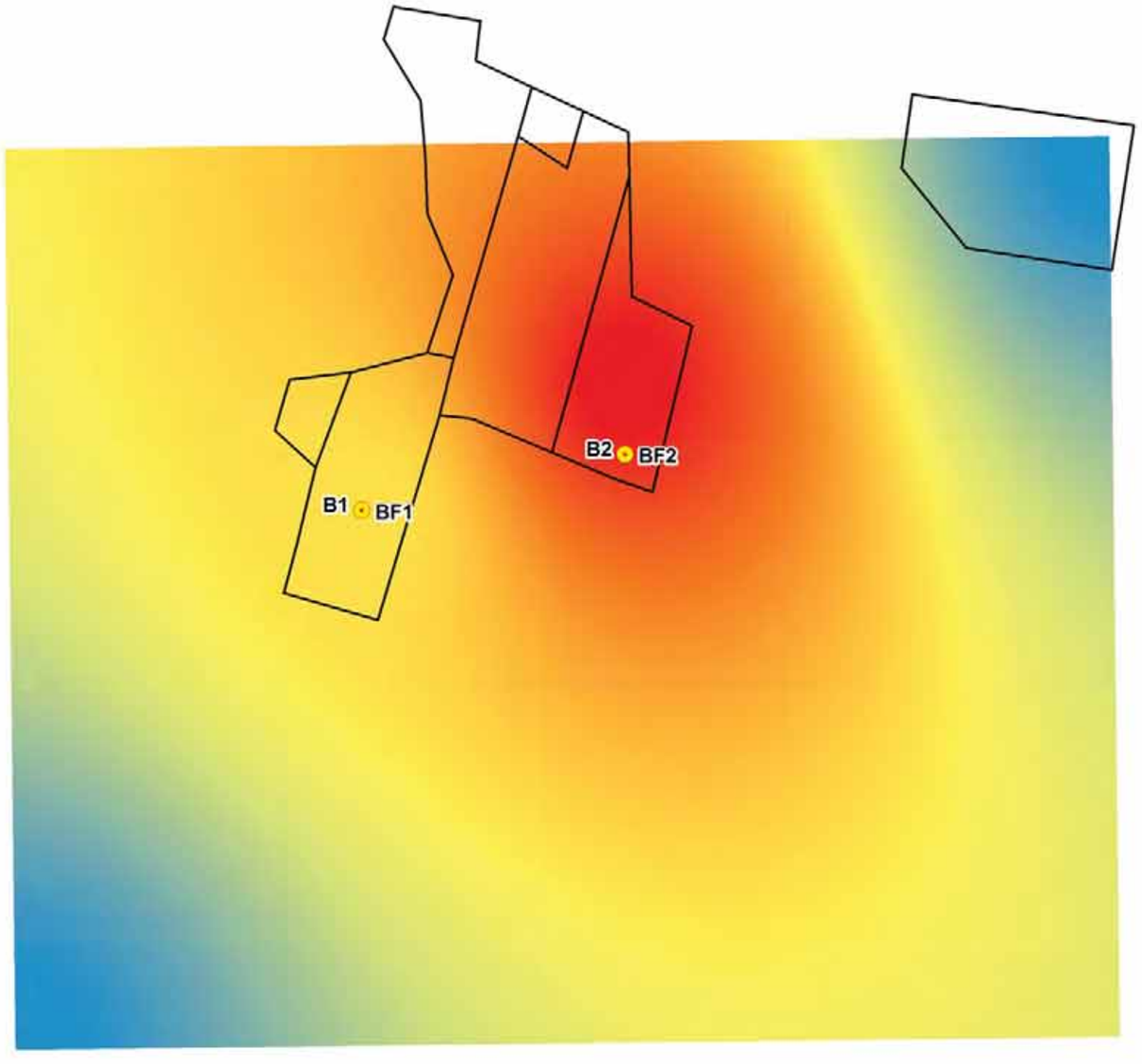
0 100 200

NSW GOVERNMENT Department of Primary Industries

Bottom Orchard

Wild Male Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Qfly Traps

Water Source

Alternative hosts

Orchard blocks

N

Meters

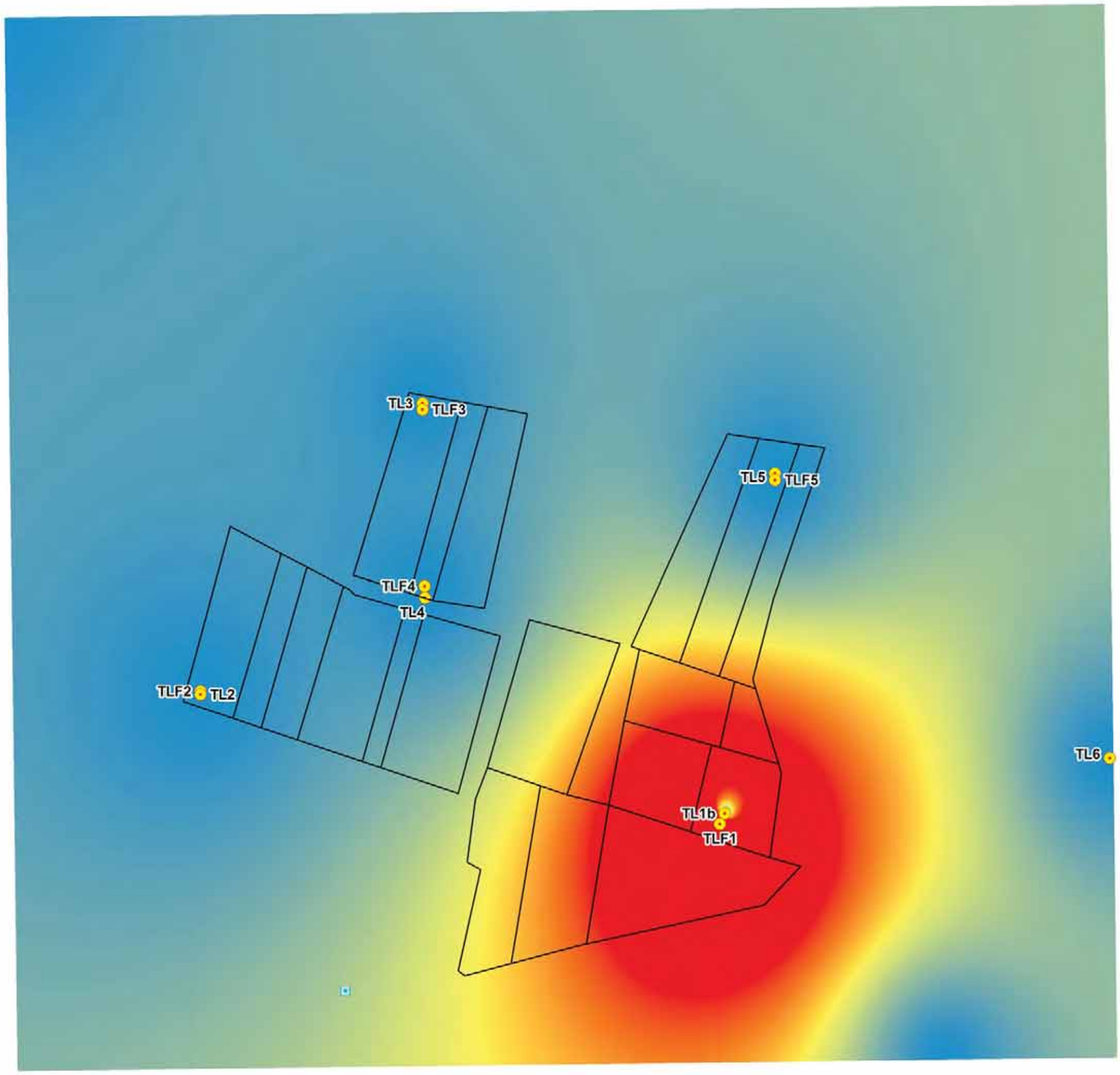
0 100 200

NSW GOVERNMENT Department of Primary Industries

Toplawson Orchard

Wild Female Queensland fruit fly 'Qfly'

2015/2016



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

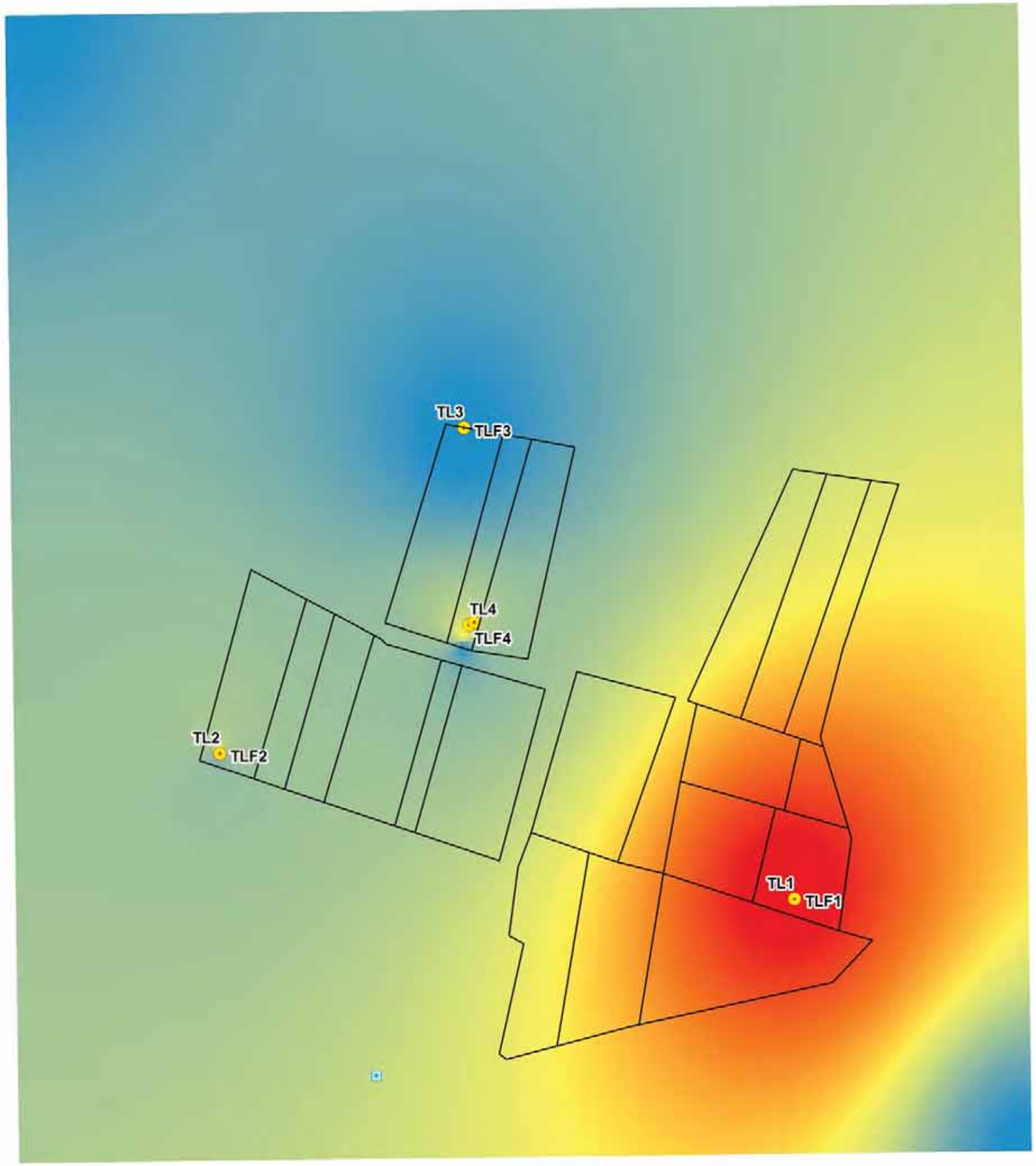
0 100 200

NSW GOVERNMENT Department of Primary Industries

Toplawson Orchard

Wild Female Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

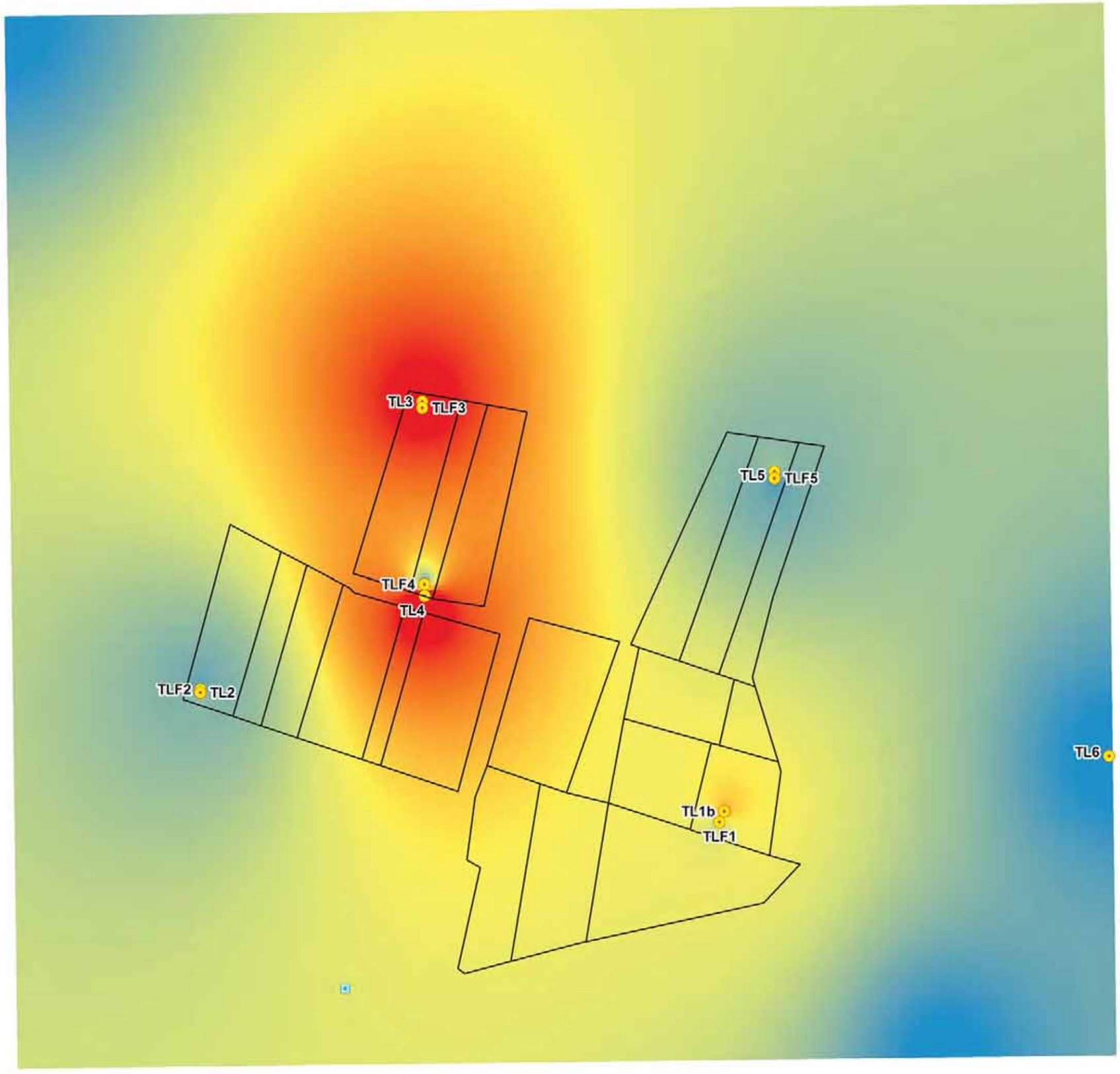
0 100 200

NSW GOVERNMENT Department of Primary Industries

Toplawson Orchard

Wild Male Queensland fruit fly 'Qfly'

2015/2016



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

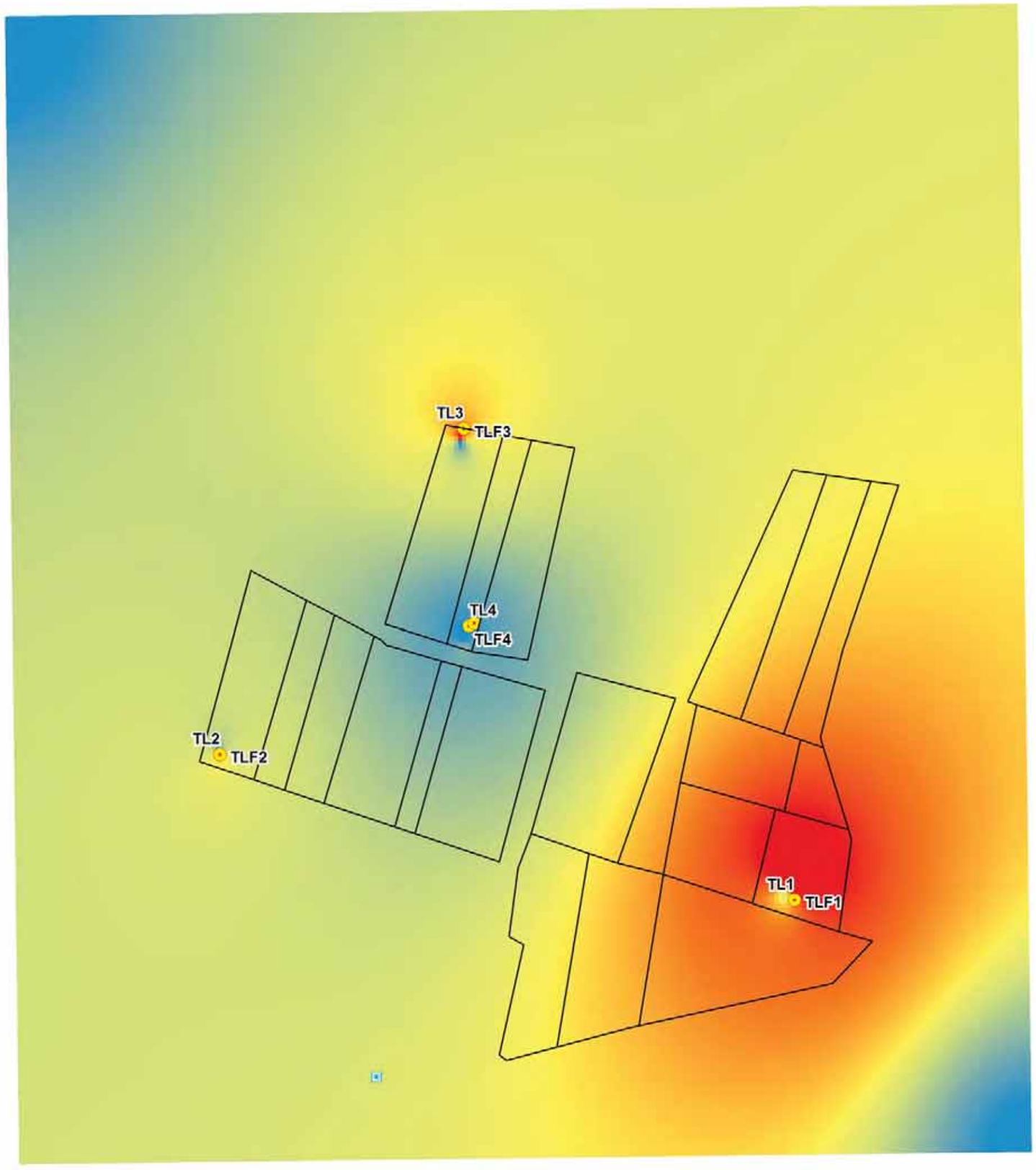
0 100 200

NSW GOVERNMENT Department of Primary Industries

Toplawson Orchard

Wild Male Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

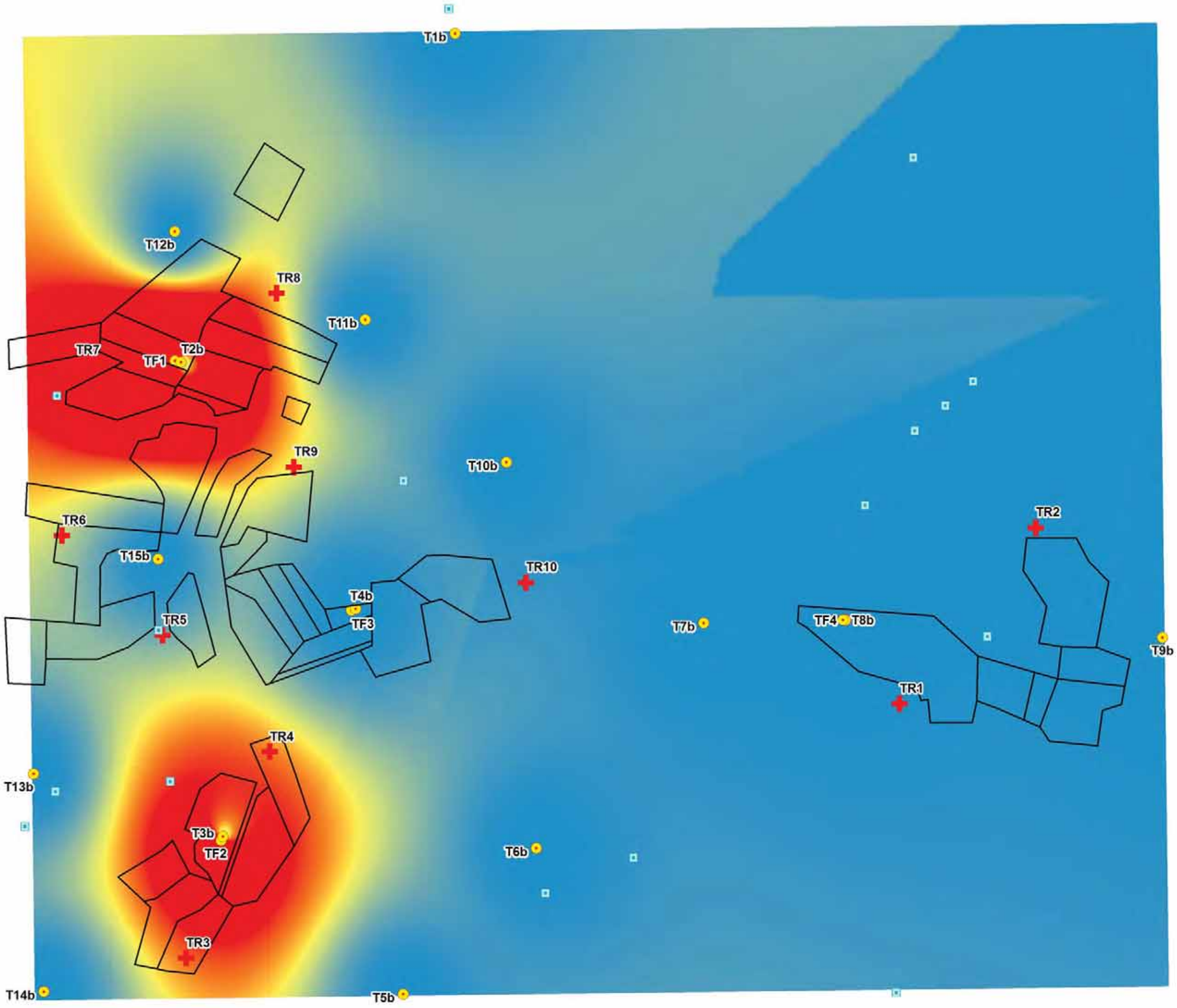
0 100 200

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Sterile Female Queensland fruit fly 'Qfly'

2014/2015



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

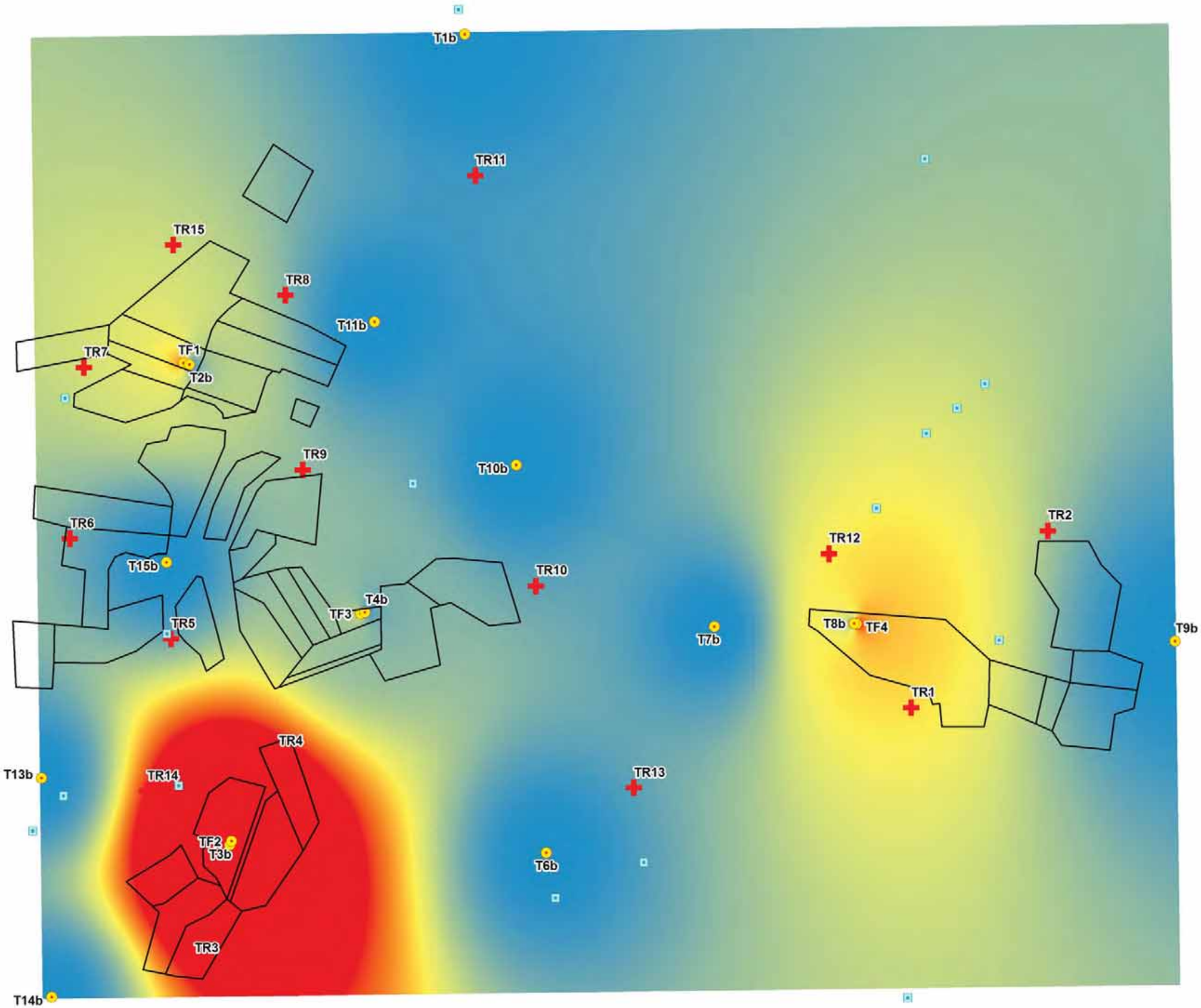
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Sterile Female Queensland fruit fly 'Qfly'

2015/2016



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

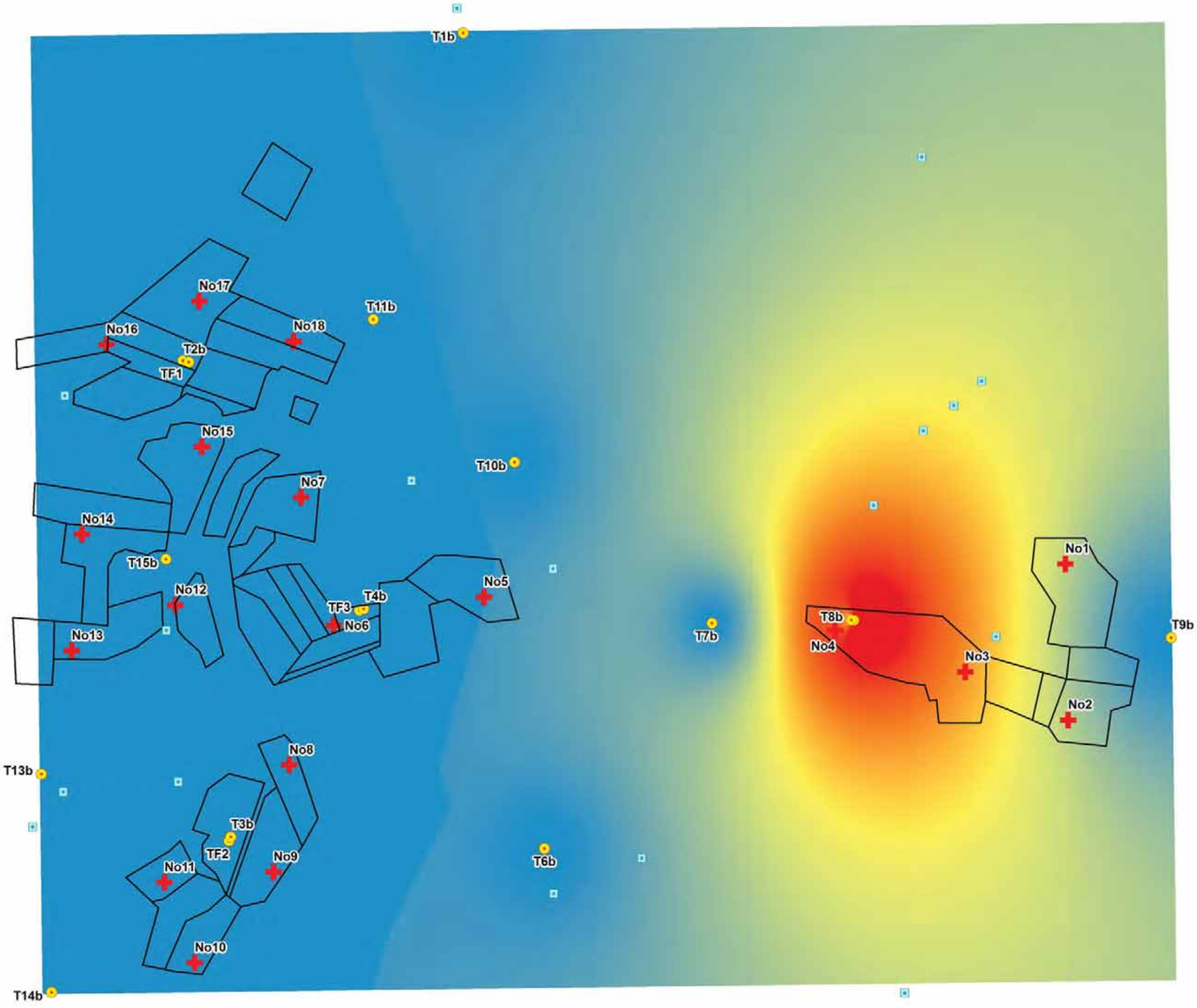
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Sterile Female Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

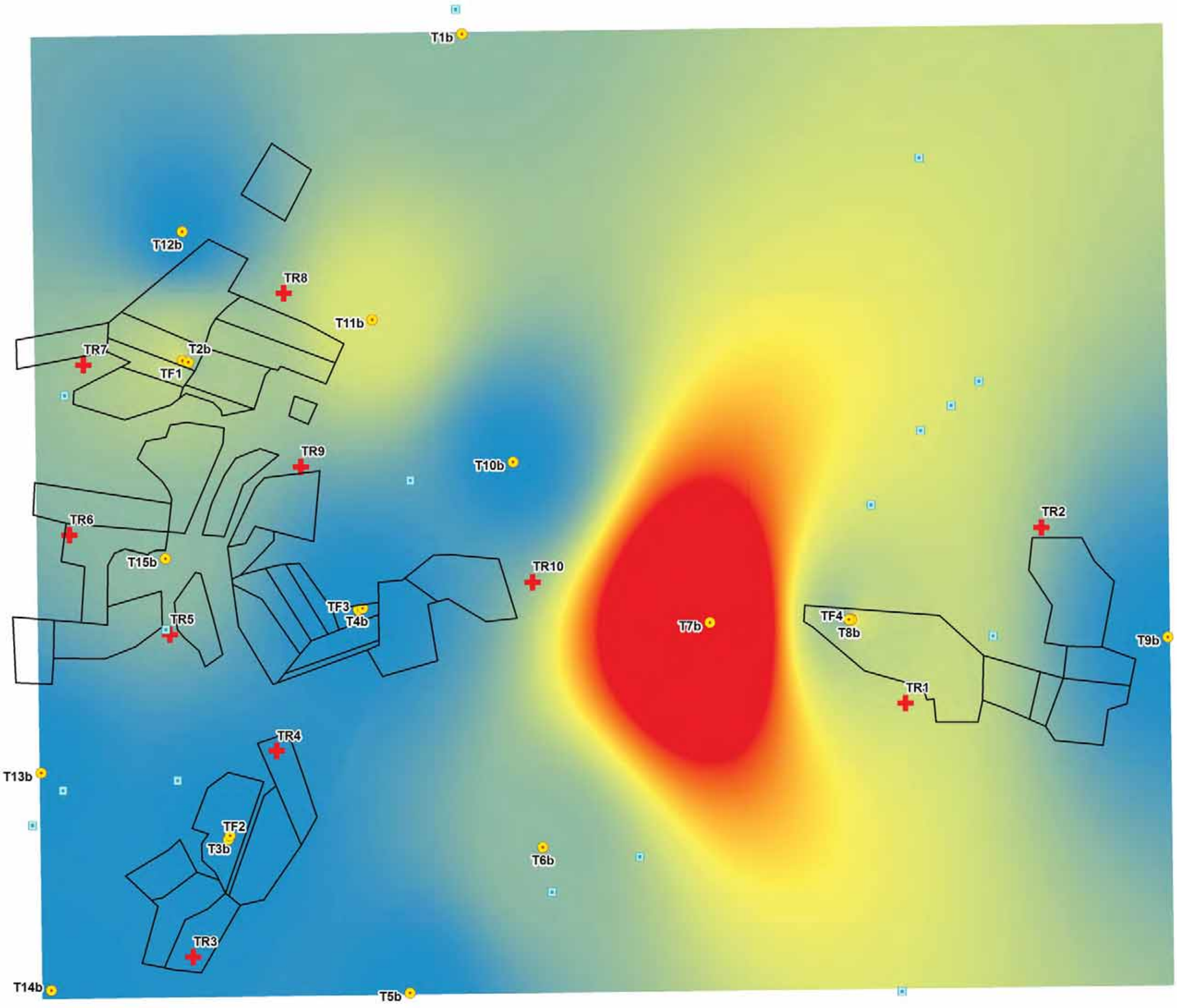
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Sterile Male Queensland fruit fly 'Qfly'

2014/2015



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

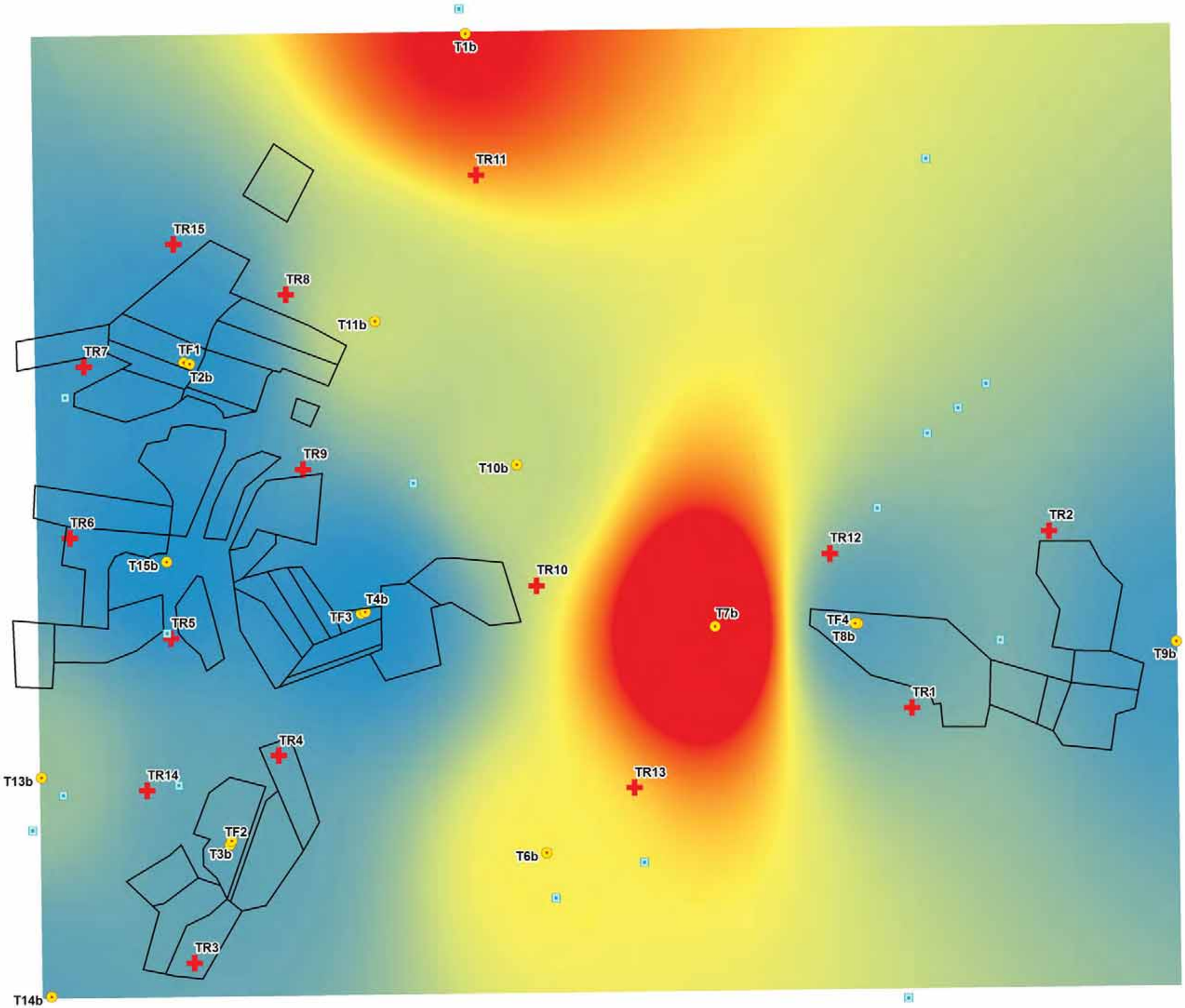
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Sterile Male Queensland fruit fly 'Qfly'

2015/2016



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

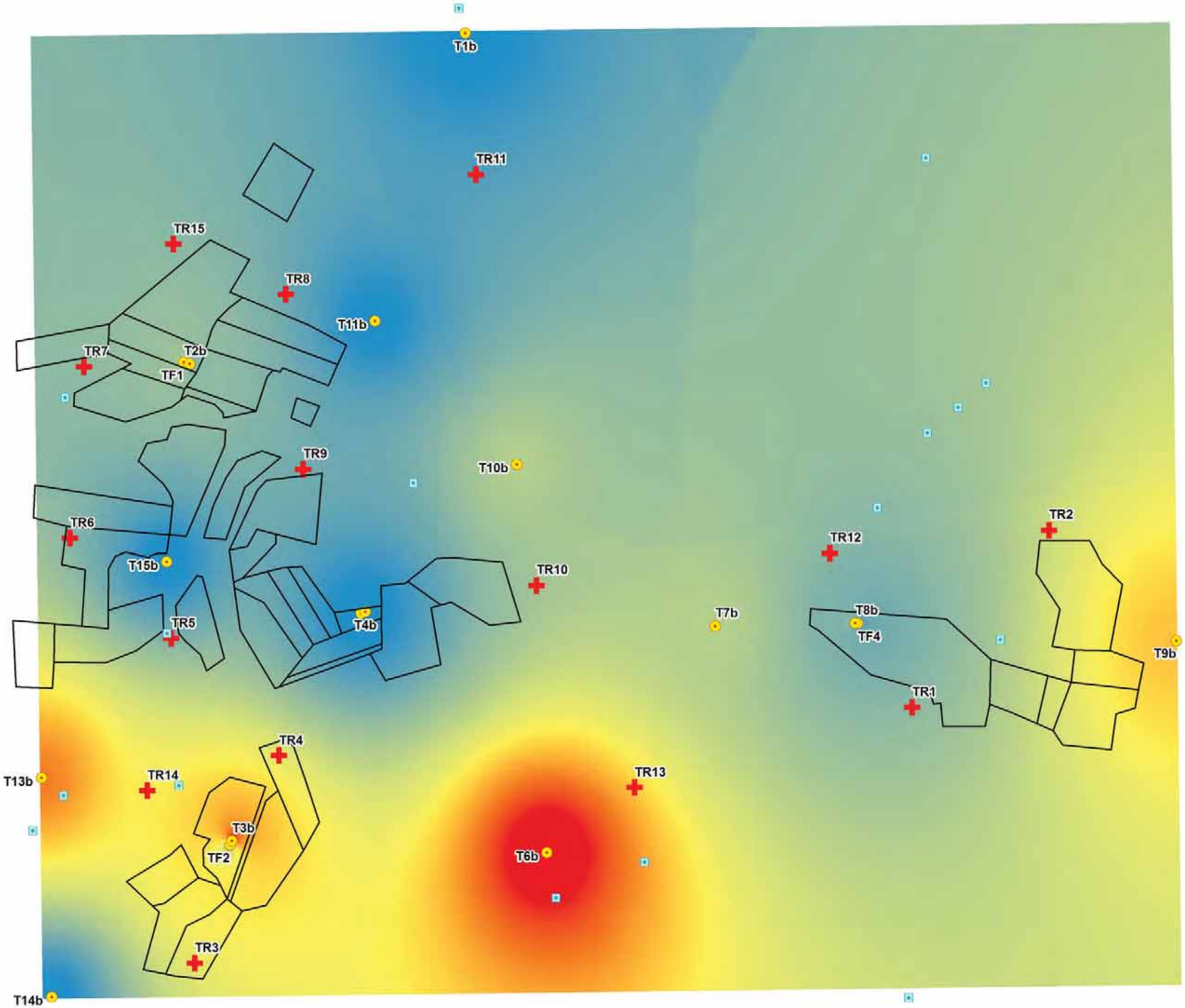
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Sterile Male Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

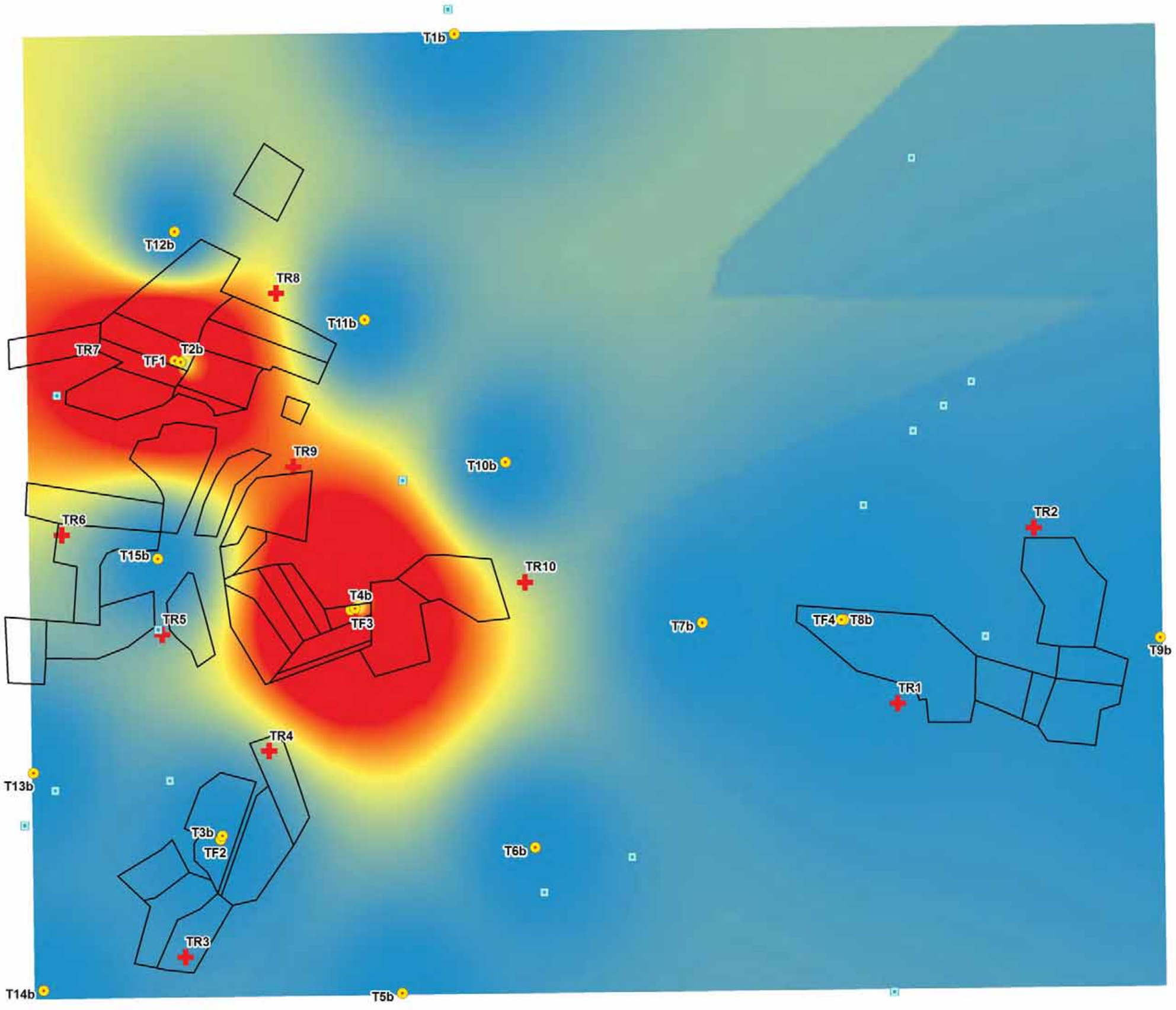
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Wild Female Queensland fruit fly 'Qfly'

2014/2015



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

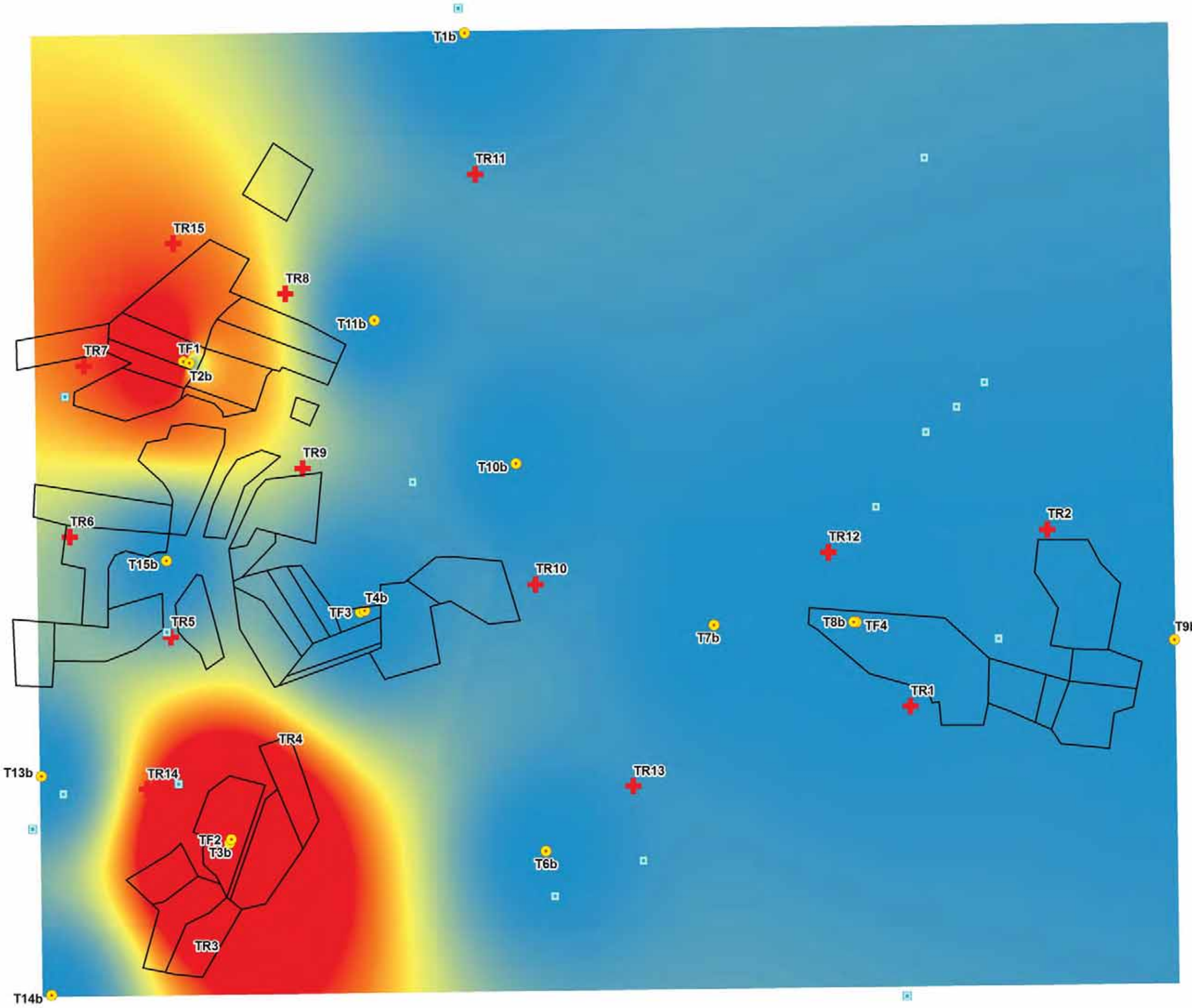
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Wild Female Queensland fruit fly 'Qfly'

2015/2016



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

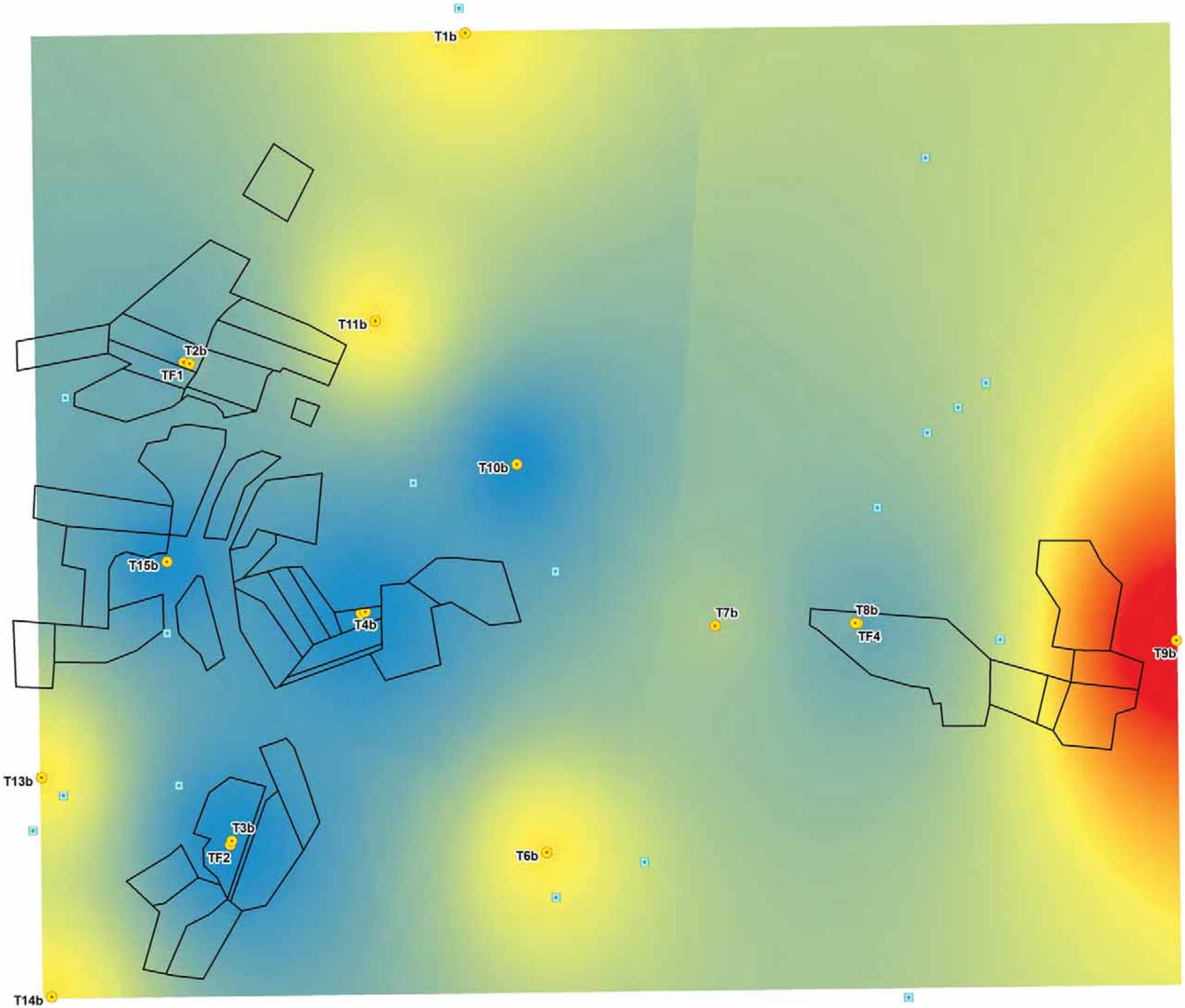
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Wild Female Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Qfly Traps

Water Source

Alternative hosts

Orchard blocks

N

Meters

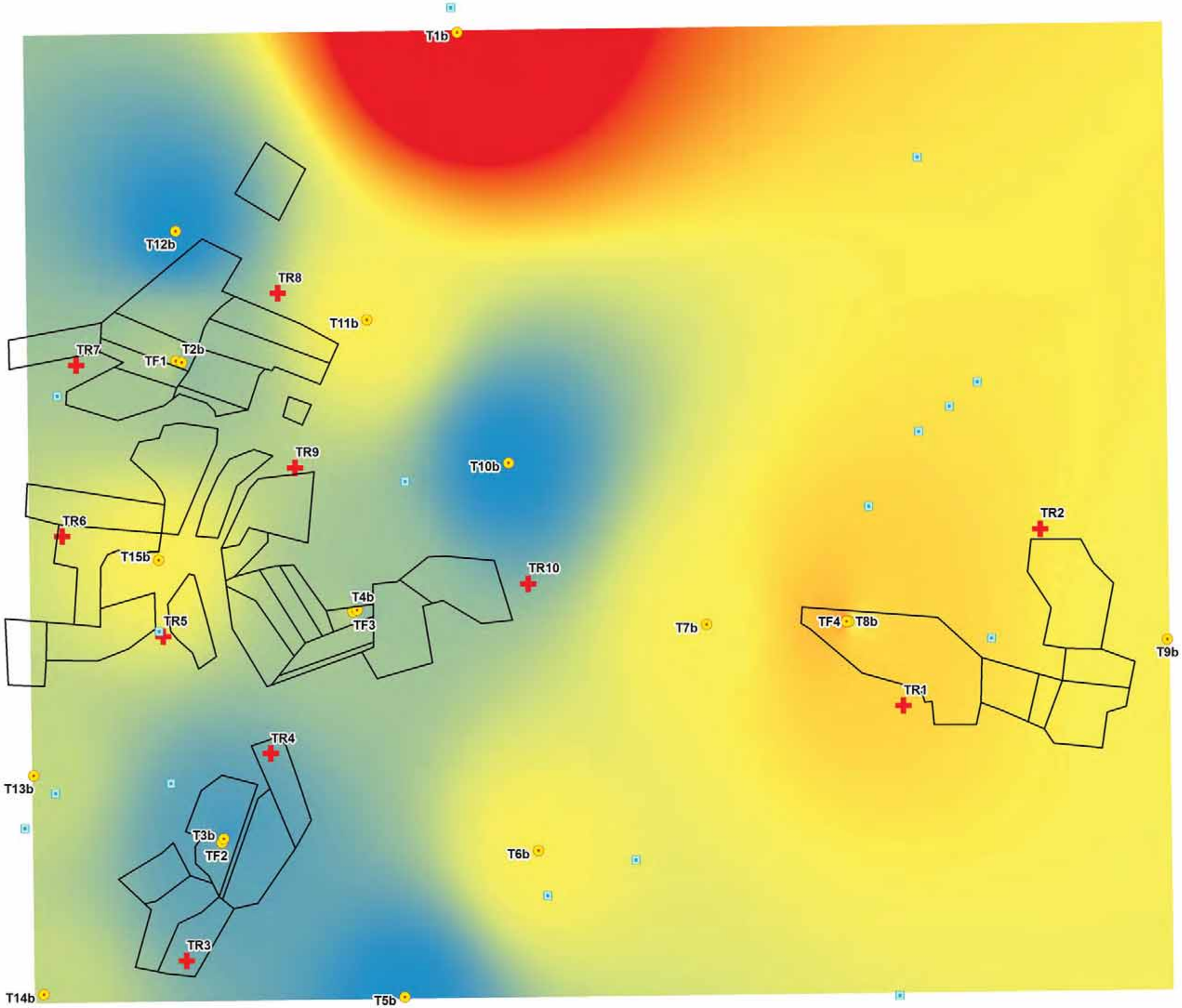
0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Wild Male Queensland fruit fly 'Qfly'

2014/2015



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

0 175 350

NSW GOVERNMENT Department of Primary Industries

Traprock Orchard

Wild Male Queensland fruit fly 'Qfly'

2015/2016

T1b
TR11
TR15
TR8
T11b
TR7
TF1
T2b
TR9
T10b
TR6
T15b
TR10
T4b
TF3
TR5
T7b
TR12
TR2
TF4
T8b
T9b
TR1
TR4
T13b
TR14
TR13
TF2
T3b
T6b
TR3
T14b

Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

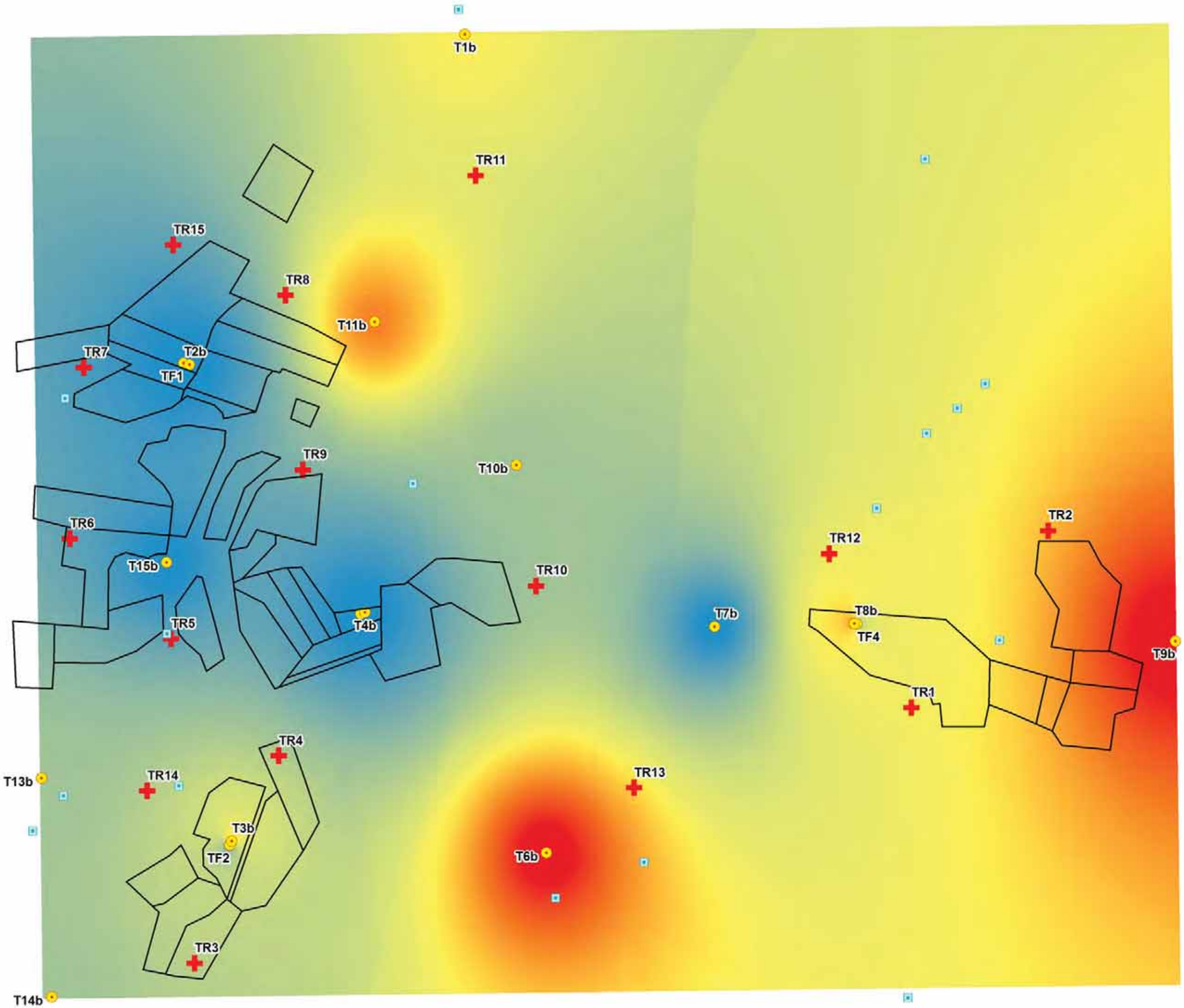
0 175 350

NSW Department of Primary Industries

Traprock Orchard

Wild Male Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Orchard blocks

N

Meters

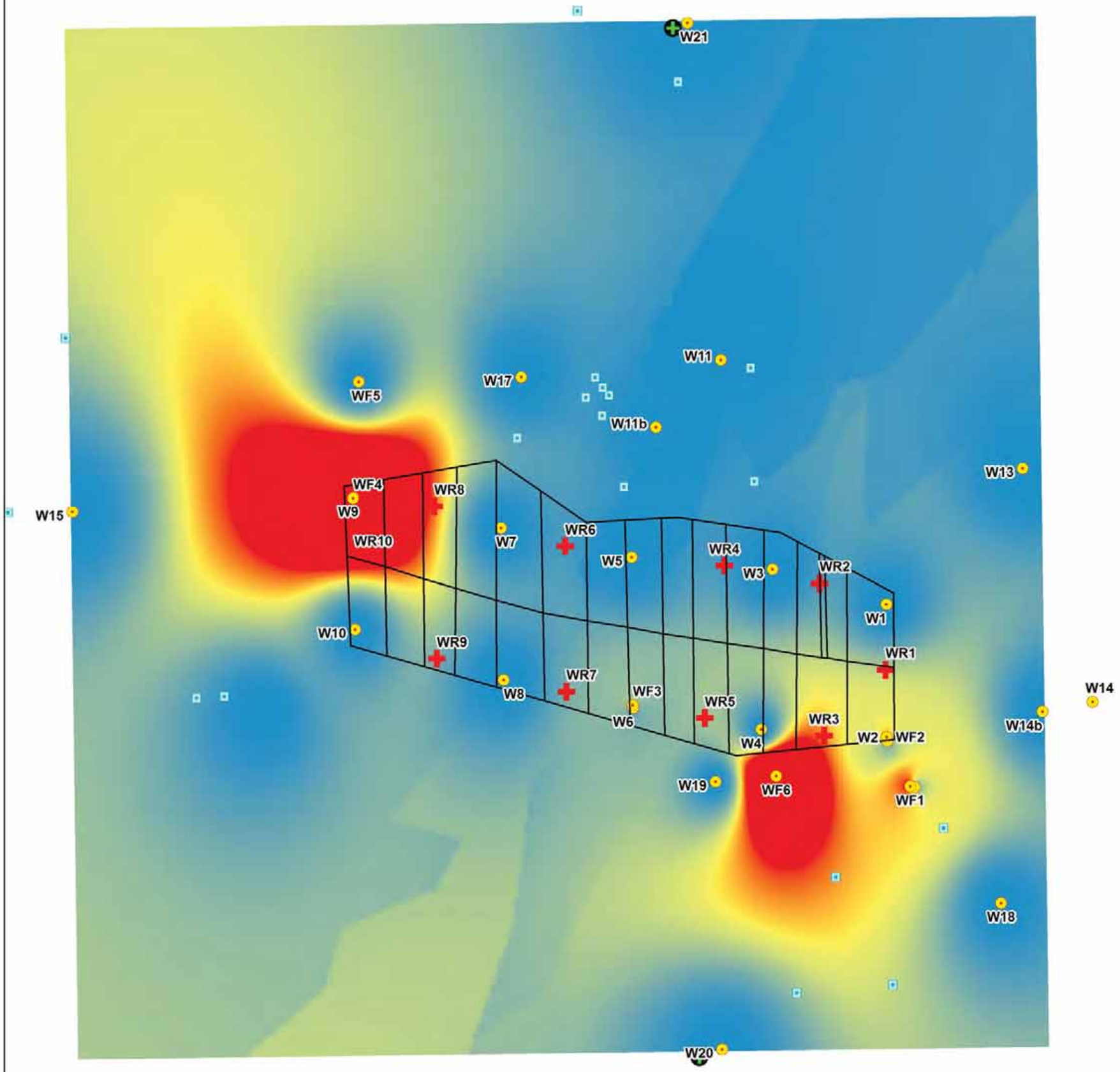
0 175 350

NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Sterile Female Queensland fruit fly 'Qfly'

2014/2015



Legend

Qfly Trapping density

High

Low

- Sterile Qfly release
- Qfly Traps
- Water Source
- Alternative hosts
- Orchard blocks

N

Meters

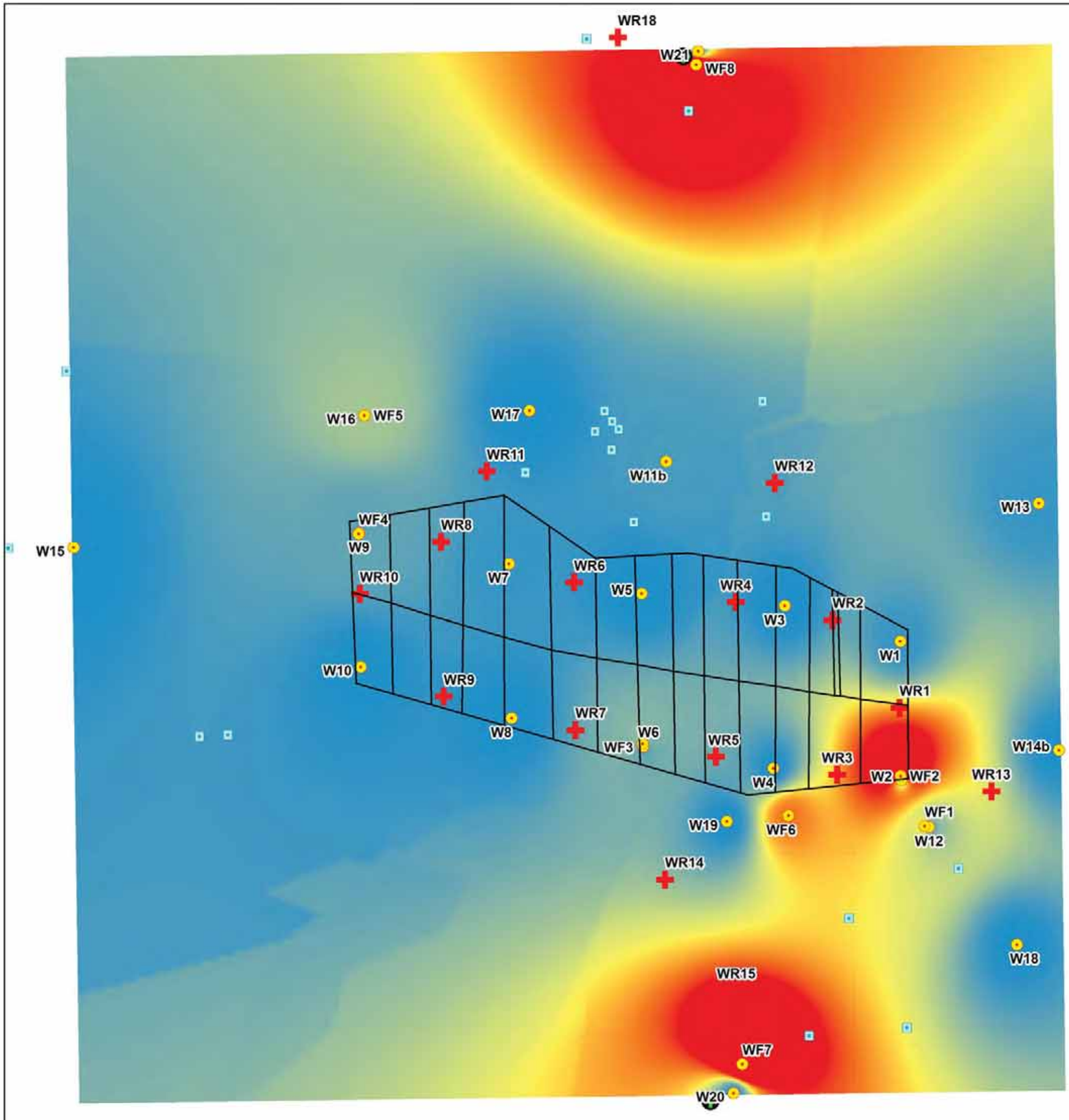
0 250 500

NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Sterile Female Queensland fruit fly 'Qfly'

2015/2016



Legend

Qfly Trapping density

High

Low

- Sterile Qfly release
- Qfly Traps
- Water Source
- Alternative hosts
- Orchard blocks

N

Meters

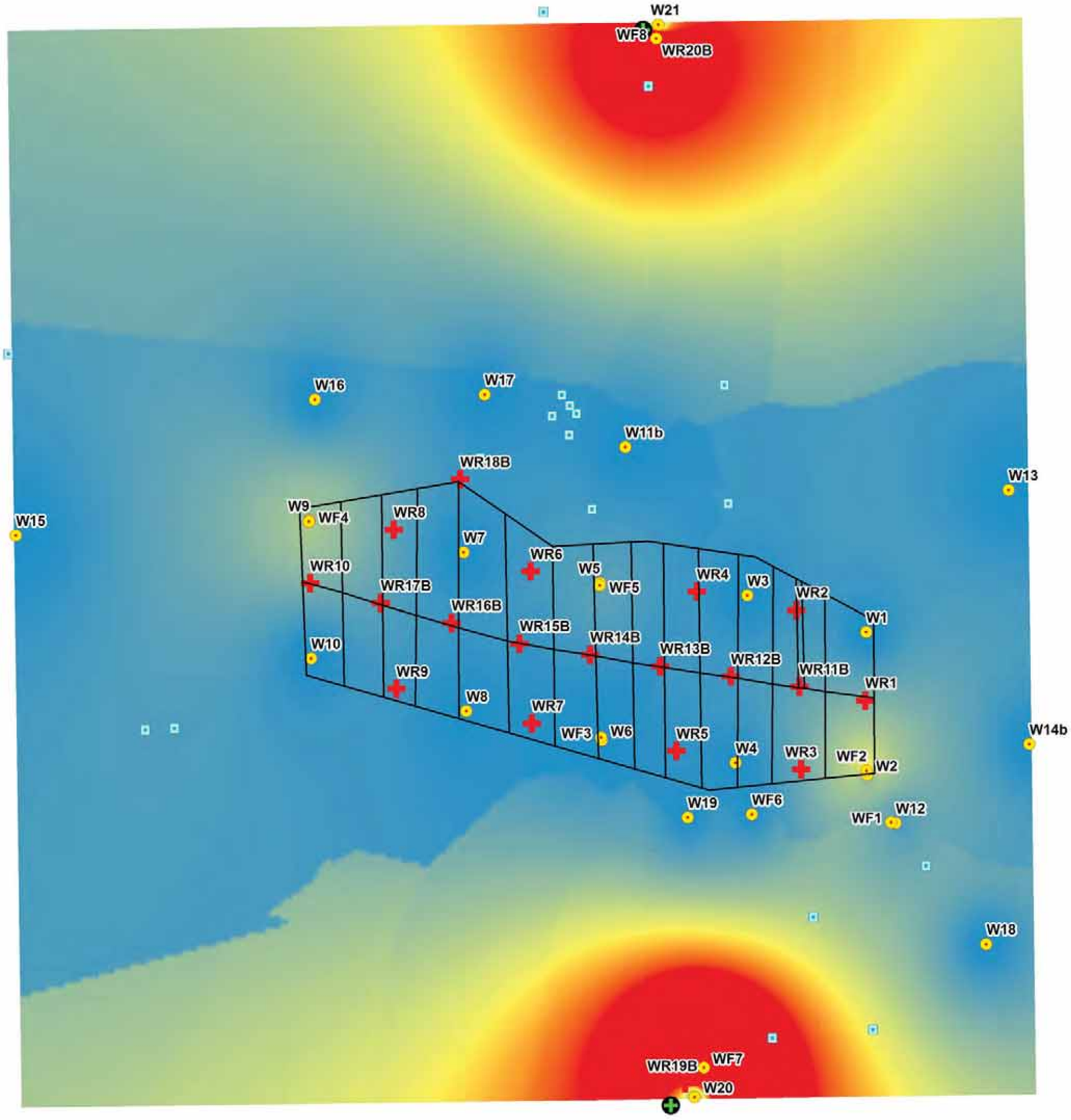
0 250 500

NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Sterile Female Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Alternative hosts

Orchard blocks

N

Meters

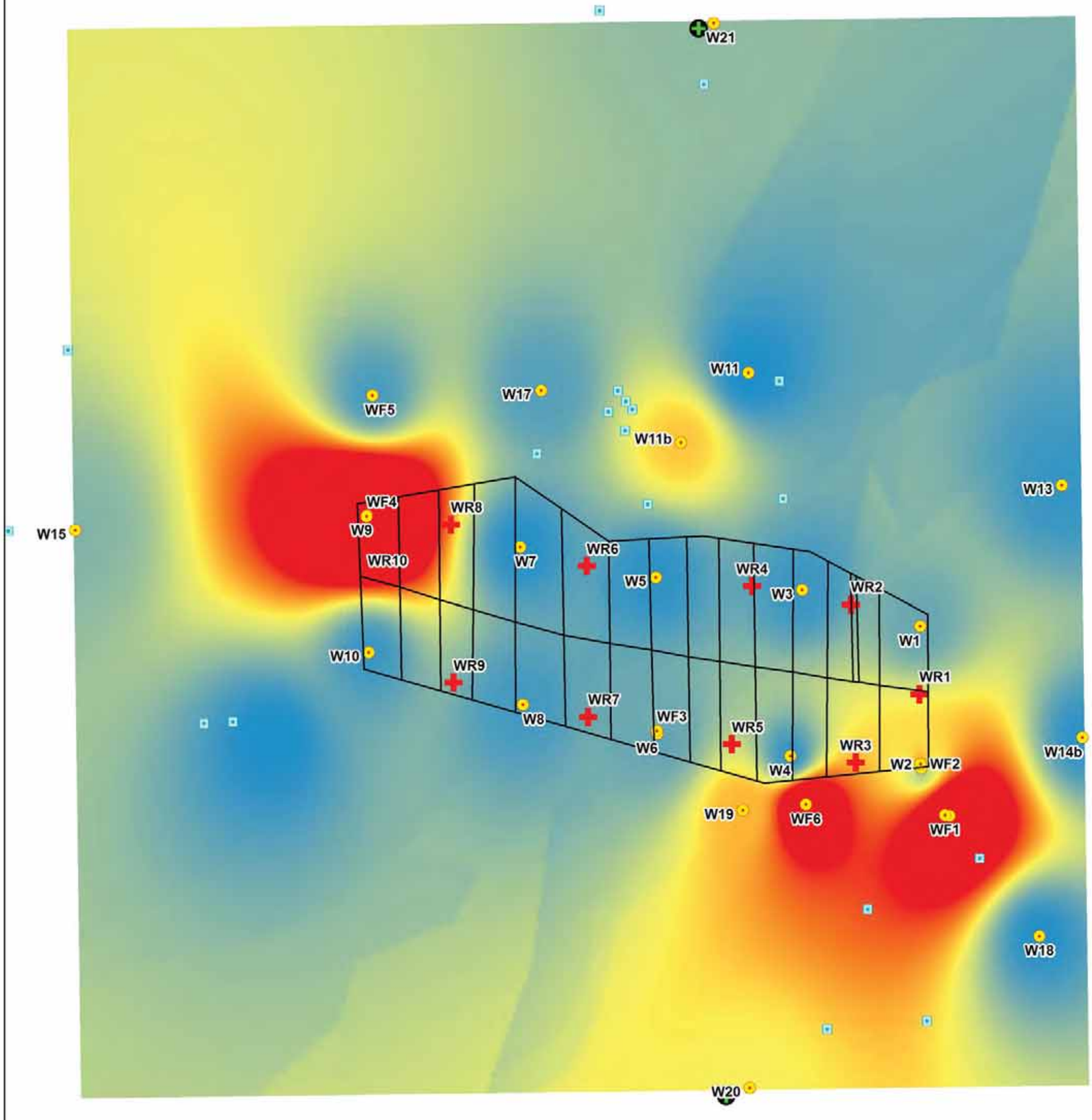
0 250 500

NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Sterile Male Queensland fruit fly 'Qfly'

2014/2015



Legend

Qfly Trapping density

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- Sterile Qfly release
- Qfly Traps
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- Alternative hosts
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N

Meters

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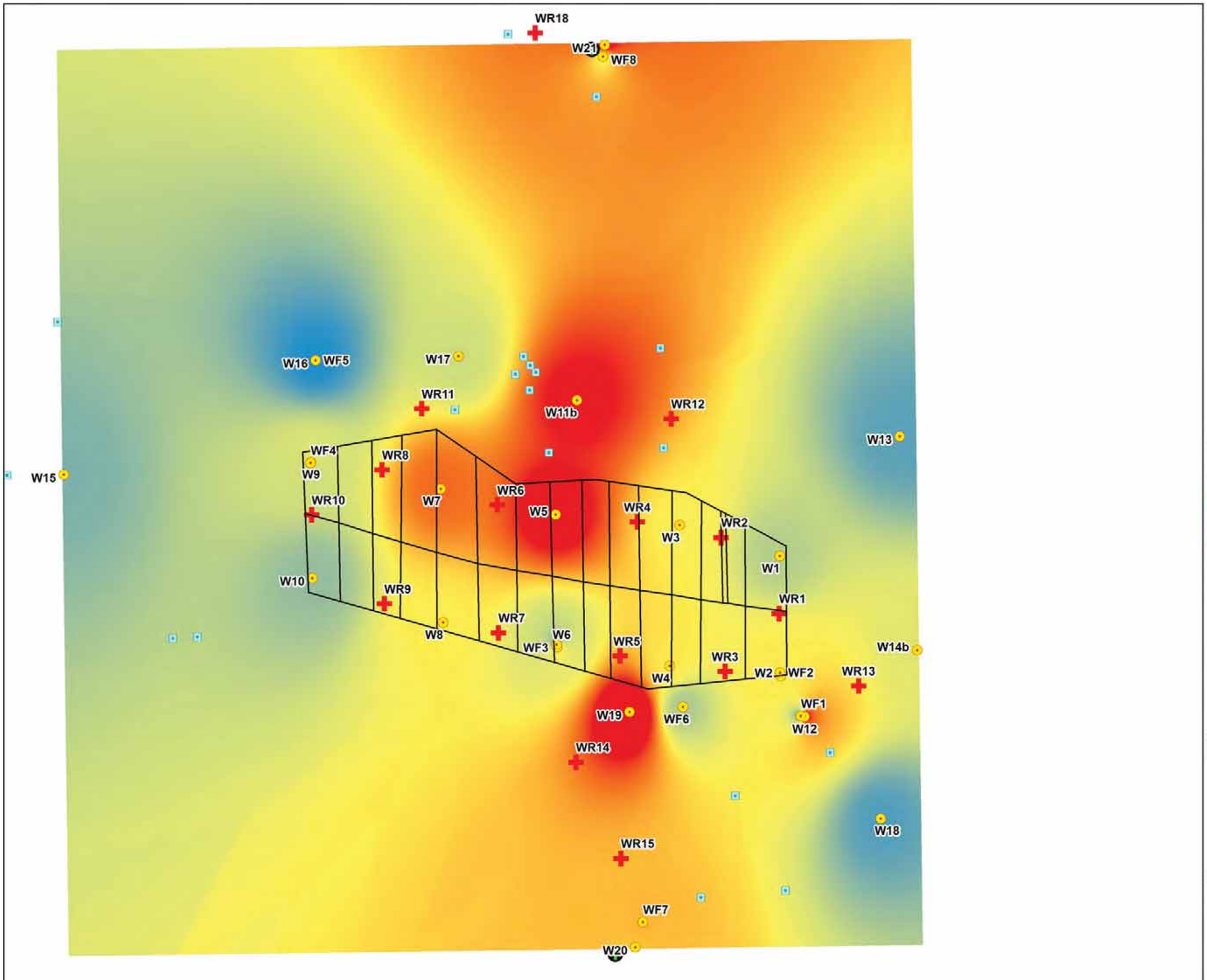
NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Sterile Male Queensland fruit fly 'Qfly'

2015/2016



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Qfly Trapping density

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- Qfly Traps
- Water Source
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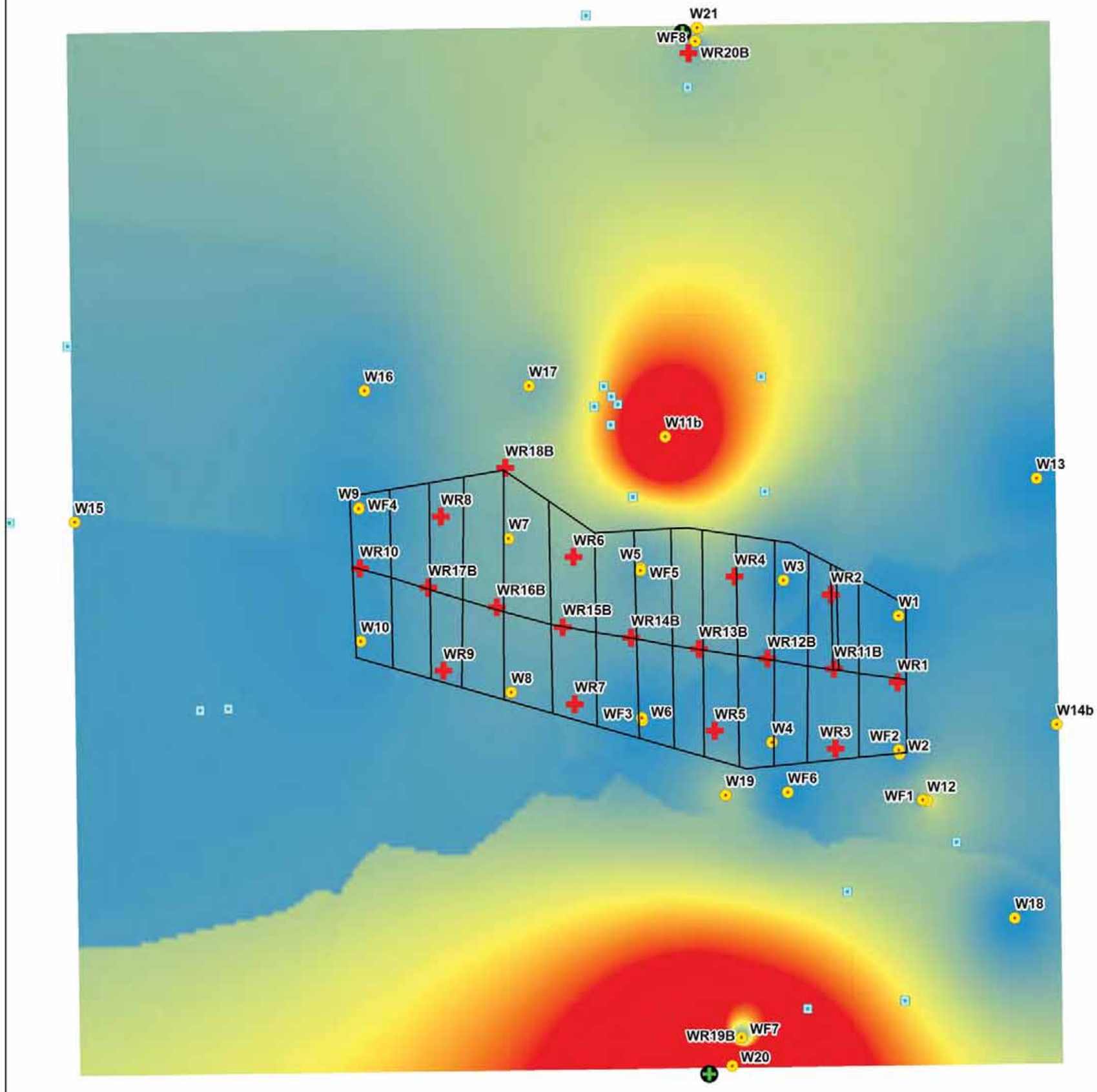
NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Sterile Male Queensland fruit fly 'Qfly'

2016/2017



Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Alternative hosts

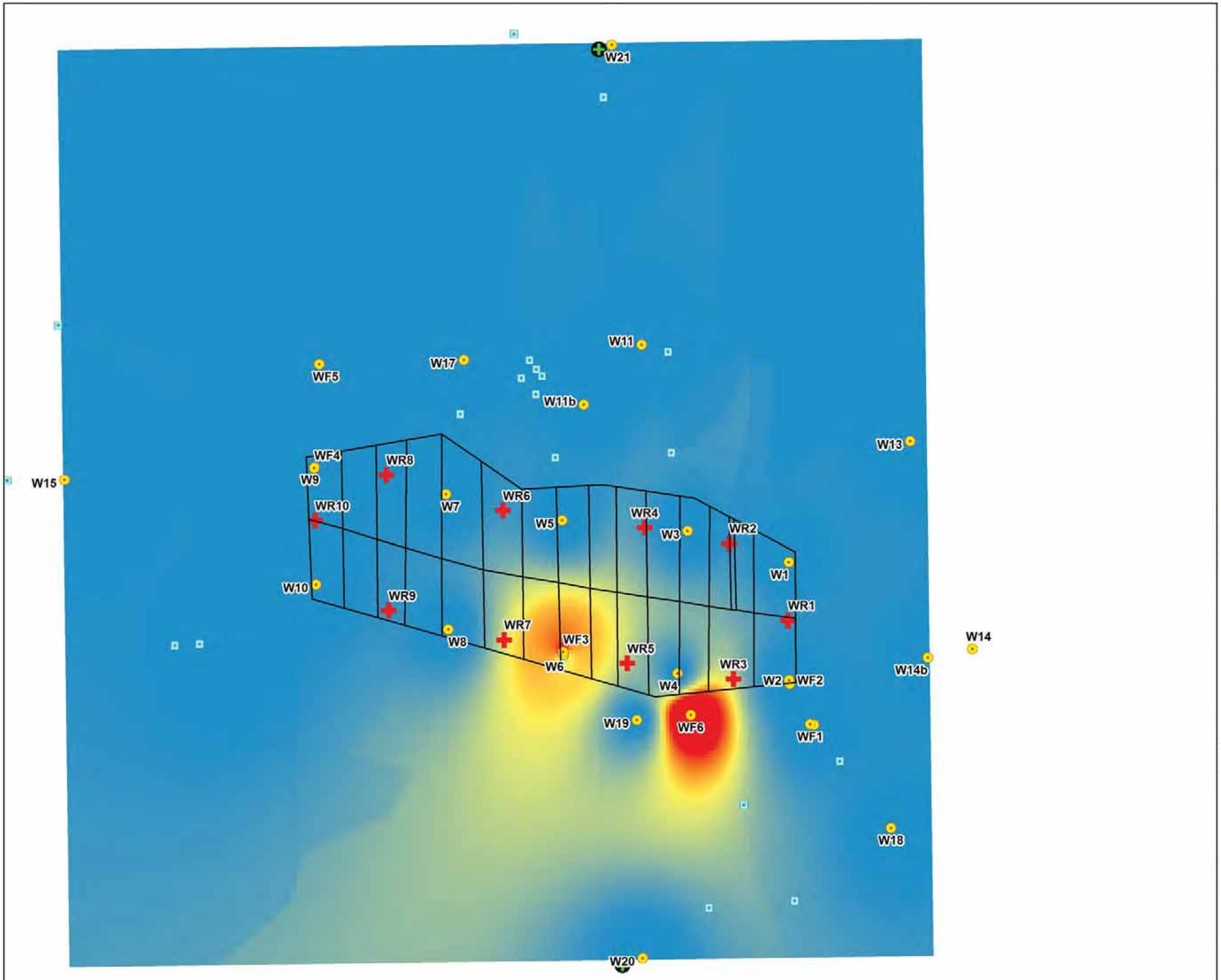
Orchard blocks

N

Meters

0 250 500

NSW GOVERNMENT Department of Primary Industries



Warroo
Orchard

Wild Female
Queensland fruit fly
'Qfly'

2014/2015

Legend

Qfly Trapping density

High

Low

Sterile Qfly release

Qfly Traps

Water Source

Alternative hosts

Orchard blocks

N

Meters

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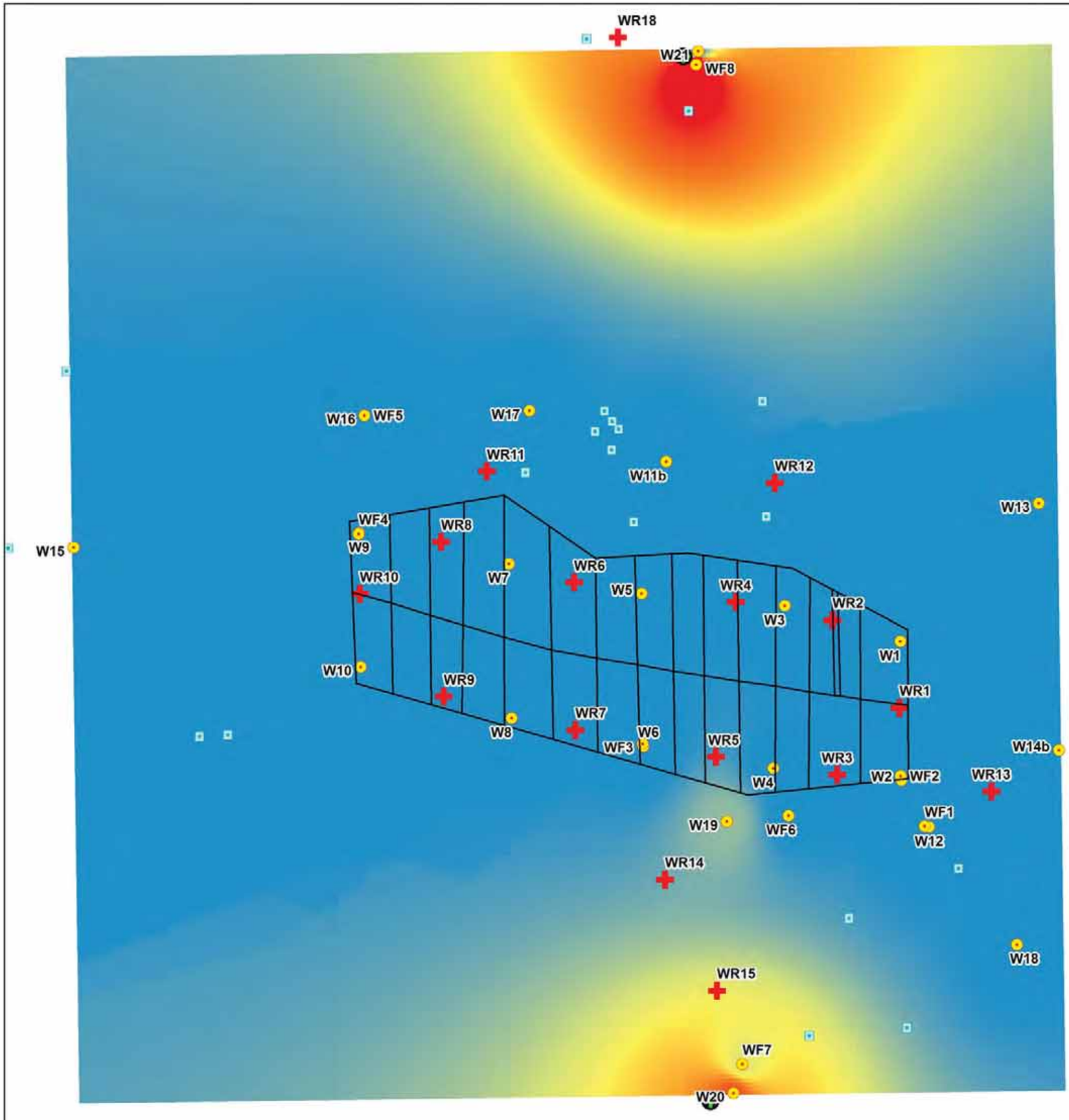
NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Wild Female Queensland fruit fly 'Qfly'

2015/2016



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- Qfly Traps
- Water Source
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Meters

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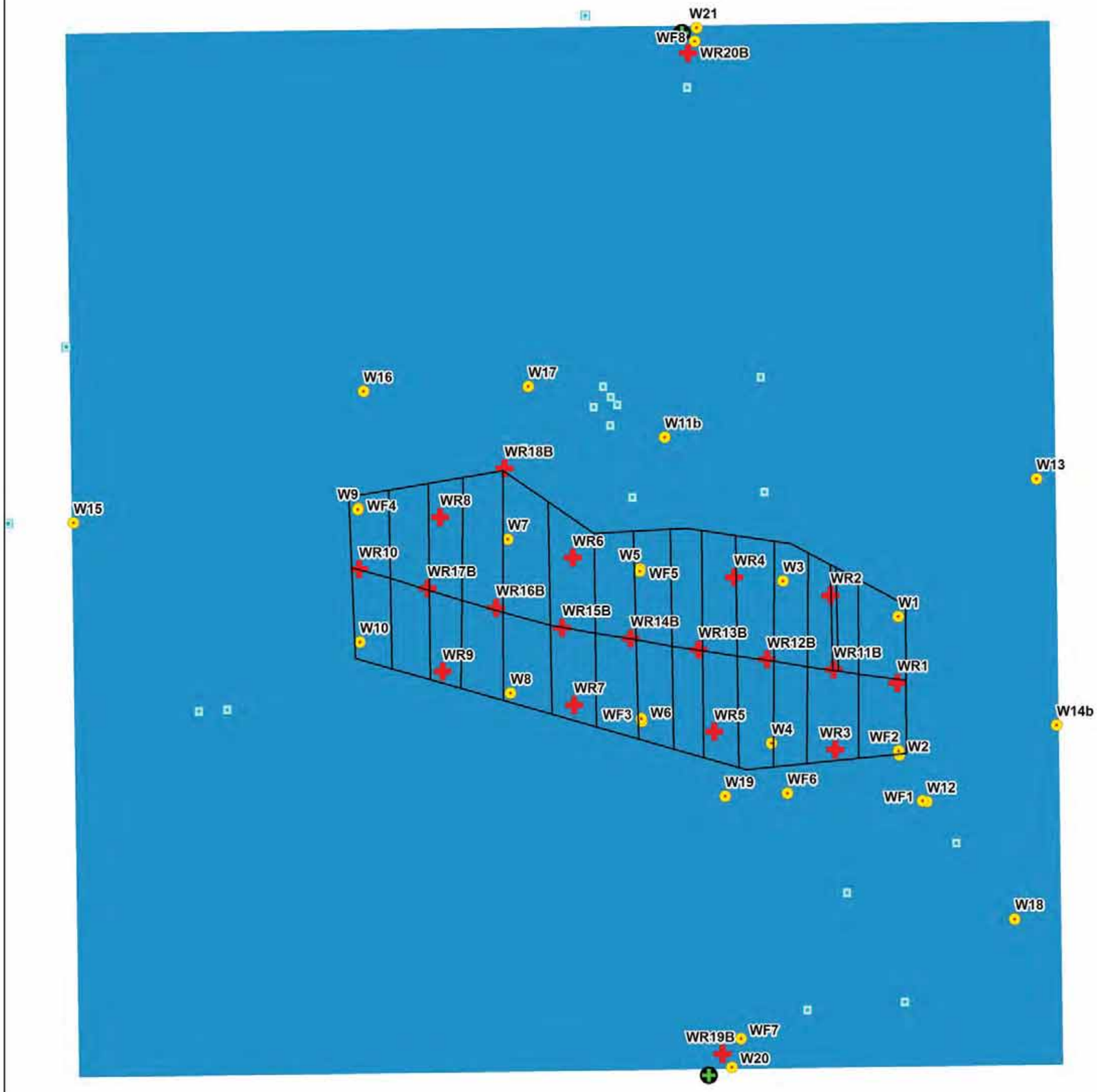
NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Wild Female Queensland fruit fly 'Qfly'

2016/2017



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Sterile Qfly release

Qfly Traps

Water Source

Alternative hosts

Orchard blocks

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Meters

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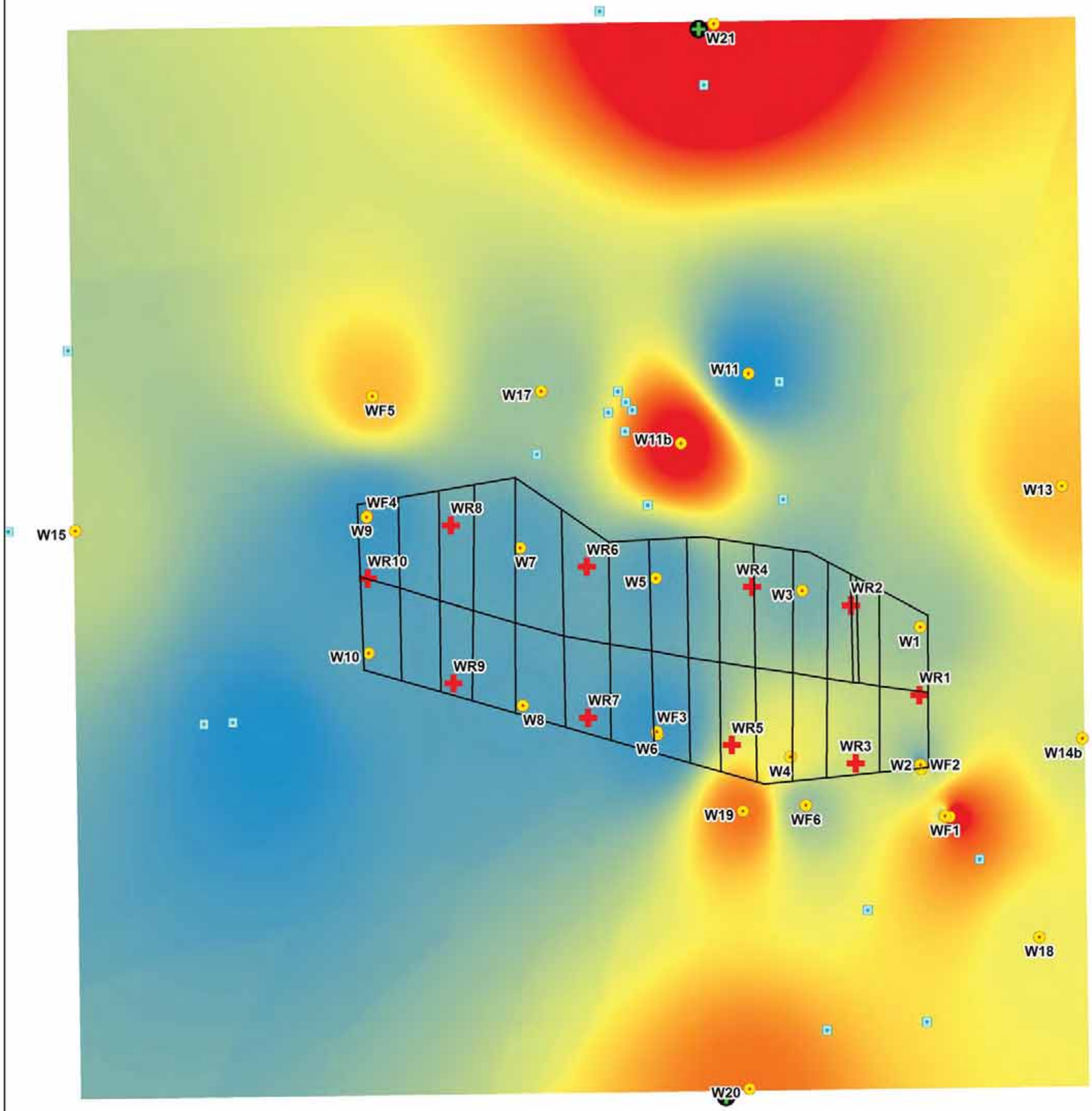
NSW GOVERNMENT Department of Primary Industries

Warroo
Orchard

Wild Male
Queensland fruit fly
'Qfly'

2014/2015



Legend

Qfly Trapping density

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Sterile Qfly release

Qfly Traps

Water Source

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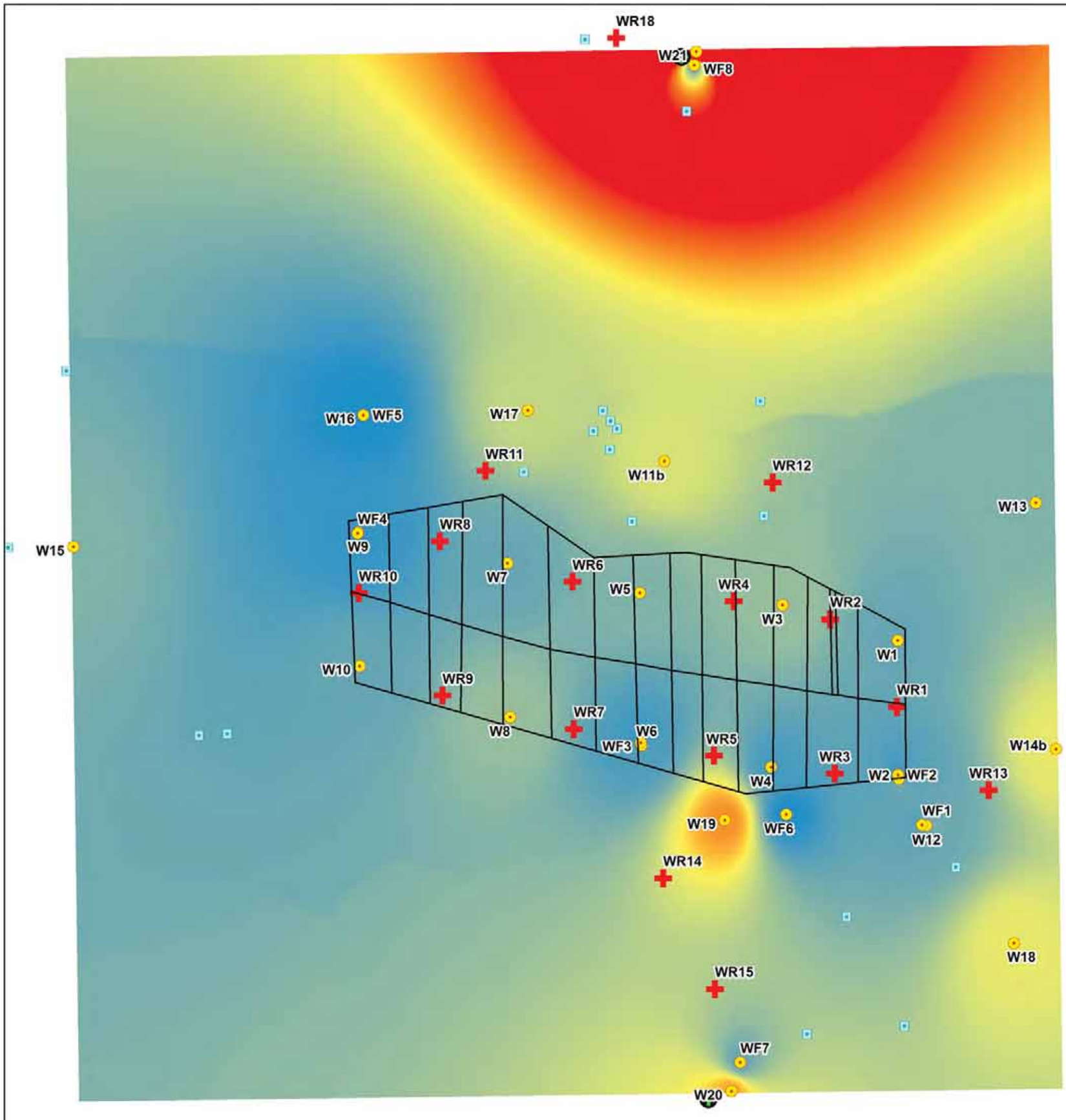
Warroo Orchard

Wild Male Queensland fruit fly 'Qfly'

2015/2016



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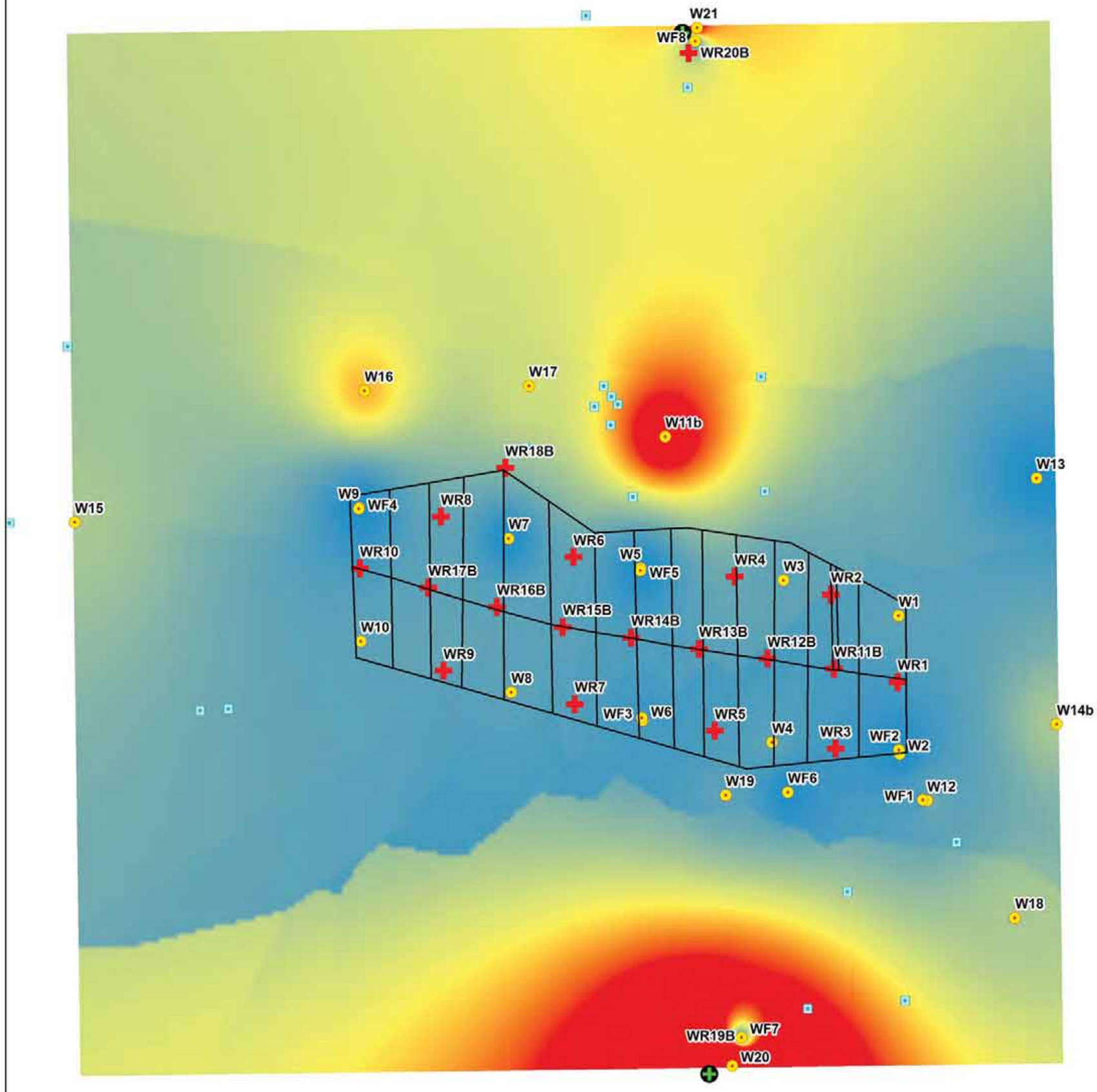
NSW GOVERNMENT Department of Primary Industries

Warroo Orchard

Wild Male Queensland fruit fly 'Qfly'

2016/2017



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N

Meters

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NSW GOVERNMENT Department of Primary Industries

Appendix C.

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Short Communication

Short Communication

Yeast: An Overlooked Component of *Bactrocera tryoni* (Diptera: Tephritidae) Larval Gut Microbiota

Ania T. Deutscher,[1,2,3] Olivia L. Reynolds,[1,2] and Toni A. Chapman[1]

[1]Biosecurity and Food Safety, NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Private Mail Bag 4008, Narellan, New South Wales 2567, Australia (ania.deutscher@dpi.nsw.gov.au; olivia.reynolds@dpi.nsw.gov.au; toni.chapman@dpi.nsw.gov.au), [2]Graham Centre for Agricultural Innovation (an alliance between NSW Department of Primary Industries and Charles Sturt University), Elizabeth Macarthur Agricultural Institute, Private Mail Bag 4008, Narellan, New South Wales 2567, Australia, and [3]Corresponding author, e-mail: ania.deutscher@dpi.nsw.gov.au

Subject Editor: Anthony Clarke

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Abstract

Yeasts, often in hydrolyzed form, are key ingredients in the larval and adult diets of tephritid fruit fly colonies. However, very little is known about the presence or role of yeasts in the diets of tephritid fruit flies in nature. Previous studies have identified bacteria but not detected yeasts in the gut of Queensland fruit fly, *Bactrocera tryoni* (Froggatt), one of Australia's most economically damaging insect pests of horticultural crops and of significant biosecurity concern domestically and internationally. Here we demonstrate that cultivable yeasts are commonly found in the gut of *B. tryoni* larvae from fruit hosts. Analysis of the ITS1, 5.8S rRNA gene, and ITS2 sequences of randomly selected isolates identified yeasts and yeast-like fungi of the genera *Aureobasidium*, *Candida*, *Cryptococcus*, *Hanseniaspora*, *Pichia*, and *Starmerella*. The prevalence of these yeasts in fruits suggests that larvae consume the yeasts as part of their diet. This work highlights that yeasts should be considered in future tephritid larval gut microbiota studies. Understanding tephritid–microbial symbiont interactions will lead to improvements in artificial diets and the quality of mass-reared tephritids for the sterile insect technique.

Key words: sterile insect technique, insect rearing, insect nutrition, Queensland fruit fly, microbial symbiont

Gut bacteria are integral to the nutrition, metabolism, longevity, reproduction, and mating success of fruit flies (Diptera: Tephritidae) (Yuval et al. 2013). In contrast to the substantial efforts investigating contributions of gut bacteria to fruit fly physiology and ecology, little consideration has been given to tephritid–yeast associations. Yeast is a major source of nutrition for fruit flies, providing sterols, vitamins, minerals, and protein; what is more, they are an important component of larval and adult diets for rearing of tephritid fruit flies, greatly influencing life-history traits (Fanson and Taylor 2012). However, very little is known about tephritid–yeast interactions in nature.

Queensland fruit fly, *Bactrocera tryoni* (Froggatt), is a major, highly polyphagous, horticultural pest in Australia, with strict quarantine regulations imposed on trade to sensitive local and international markets (PHA 2010). Restrictions or withdrawal of key insecticides for *B. tryoni* management has led to increased interest in sterile insect technique (SIT; Reynolds and Orchard 2015). A large number of insects are reared in SIT programs, which are reproductively sterilized through irradiation and released into the field, where they induce reproductive failure in pest populations (Knipling 1979). Sterile male mating performance is critical to the success of SIT, which may be improved through an increased understanding of nutrition and the roles of gut microbiota in tephritid quality and performance (Behar et al. 2009, Hamden et al. 2013, Taylor et al. 2013).

Numerous studies have described bacteria associated with *B. tryoni* in nature (Drew and Lloyd 1989), but there have been no reports of yeasts associated with this species. There was no evidence of fungal spores or yeast cells when the crop contents of adults were examined under 400× magnification (Vijaysegaran et al. 1997). The authors concluded that the labellar filtering mechanism would exclude particles greater than 0.5 μm in size. However, fungi associated with whole unsterilized adult *Bactrocera oleae* (Rossi) were recently characterized (Malacrino et al. 2015), and novel yeast species were isolated from *Anastrepha mucronota* (Stone) larvae (Rosa et al. 2009). Darby and Kapp (1934) report that yeast cells were present in the crop of *Anastrepha ludens* (Loew); the only study to identify yeasts in the alimentary canal of tephritids.

Here we present the first evidence of yeasts in the midgut of larval *B. tryoni* sampled from fruits, and we highlight that tephritid–yeast associations is an area that could lead to improvements in fruit fly rearing and performance, which will benefit SIT programs.

Table 1. Source and culture conditions of yeasts isolated from the midguts of larval *B. tryoni*

Isolate	Host (Larva ID)	Tree location	Agar plate[a]	Isolate ID
S99b	Apricot (L66)	West Wyalong	PDA	*Pichia kudriavzevii*
S112	Plum (L94)	Buronga	YDC	*Aureobasdium pullulans*
S117	Plum (L94)	Buronga	KB	*Cryptococcus saitoi*
S221	Orange (L292)	Tumut	YDC	*Pichia kluyveri/fermentans*
S272	Orange (L295)	Tumut	PDA	*Hanseniaspora thailandica/opuntiae*
S225	Yellow-fleshed peach (L259)	Tumut	PDA	*Pichia kluyveri/fermentans*
S276	Yellow-fleshed peach (L260)	Tumut	PDA	*Pichia kluyveri/fermentans*
S236	Yellow-fleshed peach (L176)	Leeton	PDA	*Pichia kluyveri/fermentans*
S268	Yellow-fleshed peach (L188)	Leeton	PDA	*Pichia kluyveri/fermentans*
S240	Apple (L302)	Applethorpe	TSA	*Pichia kluyveri/fermentans*
S262	Apple (L307)	Applethorpe	PDA	*Pichia kluyveri/fermentans*
S252	Nectarine-peach X (L215)	Leeton	TSA	*Pichia kudriavzevii*
S254	Nectarine-peach X (L215)	Leeton	YDC	*Pichia kluyveri/fermentans*
S257	Nectarine-peach X (L219)	Leeton	PDA	*Starmerella bacillaris*
S258	Nectarine-peach X (L219)	Leeton	PDA	*Pichia kluyveri/fermentans*
S269	Cherry guava (L333)	Richmond	PDA	*Hanseniaspora uvarum*
S282	Cherry guava (L333)	Richmond	PDA	*Candida quercitrusa*

[a] PDA, Potato Dextrose Agar; TSA, Tryptone Soya Agar; YDC, Yeast Dextrose Carbonate; KB, King's B.

Materials and Methods

A range of fruits infested with *B. tryoni* larvae were sourced from locations in New South Wales and from the Applethorpe Research Facility in Queensland, Australia (Table 1). A subset of larvae from each infested fruit variety was reared to the adult stage to identify fly species.

To culture gut microbiota, another subset of larvae (second–third instar) were surface sterilized by dipping in 0.5% (v/v) Tween 80, washing by inversion three times in 0.5 ml 80% (v/v) ethanol, 0.5% (v/v) sodium hypochlorite solution, and rinsing three times with sterile phosphate buffered saline (PBS). Under a stereo microscope, each midgut (cardia to above the pylorous) was extracted in a drop of sterile PBS and transferred to a separate tube containing sterile PBS. Individual midguts were ground with a sterile microfuge pestle, diluted further to a final volume of 0.75 ml, vortexed, and 50–75 µl was spread onto a range of agar plates and incubated at 25 °C, or mixed 1:1 with brain heart infusion broth with 40% (v/v) glycerol and stored at −80 °C prior to plating. Gram staining and microscopic examination of dominant isolates indicated that some were yeast-like based on cell size and the presence of budding. Yeast-like isolates were randomly selected for molecular identification (Table 1).

Isolate DNA was extracted with the MP Biomedicals FastDNA SPIN Kit and FastPrep Instrument (Santa Ana, CA) or following a modified version of the cetyl trimethylammonium bromide (CTAB) method described in Tan and Medd (2002). Modifications included resuspending a loopful of each isolate in 750 µl extraction buffer (50 mM Tris-HCl, pH 8.0, 0.7 M NaCl, 10 mM ethylenediaminetetraacetic acid (EDTA), 1% (w/v) CTAB, 1% (v/v) 2-mercaptoethanol, 60 mg RNase A) containing 0.5 and 4.0 mm sterile stainless steel ball bearings, vortexing for 10 min, followed by incubation at 65 °C for 30 min. The internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene sequence was PCR amplified using the primers ITS1 and ITS4 (White et al., 1990) and sequenced by the Australian Genome Research Facility (GenBank KX388372–KX388374 and KX686729–KX686742). Isolate identification was based on database sequence matches in the National Center for Biotechnology Information (NCBI) non-redundant nucleotide collection with 99–100% identity, high confidence (E values of 0.0), and 100% query coverage.

Results and Discussion

This is the first study showing that diverse, cultivable yeasts are frequently found in the alimentary canal of wild *B. tryoni* larvae. *Bactrocera tryoni* was the only tephritid to emerge from the infested fruits. The types and prevalence of yeasts in the alimentary canal have not been demonstrated previously, for any larval tephritid.

Yeast cells were identified in the dominant colony types isolated from 15 of 17 *B. tryoni* larval midguts. All of the isolates randomly selected for sequencing were from the phylum Ascomycota, except for *Cryptococcus* sp., a basidiomycetous yeast (Table 1). The majority of the yeast genera isolated are also commonly associated with wild Drosophila (Broderick and Lemaitre 2012). Apart from *Aureobasidium pullulans*, a yeast-like fungus, the ascomycetous yeasts were from the Saccharomycetaceae family, which includes commonly used commercially available larval diet yeasts, such as Torula yeast, *Candida utilis*, and brewer's yeast, *Saccharomyces cerevisiae*. In this study, the species of some isolates could not be determined due to low sequence variability in the region sequenced between closely related species. *Pichia* spp. were frequently isolated; however, different culturing conditions could isolate other species.

The identified yeasts are commonly found in, or on, fruit (Molnarova et al. 2014), thus the larvae probably obtained these yeast through ingestion. Vertical transmission of yeasts is possible, but the reported absence of yeasts in the alimentary canal of adult *B. tryoni* (Vijaysegaran et al. 1997) suggests this is unlikely. Preliminary results indicate yeasts are negligible in the midguts of domesticated *B. tryoni* larvae feeding on carrot diet compared to larvae from fruits (data not shown). Further investigation of this, along with whether the yeasts within the *B. tryoni* gut are symbionts and the host benefits conferred by different species, could result in improvements in the artificial diet of flies.

Consuming yeasts could be nutritionally beneficial to the larvae. Several yeasts identified in this study produce extracellular enzymes (Molnarova et al. 2014), which could break down components of the fruit into nutrients that might otherwise be unavailable to the developing larvae. Along with this, tephritid larvae may increase their nitrogen uptake by consuming yeasts, as *Drosophila mulleri* (Sturtevant) larvae can increase their nitrogen uptake 16–26 times by selectively feeding on nitrogen-rich yeasts (Starmer and Aberdeen 1990).

Yeasts can possess antagonistic activities against undesirable gut bacteria, while others enhance bacterial probiotics (Hatoum et al. 2012). Thus, tephritid gut microbiota studies should extend their focus from bacteria to also consider yeasts. Yeasts are also largely overlooked in Drosophila microbiome studies (Broderick and Lemaitre 2012). Further characterization of tephritid–microbial symbiont interactions could advance our knowledge of the functional relationships between gut microbiota, nutrition, and tephritid physiology, leading to improvements in artificial diets and, consequently, in the quality of mass-reared tephritid flies used in SIT programs.

Acknowledgments

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Appendix D.

Australian STONEFRUIT GROWER

incorporating the **Low Chill Stonefruit Grower**

FEBRUARY 2014

…Issue No. 1/14

'Australian Stonefruit Grower' is the official publication of Summerfruit Australia Ltd & Low Chill Australia Inc. – the industry bodies representing the interests of Australian stone fruit growers.

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CONTACTS –

Summerfruit Australia Ltd - ACN 105 962 196
John Moore – CEO
8/452 Swift Street, Albury NSW 2640
Ph: 02 6041 6641, Mobile: 0419 305 901,
Fax: 02 6021 0011
Email: ceo@summerfruit.com.au
Website: www.summerfruit.com.au

CONTACTS –

Low Chill Australia Inc.

ABN 283 812 712 44

Office Address:
PO Box 25, BANGALOW NSW 2479
Phone: (02) 6687 2376
Mobile: 0413 007 197
Email: president@lowchillaustralia.com.au
Website: www.lowchillaustralia.com.au
Communications Manager:
Col Scotney
PO Box 372, BURRUM HEADS QLD 4659
Phone: (07) 4129 5960; Mobile: 0407 589 445
Email: cm@lowchillaustralia.com.au
Email: australian.stonefruit.grower@aapt.net.au

Summerfruit Conference

The link to all the 2013 Combined Fruit Industry conference presentations –
http://apal.org.au/events/biennial-conference-innovate-or-real-estate/conference-presentations/

Sterile Insect Technique as part of an Area Wide-Integrated Pest Management campaign for Queensland fruit fly control

By Dr Olivia Reynolds

Queensland fruit fly 'Qfly', is a fruit fly that feeds and breeds on a variety of important crops and is the most significant biosecurity threat to Australian horticulture. This pest attacks almost all commercial fruit crops and several fruiting vegetable crops. In areas where fruit flies are native or have established populations, rigorous field control must occur to ensure the production of high quality produce.

Recently, restrictions have been placed on the use of chemicals used to control Queensland fruit fly, with few viable options remaining. A permit was issued for *Clothianidin* on 5 September 2013 (PERMIT NUMBER - PER14252) for the control of Qfly and Mediterranean fruit fly in persimmon, pome fruit and stone fruit. This permit allows for another chemical control option in a growers toolbox but we are seeing increasing restrictions placed on insecticides due largely to environmental and public health concerns.

In addition, *Clothianidin* is a *neonicotinoid* insecticide which is a group increasingly coming under scrutiny due to their alleged role in the demise of bee populations. There is a need to find alternate 'softer' in-field control options for Qfly. An **Area Wide-Integrated Pest Management** (AW-IPM) program that incorporates the sterile insect technique (SIT) is one such option.

An AW-IPM SIT program is not only a preventative control option but is intended to have a positive impact on society by improving the quality of horticultural products at a lower cost, while protecting the environment and human health. AW-IPM focuses on the preventive management of pest populations throughout a delimited geographic area. This technique has a strong emphasis on treating all habitats of the pest population preventing migrants re-establishing significant infestations, which are damaging to crops.

In contrast, conventional control focuses narrowly on protecting the crop from direct attack by pests. The SIT is a target-specific form of birth control imposed on a pest population that may be applied in the AW-IPM of insect pests of agricultural, medical and veterinary importance. The case for the SIT on an environmental, economic and biological basis is persuasive. The main objective of the study is to establish an effective AW-IPM SIT program to provide control of Qfly in an endemic area and that will inform the development of similar future campaigns.

Like most pest control techniques, the SIT is not a stand-alone technique, and in most situations requires pre-release population suppression to be effective and economically viable. There are at least 20 AW-IPM programs worldwide that have successfully incorporated the SIT to control fruit flies and include prevention, containment, eradication and suppression of these pests. The SIT is environmentally benign and can be a cost-effective component of an AW-IPM program including for the control of fruit flies of major economic importance, such as the Mediterranean fruit fly and Qfly.

The program, led by Dr Reynolds, will operate in a region identified in south-eastern Queensland, near the New South Wales border and will involve several growers and their properties, collectively known as 'Trap Rock' which are unique in that they are geographically isolated from urban centres.

With the exception of the orchards, the country is largely sheep terrain and is unsuitable fruit fly habitat. This project has been funded by **HAL** using voluntary contributions from growers **Rowan Berecry**, **Andrew & Graham Finlay**, **John & Julie Pratt**, **Duncan & Angus Ferrier** and matched funds from the **Australian Government**. This funding has resulted in a million-dollar project (MT13040) spanning 3.5 years. In order to establish an AW-IPM SIT program, a phased approach to Queensland fruit fly control will be used and will include a pre-intervention phase, population reduction phase, release phase and a maintenance phase.

There are several outcomes which are possible as a result of this work and include a reduction in the number of wild flies trapped over consecutive seasons, a reduction in fruit fly populations to below economic thresholds using AW-IPM, a reduction in pesticide use and reduced secondary pest outbreaks associated with pesticide usage and associated control costs.

Other benefits of this project may include protection of the environment and the health of farm workers resulting in savings in public health and environmental costs through reduced insecticide residues in fruit, water reservoirs and soil and strengthening research and development support of the stone fruit industry.

Photos – *'Warroo Orchard participating in the Area Wide Integrated Pest Management Sterile Insect Technique trial'.*

Product Information …

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Australian STONEFRUIT GROWER

incorporating the

Low Chill Stonefruit Grower

NOVEMBER 2015

…Issue No. 4/15

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Horticulture Innovation Australia

"This project has been funded by Horticulture Innovation Australia Limited using the summerfruit levy and funds from the Australian Government."

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CONTACTS –

Summerfruit AUSTRALIA

Summerfruit Australia Ltd - ACN 105 962 196
John Moore – CEO
8/452 Swift Street, Albury NSW 2640
Ph: 02 6041 6641, Mobile: 0419 305 901,
Fax: 02 6021 0011
Email: ceo@summerfruit.com.au
Website: www.summerfruit.com.au

CONTACTS –

Low Chill Australia Inc.

ABN 283 812 712 44

Office Address:
PO Box 25, BANGALOW NSW 2479
Phone: (02) 6687 2376
Mobile: 0413 007 197
Email: president@lowchillaustralia.com.au
Website: www.lowchillaustralia.com.au
Communications Manager:
Col Scotney
PO Box 372, BURRUM HEADS QLD 4659
Phone: (07) 4129 5960; Mobile: 0407 589 445
Email: cm@lowchillaustralia.com.au
Email: australian.stonefruit.grower@aapt.net.au

Research …

Area Wide Management Program: effective at suppressing Queensland fruit fly populations after first year

Olivia Reynolds and Terry Osborne …

This is the third article in a series on the Area-Wide Management (AWM) of the Queensland fruit fly, 'Qfly'. This AWM pilot program in the Stanthorpe region has just completed the first year of a three year Queensland fruit fly suppression program, with excellent evidence supporting the success of the program.

Despite only the first full year of trapping and suppression for five of the six stone fruit orchards, wild Qfly numbers are the lowest in the previous seven years of trapping data, with on average 0.016 flies/trap/day recorded. By international standards, this is at the lower end of the range we would expect in a suppression program.

The growers in the program used a combination of sanitation, bait sprays, male annihilation technique (MAT) and sterile flies to suppress fly numbers across their orchards.

Regular fruit monitoring played a key role in monitoring success of the program. We did not detect a single Qfly sting/egg/larvae in any of the orchards, with the exception of very low levels in two orchards at harvest. The largest orchard in the program detected no stings in fruit, nor were any wild flies trapped, in the orchard prior to a problem with sterile fly supply in late December 2014. This resulted in what appears to be only two or three wild females laying eggs in fruit in two sections of one orchard, with no more than 50 whole fruit (from a 60Ha plum orchard) detected by regular monitoring or pickers, reported as 'stung' by fruit fly. Infested whole fruit rarely had more than one Qfly larvae. It was identified that the other orchard which recorded larval fruit fly detections (unfortunately most of the infested stone fruit was inadvertently destroyed and we were unable to identify the species), had several Osage Orange (*Maclura pomifera*) trees, which were heavily infested with *B. tryoni*. These had not been removed in the sanitation process. These trees have now been removed. This was not a previously recorded host of Qfly and is the first record of this species, from this fruit. Island fly, *Dirioxa pornia*, in addition to other species has been trapped in the area and it is feasible that at least some of the larval detections were this species and not Qfly, particularly in the over ripe and very mature fruit. There were also two orchards bordering this orchard, which ceased the area-wide management of Qfly in October 2014. Fly populations from these orchards, may have also contributed to infestation in nearby orchards.

Despite no detections of infestation by regular fruit monitoring, several growers elected to put one cover spray on some of their blocks (not all) prior to harvest as an 'insurance policy'. Normally, 2-4 cover sprays would be used in a season. This is a confidence item for the growers as they adapt to a new program of control, and it should be remembered that with current available pre-harvest chemistries, these do not guarantee 100% efficacy. Overall, pesticide usage was down considerably from previous 'traditionally' managed years, with over 80% of the area under orchard receiving no cover sprays throughout the season, and only one cover spray used pre-harvest on the remaining area under orchard. One of the growers conceded, that he probably didn't require the single cover spray.

The growers reported no problems with market access. None of the fruit was post-harvest disinfested. There was not a single reported detection of infested fruit in any of the fruit sent to market for the 2014/15 season for any of the orchards. All orchards sold their fruit domestically to Woolworths and Harris Farms, with the exception of three orchards. Approximately 70% of the fruit from the largest orchard was marketed domestically, as either pre-packs or loose through Woolworths in Queensland, New South Wales and Victoria, with a small percentage that went through the Brisbane Market. The remaining 30% was sent to Singapore, Hong Kong, Malaysia, Indonesia and the United Kingdom (UK). Every single piece of fruit sent to the UK was individually inspected. The majority of peaches and all plums from the smaller two orchards went to the domestic market (Brisbane Markets: ~40%; Woolworths & Coles ~30% each), with 1000kg of the peaches going to the Malaysian market.

Reducing chemical usage in orchard can lead to a host of benefits, including improved human and environmental (including tree) health and a reduction in the number of secondary pest outbreaks, or 'flare-ups'. Numerous beneficial

insects have been reported in the orchards, since the program was implemented. One grower claimed that he did not have to spray for anything that 'bites, sucks or chews', which he would have otherwise. Overall, the program, even in its first year, is looking very effective at suppressing Qfly populations, in endemic areas.

Osage orange: a new host for the Queensland fruit fly

Osage orange, *Maclura pomifera*, has been identified as a host of Queensland fruit fly. This has management implications for this pest. Removal of the fruit from this tree or of the tree itself, in horticultural production areas should be considered to prevent fly populations breeding up in these hosts and/or this tree acting as a successive host for this pest.

Acknowledgements

This project has been funded by **Horticulture Innovation Australia Limited**, using the Summ**erfruit industry levy** with co-investment from **Traprock Group** and **NSW Department of Primary Industries** and funds from the **Australian Government**.

Australian Stonefruit Grower

incorporating the Low Chill Stonefruit Grower

- 2016 Publication Timetable -

Contributions are invited for the next scheduled publication - **FEBRUARY 2016**

FEBRUARY	MAY	AUGUST	NOVEMBER
Advertising Deadline **7 February**	*Advertising Deadline* **21 April**	*Advertising Deadline* **31 July**	*Advertising Deadline* **31 October**
Copy Deadline **10 February**	*Copy Deadline* **28 April**	*Copy Deadline* **21 August**	*Copy Deadline* **7 November**

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CONTACT –

National Producer/Editor

***Australian Stonefruit Grower* – Email:** cm@lowchillaustralia.com.au

Communications Manager

Low Chill Australia Inc. – *Email:* cm@lowchillaustralia.com.au

Pilot program in Stanthorpe suppresses fruit fly

Olivia Reynolds and **Terry Osborne**, *NSW DPI, Menangle, NSW*

The Area-Wide Management (AWM) pilot program incorporating the sterile insect technique (SIT) to control the Queensland fruit fly (Qfly) has now entered its third and final year.

This model AWM program in the Stanthorpe region, aimed at suppressing wild Qfly populations to greatly reduce the risk of fruit damage, has demonstrated ongoing success.

This program uses sterile male and female Qflies, with field studies demonstrating sterile females have no impact on stone fruit quality.

Suppression across approximately 100ha of stone fruit in 2015-2016 in an endemic region in Queensland has demonstrated that wild Qfly numbers remain at the same very low levels (<0.05 flies/trap/day; 400m spaced trapping grid), since the program was implemented in 2014-2015.

The growers in the program continue to use a combination of techniques to suppress fly numbers across their orchards.

These include bait sprays, male annihilation technique (MAT), sanitation through stripping fruit left post-harvest and managing non-commercial fruit trees and vegetable gardens, fruit monitoring and sterile flies used at approximately 4000 sterile males/ha.

Cover sprays are an option, if felt necessary.

Harvest for the largest orchard in the program comprising 60ha of plums commenced on 15 January 2016 and was completed two weeks later.

Harvest for the smaller 30ha orchard began on 6 October 2015 and was completed by 18 December.

There was not a single reported detection of infested fruit in any of the fruit sent to market for the 2015-16 season for either orchard.

The largest orchard in the trial sold a total of 384 tonnes of Queen Garnet plums, including 120 tonnes to Woolworths, three tonnes to Singapore, 250 tonnes through domestic markets in Brisbane, Sydney and Melbourne, and several tonnes to Adelaide after irradiation. Some fruit was stored for juice or nectar (21 tonnes to juice), with a very small amount of waste of 10 tonnes.

Harvest was down this year from 720 tonnes in 2014-15, due to a chill problem caused by warm weather in winter. For example, the orchard experienced a 25°C on 20 June 2015.

Poor pollination was also an issue.

However, the plums were of excellent quality, and according to Andrew Finlay, chair of Summerfruit Australia, the best they had seen so far from the orchard.

A total of 175 tonnes of plums, peaches and nectarines was harvested from the 30ha orchard in 2015.

The quantity of fruit was down from the previous year due to two severe hailstorms in March 2015 which caused premature flowering and significant damage across the entire orchard, with two-thirds of the orchard more significantly impacted than the remainder.

All harvested fruit was sold domestically.

Western flower thrips and plague thrips caused some problems at this orchard from mid July 2015 through to late November 2015, with most fruit blocks sprayed one to three times during the season with Mavrik (22 mL/100L) and/or Delegate (20 g/100L) to control thrip.

It is likely that these sprays impacted the sterile fly populations in the orchards and would have disrupted the beneficial insects.

Unfortunately, there is scant research demonstrating the effectiveness of other control options including biological control agents for managing thrips in tree crops.

Addressing concerns around managing other pests within the system, while minimising disruption to beneficial insects and sterile Qfly, is a challenge for all pest management programs.

Regular fruit monitoring detected an extremely low number of fruit fly stings in both orchards (e.g. estimated at less than 100 whole fruit across 60ha).

Fruit that had been damaged during a hail storm was noted to be particularly susceptible.

Overall, after a second consecutive season, the program continues to be very effective in suppressing Qfly populations in endemic areas with no market access issues.

Area Wide Management is a very effective management tool for mobile pests, such as Qfly.

Acknowledgements

This project has been funded by Horticulture Innovation Australia using the summerfruit industry levy with co-investment from Traprock Growers and NSW Department of Primary Industries and funds from the Australian Government, as part of the SITPlus Initiative.

Olivia Reynolds (pictured) and Terry Osborne from NSW DPI **have been investigating Qfly control.**

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High-antioxidant PLUM DEVELOPED and grown in Queensland

Bim Goodrich
Warroo Station
Warroo, Queensland

New "super plums" being commercially grown at Warroo Station west of Stanthorpe by Bim Goodrich and his Manager Rowan Berecry are being promoted as obesity fighters.

The Queen Garnet plums have up to five times the level of the pigment antioxidant, anthocyanin, as normal plums (up to 250 mg/g compared to about 50mg/g). Numerous scientific studies suggest that anthocyanins are very effective anti-inflammatory agents in the fight against obesity, defined by health scientists as a chronic, low intensity inflammatory disorder.

Dr Kent Fanning at the Queensland Department of Agriculture Fisheries and Forestry (QDAFF) has been the main collaborator working to maximise anthocyanin production. Dr Michael Netzel from CSIRO examined the bioavailability of the anthocyanins in humans with excellent results. Prof. Lindsay Brown at the University of Southern Queensland, successfully tested the anti-inflammatory properties of anthocyanin in rats and also saw major improvements in blood pressure and heart muscle stiffness. Dr Indu Singh at Griffith University has had positive results with thrombosis in humans.

Apart from their impressive scientific pedigree, the plums are also very good to eat. The new variety is large with very dark red flesh, almost black skin and a rich, sweet flavour.

Bim Goodrich, a fifth-generation wool grower and President of the Traprock Group, chose to diversify his operation with a horticulture venture at his property, Warroo Station.

"Bim was going to lose his water licence if he didn't use it so he sought advice from a consultancy mate who knew about the plum. Bim wanted a niche product, not another commodity he would struggle to sell profitably," Rowan Berecry said.

The variety is owned by QDAFF but Bim and his brother Rick, along with three non-growers, established Nutrafruit which holds the licence worldwide.

"Nutrafruit has control over who can grow it. We've got 75 000 trees over 60 hectares and Rick has 10 000 at Deniliquin. Nobody else can grow for processing until such time that we can't meet the market demand," he said.

"We probably won't have the water here to increase the number of trees so we're looking for others to grow for the fresh market through a controlled process."

One of the issues with growing the plums is early picking because of the misleading sugar content.

"They look as though they're ready to eat up to six weeks early and their sugar content is already 20 brix – a normal plum has 12 when it's ripe. We need to keep an eye on this because they aren't edible at this stage and it could destroy the market if they are picked and sold too early," he said.

The first commercial harvest recently finished but it only reached half of its potential.

"The recommended pollinator was incompatible so we've had to find compatible varieties that will flower at the same time and are high in anthocyanins, and change them over. We've now got four successful varieties. It can take 10 years to work it out but we got on to a researcher in Japan who trained a QDAFF researcher to assess DNA compatibility. That saved millions of dollars and a lot of

heartache. By next year we expect to be on track with a 1000 tonne harvest and work up from there," he said.

Bim's long-term plan is to process all of his plums, to be made into a range of value-added health products. Selling to the fresh market this year was about raising the plum's profile.

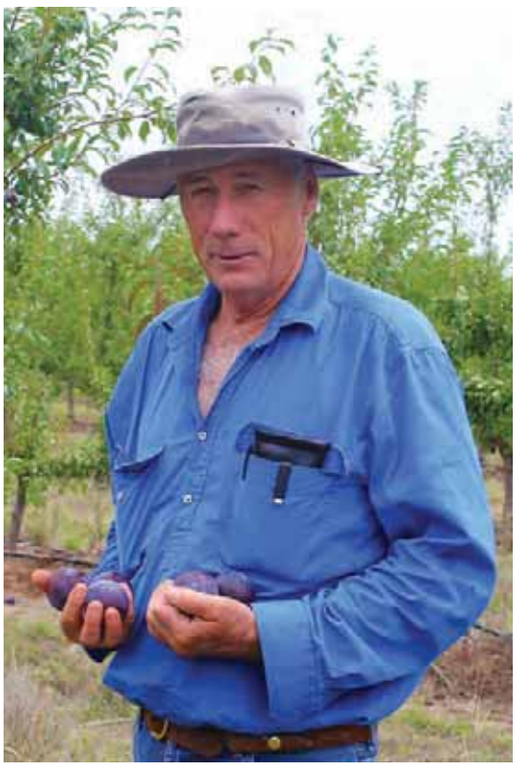
"The plums could be used in supplements, juices, yoghurt, powders – the list goes on. There's a push for natural products and this is one of the highest sources of anthocyanins in the fruit game. Blueberries are renowned for it but these plums contain higher levels, and we can produce them for a fraction of the cost," he said.

Rowan previously owned his own stone fruit orchard but had moved to Moree to work in agronomy when the nursery owner growing the plums in Goondiwindi contacted him with some problems.

"He heard there was a bloke in Moree that knew something about fruit trees so I went and fixed them. When Bim heard he asked me to look at his orchard, and offered me a job here on the farm," he said.

Rowan's wealth of experience and knowledge also led to the decision to grow the plums biologically.

"On my orchard I was as conventional as they come, using everything people told me I should. We were getting wiped out by brown rot, beetles and mites – you name it, we had it. The state ag



Above: Warroo Station farm manager Rowan Berecry.

department's brown rot research was done with us because of the plentiful supply. Within three years of converting to biological farming we had no brown rot or insect issues, production was up 30 per cent and quality had improved out of sight. I've transposed that here to Bim's farm," he said.

"When I arrived Bim was having fungal and trunk canker problems with the first 10 000 trees but that all disappeared when we changed over.

"It's a different mentality – we're feeding the bugs in the soil which feed the tree. There's been no artificial fertiliser use. We're using compost which we're getting made specifically, and through irrigation we apply a brew of fish, kelp, aloe vera, azotobacter and a range of other things. The only spraying we have done has been calcium, kelp and desalinated sea water.

"We've got a super healthy orchard without chemical use which is what everyone should be trying to achieve. Initially the cost is probably on par but long-term it's much cheaper than spraying umpteen pesticides and insecticides."

Rowan believes it will take a huge mindset change for growers to convert to biological farming.

"It's peer pressure as much as anything else. You get ridiculed by your neighbours and you're on your own. It's easier to stay in mainstream because there's a feeling of safety in numbers," he said.

Another element to biological farming is the elimination of spraying to control Queensland fruit fly.

"We're releasing sterile male Queensland fruit flies to mate and wipe out the local population. Horticulture Australia Limited is on board now and we're doing nearby farms so it will be an area-wide program over the next three to five years. It's a potential model for the country if we pull it off. We only found two bits of fruit attacked through harvest so it's been a raging success," he said.

"We have cages where we put infertile male fruit fly pupae, hatch them, feed them for a few days, hit them with pheromones and then release them so all they want to do is chase females."

This year the farm had seven full time staff members with an additional 20 hired for harvest, but Bim and Rowan have much bigger plans to increase productivity.

"We're looking at technology to mechanise the picking process and eliminate some of the labour costs which make up a huge part of on-farm expenditure. Instead of 200 people, we'll have five people on the picking machines and tractors," he said.

"We're also looking at mechanising the pruning. We've had a French consultant here promoting an almost 100 per cent mechanised method for pruning that has been trialled successfully. Instead of 20 people pruning we'll put the hedger through and it might take three people to clean up.

"The orchard has been designed to mechanise. We have a 2 cm auto steer on the tractors, so drivers basically just need to push a button, and we have a three-row sprayer for the little spraying we do."

This season, the exclusive sale of the plums has been given to six high-end retailers in Brisbane, Woolworths in Victoria and Harris Farm Markets in New South Wales. Some have also been exported to Singapore.

Currently there are trees in Stanthorpe, Western Australia, Spain, the United States and South Africa.

Good Rich Fruit Company manager Rowan Berecry has had success with using sterile fruit flies to control populations in the orchard. **Ella Archibald-Binge**

A LANDMARK research co-investment of $20.5million between Macquarie University and Horticulture Innovation Australia is set to focus on the management of Queensland fruit fly (Qfly) through Sterile Insect Technique.

A research team lead by Associate Professor Phil Taylor will focus on developing an effective SIT program to curb the prevalence of fruit flies in Australia.

The Good Rich Fruit Company near Inglewood has had some success with using sterile flies to control numbers in their plum orchards.

Manager Rowan Berecry said this method of controlling populations had the potential for widespread use on Australian farms.

"The property here we've been doing it for three years and releasing sterile fruit flies," Mr Berecry said.

"It's looking as though it has potential but, at the moment, it's still at the research phase.

"They haven't got the capacity to breed more elsewhere yet but the program is still being used as model for a big chunk of Australia and there's hope it can be superimposed elsewhere."

By releasing the sterile males, Mr Berecry said the population of wild flies was drastically reduced because they were unable to reproduce and infest fruit in the orchard.

"We have found we release the sterile flies and bait them as well that in those three years we've reduced the wild population more than 20 fold," he said.

"If you don't do anything about it can be devastating because you get the maggots in the fruit and that's the end and because we're exporting we have to ensure we don't have any going overseas with maggots."

Mr Berecry said that although this method of control was expensive, the money invested in this technology signified changes in the industry moving away from harmful insecticides.

The Qfly presents the most difficult and costly biosecurity challenge to market access for most Australian fruit producers, and threatens Australian crops valued at $9billion.

The SIT technique introduces sterile flies into the environment with the intention of these flies mating with the wild population and ensuring they cannot reproduce.

"Fruit flies, especially the Queensland fruit fly, present a truly monumental challenge to horticultural production in Australia," Associate Professor Taylor said.

"For generations, Australia has relied on synthetic insecticides to protect crops, but these are now banned for many uses."

Bush Telegraph

Warwick

TOPICS: BUSH TELEGRAPH, FRUIT FLIES,FRUIT FLY RESEARCH, GOOD RICH FRUIT COMPANY,HORTICULTURE, INGLEWOOD, RURAL NEWS, WARWICK

the INNOVATOR

Charles Sturt University

NSW GOVERNMENT Department of Primary Industries

SUMMER 2015-16 EDITION

The Newsletter from the **GRAHAM CENTRE** for Agricultural Innovation

From the Director

Welcome to the Summer edition of ***the Innovator*** for 2015-2016. In my last column I mentioned we had compiled our submission to the review of University Research Centres, and we have now received feedback.

The panel were strongly supportive of the CSU-DPI alliance, acknowledging that while transaction costs were increased, the combined strengths of the alliance greatly increased our ability to undertake multi-disciplinary agricultural research and development. Our strong links with industry, in particular the farming systems groups, was viewed as a key asset. The Graham Centre was responsible for 26% of the University's research income between 2010-14, making it the biggest Centre by far, and the panel noted there was considerable potential to increase this further. We should celebrate our achievement in this regard.

In reviewing all CSU research centres, the panel assessed the alignment with the CSU Research Narrative. A key recommendation in the process was a need for the University to review the Research Narrative to strengthen the implementation component. This will be completed by March 2016. Each research centre is required to review its mission for alignment with the University Research Strategy. This is timely for the Graham Centre, as we will be preparing our new strategic plan for 2016-2020, for approval by the Board of Management. Other tasks in response to the centre review include a reviewal of membership arrangements (it was noted we had a very large membership base) and implementation of strategies to improve publication rates. These are linked - membership entitles our members to apply for internal grants, but it is expected in turn that our members secure grant income in areas of alignment with the Centre's mission and publish the work. Our membership criteria will be revised to better reflect this.

CSU and DPI have also reviewed the current governance arrangements for the Centre as part of a new alliance agreement. It is expected the new alliance agreement will be signed before Christmas, and I will be able to report on details of this in the New Year. Suffice to say, the new alliance will create a governance structure that will allow greater engagement from senior managers within both CSU and DPI, yet retain the independence of our industry representation.

As a final note, I am pleased to report the University's significant improvement in our Excellence in Research

Continued on Page 2

THIS ISSUE

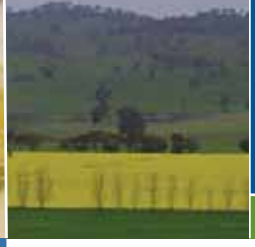
quick links

Graham Centre website

CSU website

DPI website





and exploring the region and the Yucatan culture, including the food and drinks.

Contact: Dr Marta Hernandez-Jover,
T: 02 6933 2086, E: mhernandez-jover@csu.edu.au

Probiotics to improve the fitness and performance of sterile fruit flies

Restrictions on the use of agrichemicals used to manage Queensland fruit flies (Qflies) (*Bactrocera tryoni* Froggatt) means fruit and vegetable crops are more susceptible to Qfly damage. The sterile insect technique (SIT) is an internationally accepted tool used to manage Qfly populations, however, mass-reared sterile fruit flies do not necessarily perform as well as wild male flies. Seminal contributions from around the world have shown the importance of microbial symbionts, particularly symbiotic bacteria, in the performance and mating success of pest fruit flies. Knowledge and an understanding of the microbial symbionts that co-exist with fruit flies, may lead to improved quality of mass-reared sterile flies. NSW DPI researcher Dr Ania Deutscher, together with colleagues Dr's Toni Chapman and Olivia Reynolds, is investigating the use of beneficial microorganisms to increase the quality and performance of mass-reared Qflies for SIT, as part of the SITplus project 'Area-wide integrated pest management using the sterile insect technique to control the Queensland fruit fly'. This project is funded by Horticulture Innovation Australia Limited (HIA) using the Summerfruit levy with co-investment from NSW DPI, the Traprock Group and funds from the Australian Government.

Supported by the Graham Centre and HIA, Ania travelled to Guatemala in October to attend a workshop and meeting on the *'Use of Symbiotic Bacteria to Reduce Mass-Rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application'*. The workshop and meeting were coordinated by the International Atomic Energy Agency and the Food and Agriculture Organisation of the United Nations. The meeting was attended by experts in fruit fly ecology, microbiology, and molecular biology, fruit fly mass-rearing facility staff, research fellows and PhD students.

Ania gave an oral presentation titled *'A Novel Molecular Sequencing Technique to Determine the Effect of Mass-rearing on the Queensland Fruit Fly Larval Gut Microbiome'*. She is testing a novel next-generation sequencing technique developed by Associate Professor Aaron Darling and Dr Catherine Burke from the University of Technology Sydney, to identify potential bacterial probiotic candidates. This method results in close to full length 16S rRNA gene sequences and reductions in errors typically introduced during amplification. Early results suggest low bacterial diversity in mass-reared Qfly larval gut bacterial communities compared with their wild counterparts, the former which may therefore benefit from probiotic supplements.

NSW DPI researcher Dr Ania Deutscher presented her research findings on the use of beneficial microorganisms to increase the quality and performance of mass reared Qflies for the sterile insect technique at an international meeting in Guatemala during October.

Appendix E.

MANAGE QUEENSLAND FRUIT FLY

JULY 2015

IT'S YOUR RESPONSIBILITY

...and it's as easy as A B C

A ATTRACT AND KILL WITH PROTEIN BAIT SPRAYS

B REDUCE MALES WITH MALE ANNIHILATION TECHNIQUE (MAT)

C PRACTISE GOOD SANITATION

Horticulture Innovation Australia

NSW Department of Primary Industries

BUGS FOR BUGS

Olivia Reynolds (NSW DPI) Dan Papacek (Bugs for Bugs)

MANAGE QUEENSLAND FRUIT FLY

IT'S Y

A PROTEIN BAIT SPRAYS

WHY

- A protein + toxicant attracts and kills flies
- Fruit flies require protein before they can sting fruit

HOW

- Mix protein lure with toxicant at the recommended rate
- Apply as a spot or band to the host trees

WHEN

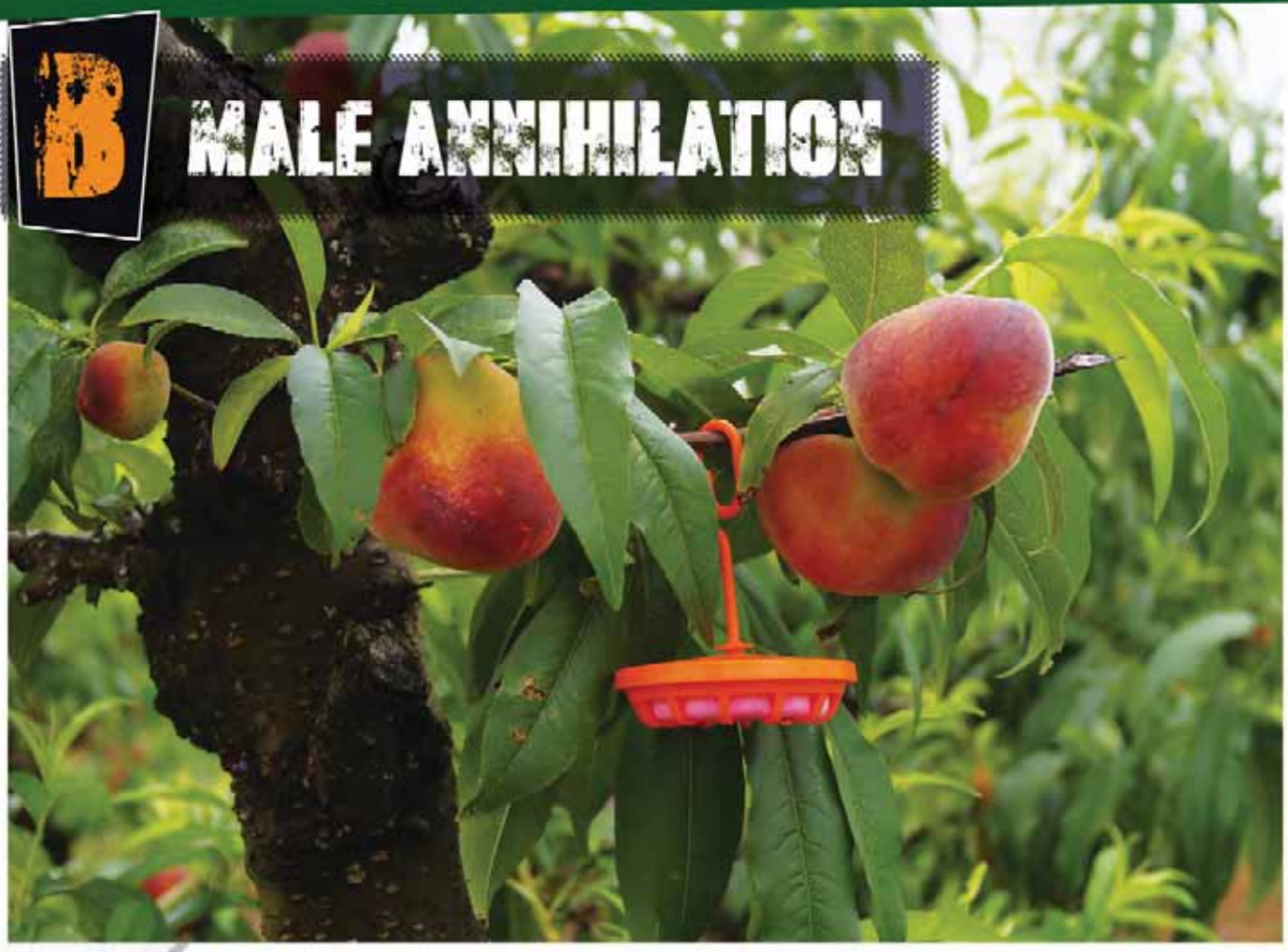
- Start early and apply weekly until at least 3 weeks after harvest
- Apply more often if you see signs of fruit fly damage or increased activity

ALSO

- Protein may cause fruit burn – test before use – minimise fruit contact
- Treating larger areas including non fruiting blocks and surrounds will improve results

YOUR RESPONSIBILITY ...and it's as easy as A B C

B MALE ANNIHILATION



WHY
- Reducing the male population will help improve fruit fly control

HOW
- Place MATs throughout your orchard at 10 – 20 per hectare

WHEN
- Apply three times per year
- Leave individual MAT out for a full 12 months

ALSO
- Use MAT as well as protein baiting (not instead of)
- Works best when used over large areas leading to improved control over time

MANAGE QUEENSLAND FRUIT FLY

IT'S YOUR RESPONSIBILITY ...and it's as easy as A B C

C SANITATION

WHY

- Surviving flies from last year are major contributors to spring populations
- Minimise fruit fly breeding for best results

HOW

- Remove unwanted hosts including feral and neglected trees
- Remove all residual fruit following harvest
- Destroy any fallen fruit if damaged by fruit fly

MONITORING

- Fruit fly traps monitor male population trends
- Replace wicks every three months
- Treat trap counts as a guide only
- Do not rely on trap counts to decide whether or not to apply protein bait sprays and MAT
- Regularly inspect your crop for any sign of fruit fly damage

All photos: Dan Papacek

For more information go to: www.dpi.nsw.gov.au or www.bugsforbugs.com.au

Appendix F.

Help us control Queensland fruit fly (Qfly)

Qfly is a fruit fly pest that costs Australian horticultural producers ten's of millions dollars annually.

The stone fruit orchards in the Traprock are participating in a trial to control Qfly without cover sprays. The reason for this trial is the APVMA are phasing out the use of these sprays and we need to find alternate control methods.

The trial will run for 3.5 years from January 2014.

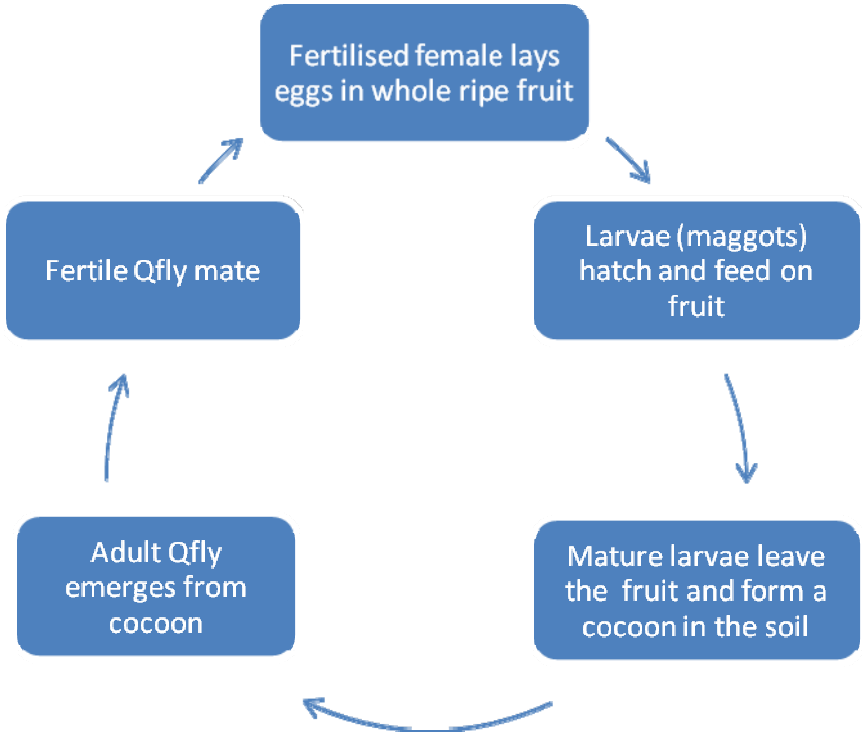
What is Qfly?

Queensland fruit fly is a species native to eastern Australia.

It lays its eggs in whole ripe fruit, rendering the fruit unsaleable, and breeding up very quickly if left unchecked.

Image: Queensland fruit fly (about the size of a domestic house fly)

What is the Qfly life cycle? 4-5 WEEKS



What fruit can Qfly infest?

Qfly is known to infest over 240 native and commercial fruits and vegetables. These include stone fruit, apples, pears, figs, grapes, citrus, chilli, capsicum, eggplant and prickly pear.

Researchers are determining if Qfly can infest tree pear.

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What can you do to help?

DO's

- Dispose of over ripe fruit/veggies correctly (this includes all uneaten fruit/veggies purchased or grown for consumption) – put them in a sealed plastic bag in the sun for 3 days before putting it in the bin or compost
- Keep your veggie garden clean of fallen fruit
- Manage fruit trees with adequate pruning – so you can reach all the ripe fruit
- Hang fruit fly baits and a Qfly trap in your fruit trees or around your veggie garden
- Let your neighbours know if you trap a Qfly or if you think your fruit/veggies are Qfly infested

DONT'S

- Leave fallen fruit/veg on the ground in your garden
- Throw whole ripe fruit/veg over the fence or into a compost bin
- Leave rotten fruit on the tree or excess veggies in the garden

Who do I contact for further information?

John & Julie Pratt, 07 4685 6144
or 07 4685 6031
Andy Finlay, 07 4685 6171
Graham Finlay, 07 4685 6160
Angus Ferrier, 07 4685 6154
or 0438 856 154
Rowan Berecry, 07 4652 4193
or 0418 216 889
Olivia Reynolds (Project leader, NSW DPI)
02 4640 6200

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Appendix G.

Diet Influences the Gut Microbiome of Queensland Fruit Fly Larvae:

Understanding Gut Microbiota to Improve the Quality of Mass-Reared Flies for the Sterile Insect Technique

Dr. Ania Deutscher

Dr. Toni Chapman & Dr. Olivia Reynolds (NSW DPI)

Dr. Catherine Burke & A/Prof. Aaron Darling (University of Technology Sydney)

A/Prof. Markus Riegler (Western Sydney University)

Queensland Fruit Fly (Qfly)
Bactrocera tryoni

- An economically significant horticultural pest in Australia;
- Native to eastern Qld and north eastern NSW; however, spread to urban and horticultural areas in Qld, NSW, VIC and NT;
- Restrictions on or withdrawal of the most effective pesticides → growers need alternative tools to control Qfly

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Qfly Sterile Insect Technique (SIT)

- Environment friendly, species-specific pest management tool;
- Process:
  1. mass production
  2. sterilization
  3. release of sterile flies at high densities
  4. sterile males transfer non-viable sperm to wild female flies
  5. suppression of fruit fly population
- The quality and performance of the released sterile insects is critical to the efficiency and effectiveness of SIT programs.

Adult Qfly cages at the Elizabeth Macarthur Agricultural Institute (EMAI) Fruit Fly Production Facility (FFPF) in Menanagle, NSW

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Gut Microbiota Matters

- Gut microbiota can influence various aspects of insect health, fitness and behaviour;
- Fruit flies reared under artificial conditions are no longer exposed to the microorganisms present in their natural environment;
- Reported shifts in bacterial types and relative abundances present between laboratory and wild adult tephritid fruit flies (Ben-Ami et al., 2010; Estes et al., 2012; Morrow et al., 2015);
- Very little is known about the bacteria present in the gut of Qfly larvae and the influence of artificial fruit fly rearing on the larval gut microbiome.

1. Qfly Larval Collections

Qfly larvae inside a white-fleshed peach

Qfly larvae feeding on carrot diet

- Wild populations from peaches from two locations; and
- Three domesticated colonies feeding on an artificial carrot diet

Suffix used in this Study	Wild or Domesticated (Generation)	Substrate	Source	Collection Date	Number of Larvae Analysed
Bux_P	Wild	Peach	Residential property, Buxton, NSW	Jan 2015	20 (from 7 fruit)
Tum_P	Wild	Peach	Residential property, Tumut, NSW	Feb 2015	15 (from 5 fruit)
GPII_Col	Domesticated Colony (F24)	Artificial carrot diet	Gosford Primary Industries Institute, Ourimbah, NSW	Dec 2014	9
FFPF_Col	Domesticated Colony (F78-81)	Artificial carrot diet	Fruit Fly Production Facility, Menangle, NSW	Dec 2015	8
MQ_Col	Domesticated Colony (unknown)	Artificial carrot diet	Macquarie University, North Ryde, NSW	June 2015	6

2. Sample Processing & Sequencing

- Each larva was surface sterilised and dissected → midgut collected → DNA extracted;
- 16S rDNA libraries were constructed and sequenced on an Illumina MiSeq following the near full-length 16S method by Burke and Darling (2016) https://peerj.com/articles/2492/:
  - single molecule dual tagging scheme that helps with:
    - ✓ reassembly of near full-length sequences
      - ➢ better species resolution
    - ✓ offers large reductions in:
      - ➢ base calling errors
      - ➢ chimera (in vitro-recombination) detection
      - ➢ reduced amplification bias

Qfly larva mid and hindgut

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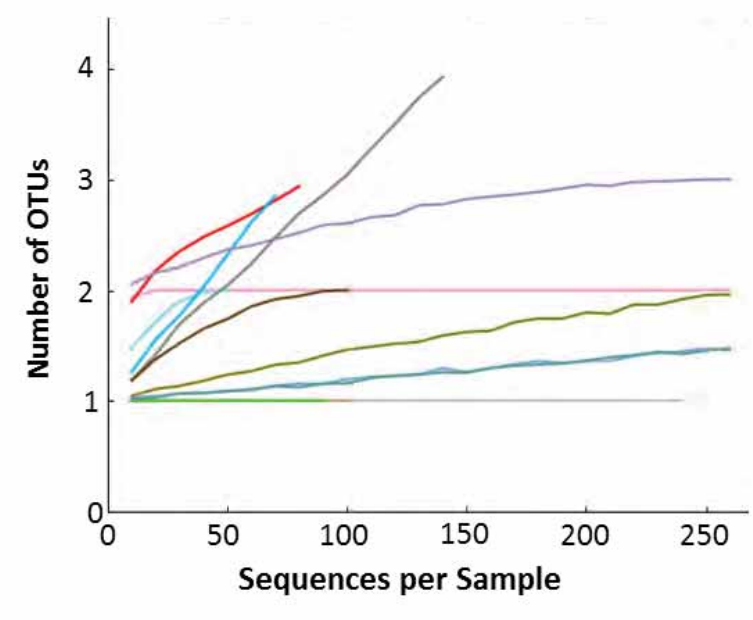
Results

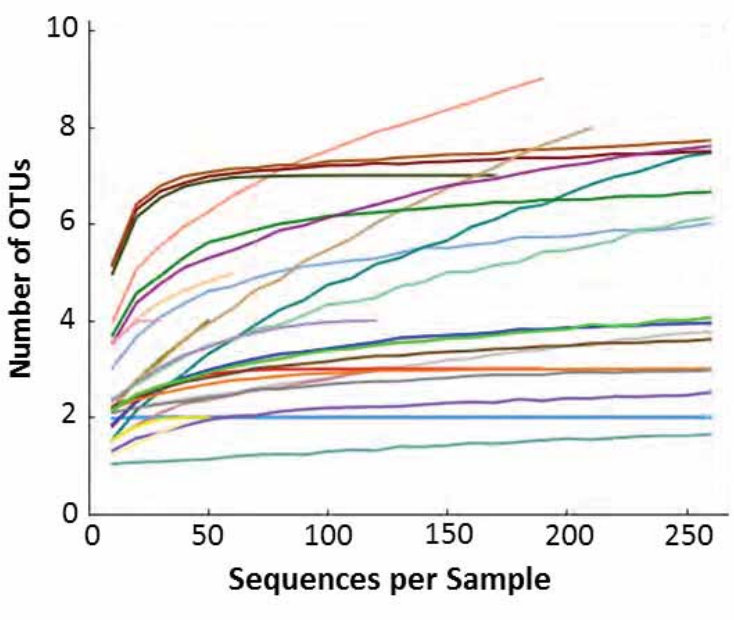
- 32,337 sequences assembled based on the molecular tags from 5,160,368 Illumina read pairs;
- 14,710 16S rRNA gene sequences (>1300 bp) were analysed; the number of sequences differed per sample;
- Near full-length sequences clustered at 99% similarity;
- Low gut bacterial diversity: max. 13 OTUs per larva;
- Gut bacterial diversity significantly reduced in domesticated larvae ($p<0.0005$).

Results

- Rarefaction analysis showed that we captured the bacterial diversity for the majority of samples.



Domesticated Larval Samples



Wild Larval Samples

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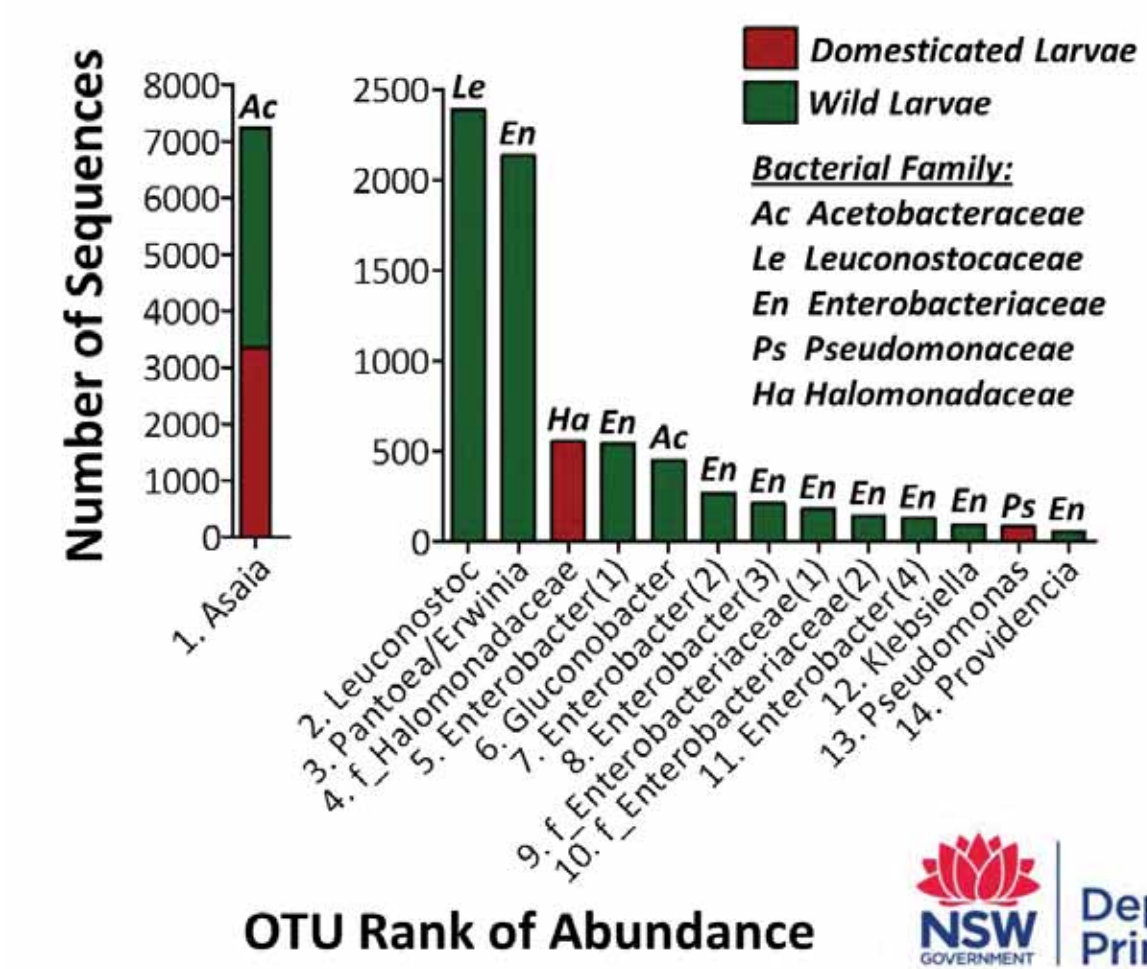
Results

- Very few OTUs were common to both wild and domesticated larvae.



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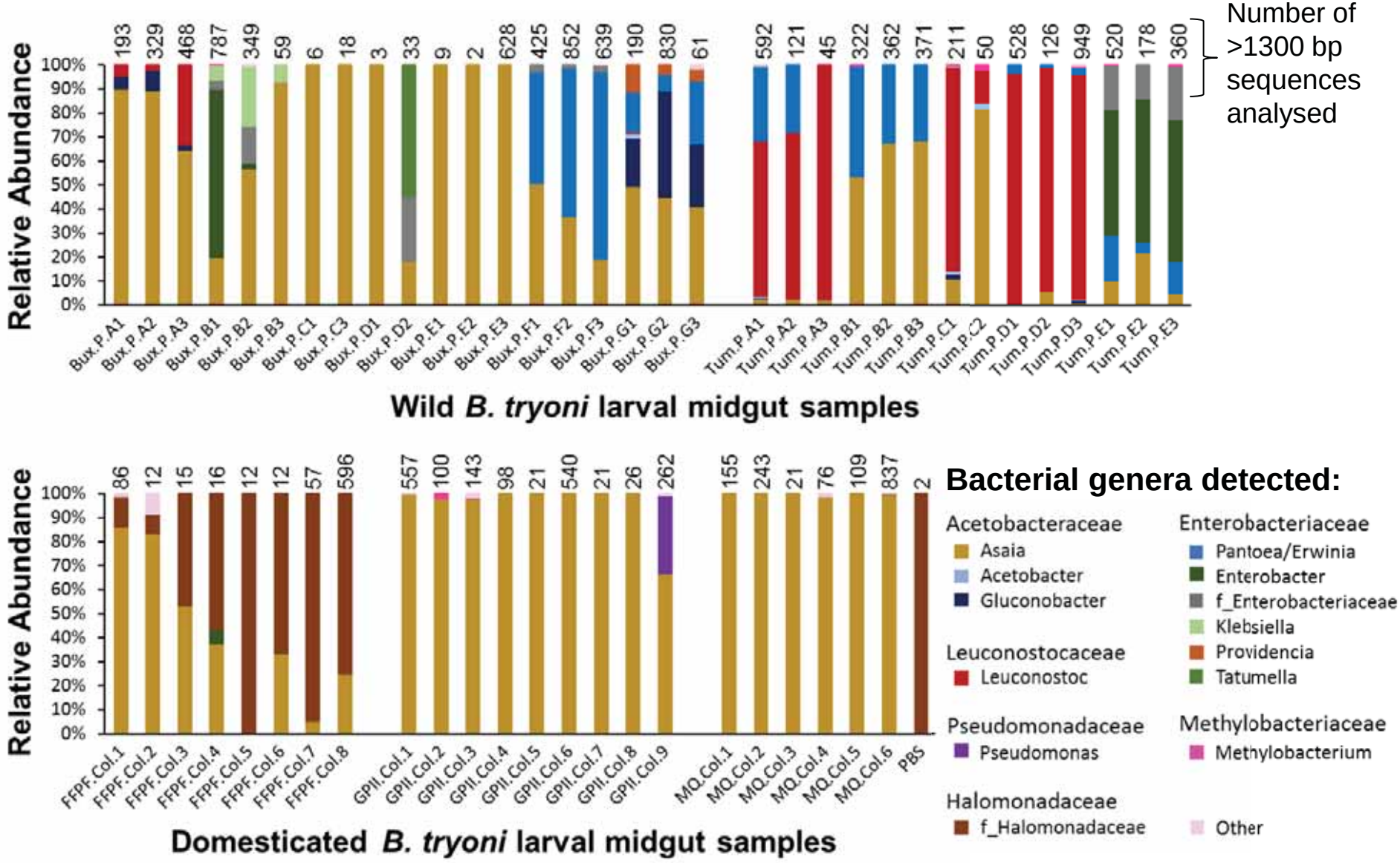
Relative Abundance of Midgut Bacteria



Future Directions

- Understanding what drives the microbial changes observed in domesticated Qflies;
- Determining the functions of the bacteria; and
- Better understanding of the vertical transmission of bacteria.

→ eco-engineering of diets that encourage ‘beneficial’ microbial communities, which may restore gut microbial diversity, and improve the quality and performance of the released sterile flies

Postdoc position available in this research area. If interested, please send me an email at ania.deutscher@dpi.nsw.gov.au.

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Acknowledgements

This project was funded by Horticulture Innovation Australia using the summerfruit industry levy with co-investment from NSW Department of Primary Industries and funds from the Australian Government as part of the SITplus initiative.

Collaborators
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Toni Chapman (NSW DPI)
Aaron Darling (UTS)
Olivia Reynolds (NSW DPI)
Markus Riegler (WSU)

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David Cruickshank
Bernie Dominiak
Andrew Jessup
Jeanneth Perez
Peter Treloar

Technical assistance
Nicholas Deutscher
Michael Liu
Narit Thaochan
Paul Worden

Contact Details:
Ania Deutscher: ania.deutscher@dpi.nsw.gov.au

Gut Microbiota to Improve the Quality of Mass-Reared Queensland fruit fly under the Sterile Insect Technique

Ania Deutscher and Lucas Shuttleworth (NSWDPI)

Olivia Reynolds, Toni Chapman, Damian Collins & Terry Osborne (NSW DPI)

Catherine Burke & Aaron Darling (University of Technology Sydney)

Markus Riegler (Western Sydney University)

Queensland fruit fly

- *Bactrocera tryoni* Froggatt
- Polyphagous; native to Australia
- Australia's most significant insect biosecurity threat to our >$9 billion horticulture industry
- SIT



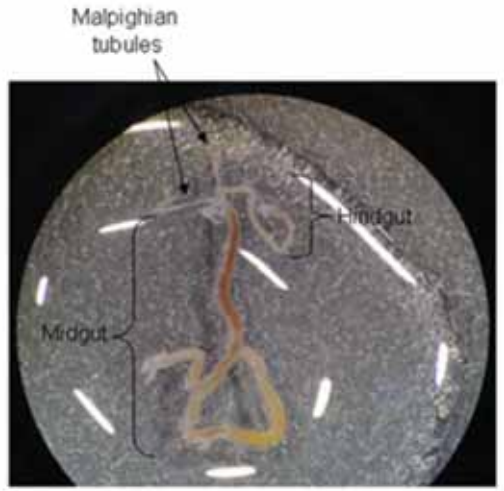
Aim

- To determine the difference in abundance and diversity of microbial symbionts of mass-reared and wild-collected larval *B. tryoni* as part of the SIT
- To determine the difference in larval and adult quality & performance traits between mass-reared probiotic fed *B. tryoni* larvae and non-probiotic fed larvae (control)

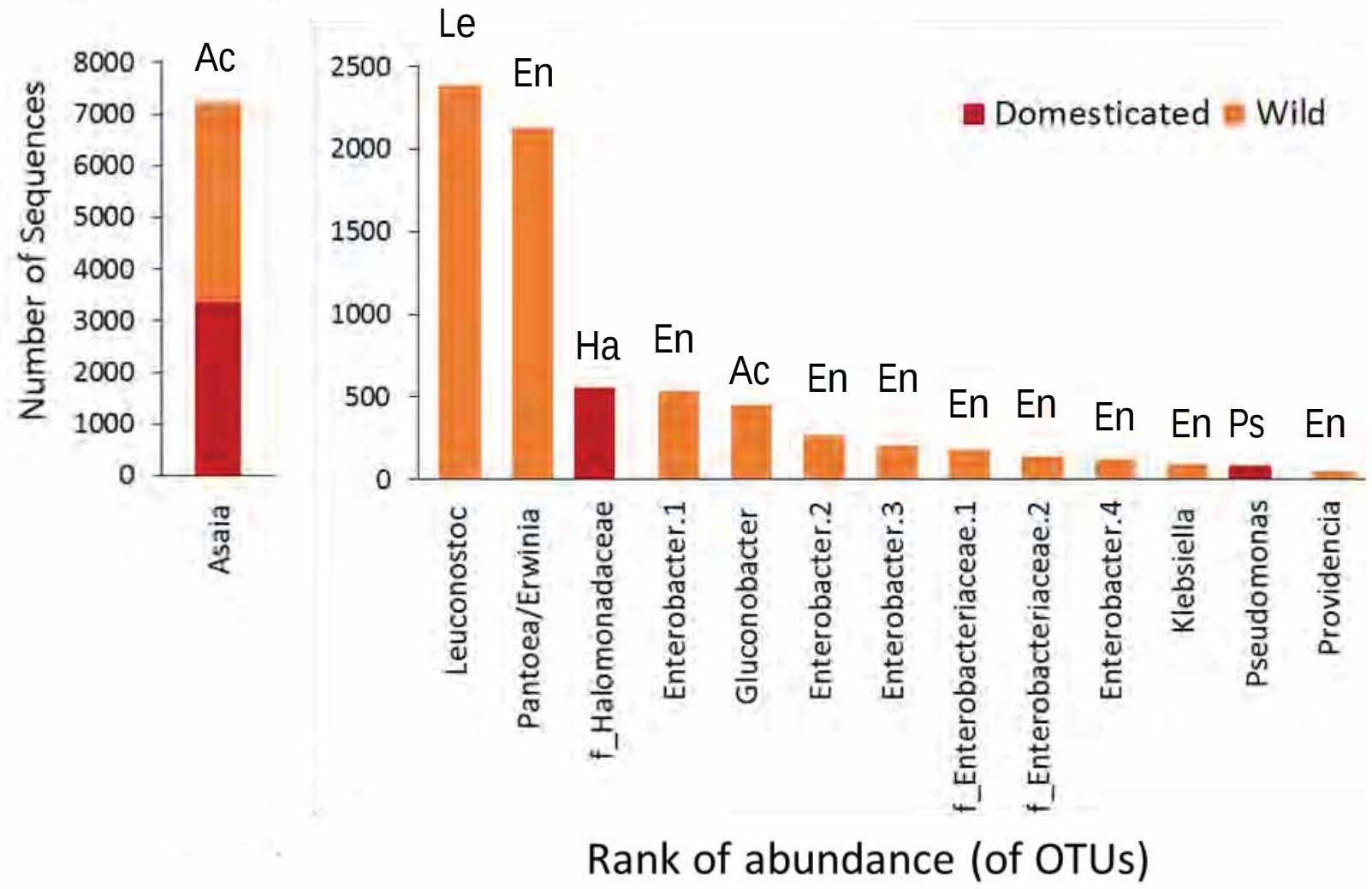
LARVAL MICROBIAL CHARACTERISATION

Method

- 16S rRNA gene amplicon NGS (Burke and Darling, 2014); produces near full-length 16S sequences, to analyse the midgut bacterial community of wild and domesticated *B. tryoni* larvae



Rank Abundance of OTU's



> 50 near full-length sequences

- *Asaia sp.*
  - dominant in both domesticated and wild larvae
- Greater abundance and diversity in wild larvae

Ac – Acetobacteriaceae

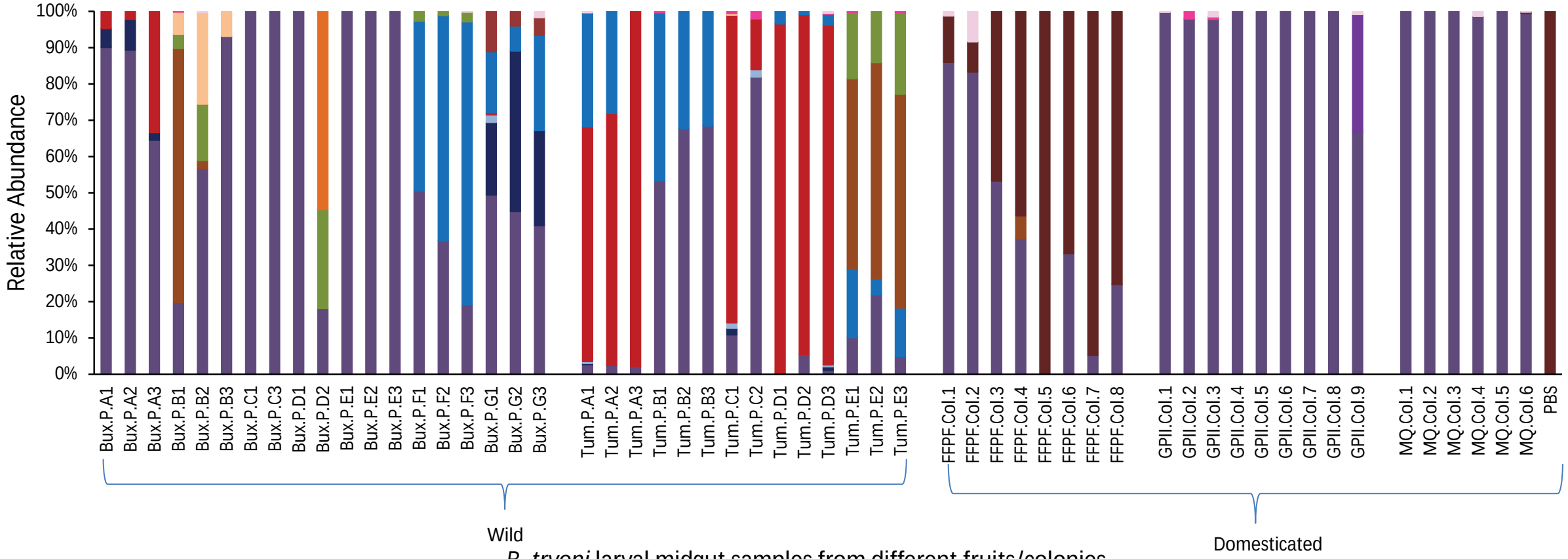
Le – Leconostocaceae

En – Enterobacteraceae

Ha – Halomonadaceae

Ps - Pseudomonaceae

Bacteria - larval midgut



Diversity and relative abundance of bacterial taxa in *B. tryoni* larval midguts

- Overall, gut bacterial diversity is low; significantly lower in domesticated larvae
- Bacteria commonly associated with fruit (Acetobacteraceae, Enterobacteriaceae and Leuconostocaceae) detected in wild larvae; largely absent from domesticated larvae
- *Asaia*, an acetic acid bacterium, detected in wild and domesticated populations (55/56 larval gut samples)
- Larvae from the same single peach shared a similar gut bacterial profile, whereas larvae from different peaches collected from the same tree had different gut bacterial profiles
- Clustering of the Asaia near full-length sequences at 100% similarity showed that the wild flies from different locations had different *Asaia* strains

Fungi - Larval Midguts

- Diverse range of yeasts and yeast-like fungi cultured from wild *B. tryoni* larval midguts (genera: *Aureobasidium, Candida, Cryptococcus, Hanseniaspora*, *Pichia*, *Saccharyomcyes* and *Starmerella*)
  - Cultured yeasts from 15 out 17 wild larvae
  - Yeasts were amongst the dominant colony types
  - Yeasts present in midguts from larvae on diverse diets: apples, oranges, peaches, cherry guavas, plums
- Negligible cultivable yeasts in domesticated compared to wild larvae (preliminary results)

Gram stain of *Aureobasdium pullulans*

Journal of Economic Entomology, 2016, 1–3
doi: 10.1093/jee/tow262
Short Communication

Short Communication

Yeast: An Overlooked Component of *Bactrocera tryoni* (Diptera: Tephritidae) Larval Gut Microbiota

Ania T. Deutscher,[1,2,3] Olivia L. Reynolds,[1,2] and Toni A. Chapman[1]

[1]Biosecurity and Food Safety, NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Private Mail Bag 4008, Narellan, New South Wales 2567, Australia (ania.deutscher@dpi.nsw.gov.au; olivia.reynolds@dpi.nsw.gov.au; toni.chapman@dpi.nsw.gov.au), [2]Graham Centre for Agricultural Innovation (an alliance between NSW Department of Primary Industries and Charles Sturt University), Elizabeth Macarthur Agricultural Institute, Private Mail Bag 4008, Narellan, New South Wales 2567, Australia, and [3]Corresponding author, e-mail: ania.deutscher@dpi.nsw.gov.au

Subject Editor: Anthony Clarke

Received 27 June 2016; Editorial decision 17 October 2016

Abstract

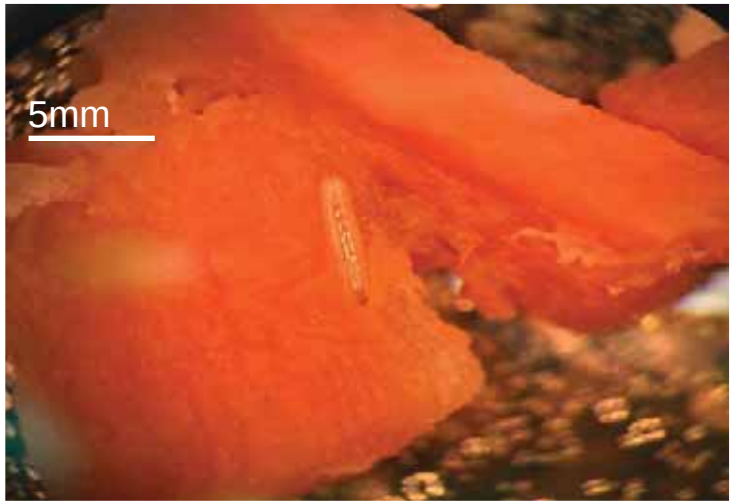
Yeasts, often in hydrolyzed form, are key ingredients in the larval and adult diets of tephritid fruit fly colonies. However, very little is known about the presence or role of yeasts in the diets of tephritid fruit flies in nature. Previous studies have identified bacteria but not detected yeasts in the gut of Queensland fruit fly, *Bactrocera tryoni* (Froggatt), one of Australia's most economically damaging insect pests of horticultural crops and of significant biosecurity concern domestically and internationally. Here we demonstrate that cultivable yeasts are commonly found in the gut of *B. tryoni* larvae from fruit hosts. Analysis of the ITS1, 5.8S rRNA gene, and ITS2 sequences of randomly selected isolates identified yeasts and yeast-like fungi of the genera *Aureobasidium*, *Candida*, *Cryptococcus*, *Hanseniaspora*, *Pichia*, and *Starmerella*. The prevalence of these yeasts in fruits suggests that larvae consume the yeasts as part of their diet. This work highlights that yeasts should be considered in future tephritid larval gut microbiota studies. Understanding tephritid–microbial symbiont interactions will lead to improvements in artificial diets and the quality of mass-reared tephritids for the sterile insect technique.

Key words: sterile insect technique, insect rearing, insect nutrition, Queensland fruit fly, microbial symbiont

LARVAL PROBIOTIC

Methods

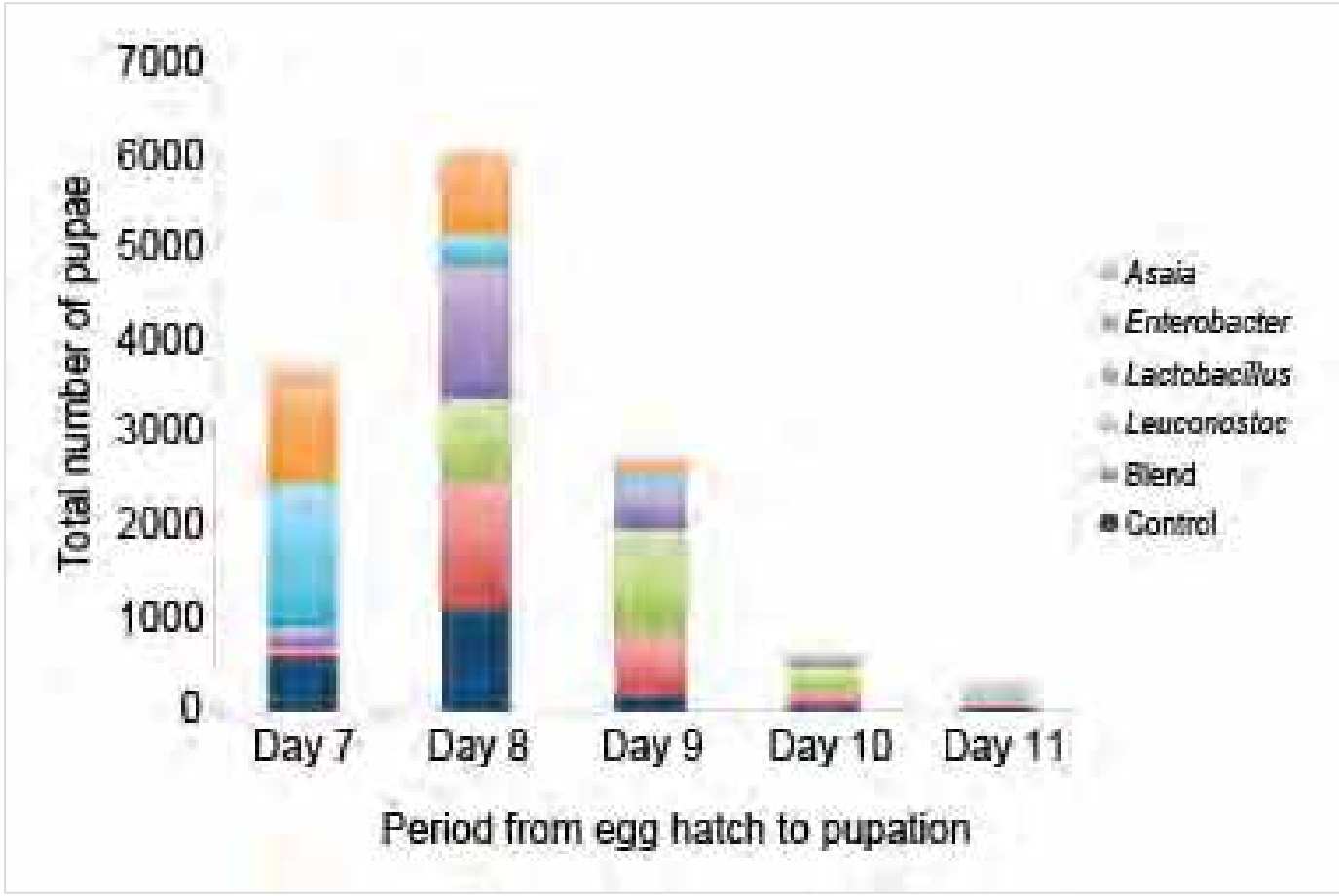
- Four probiotic candidates selected
  - *Asaia* sp., *Enterobacter* sp., *Lactobacillus* sp., *Leuconostoc* sp.
- Six treatments; each singularly, blend of all four, and control
- 1 x 10^8 bacterial cells per gram of diet
- Development times, pupal-weight, flight-ability, sex-ratio, and locomotor activity.
- Survival (field cage), and mating currently underway



Second instar *B. tryoni* larva feeding on probiotic enriched carrot diet

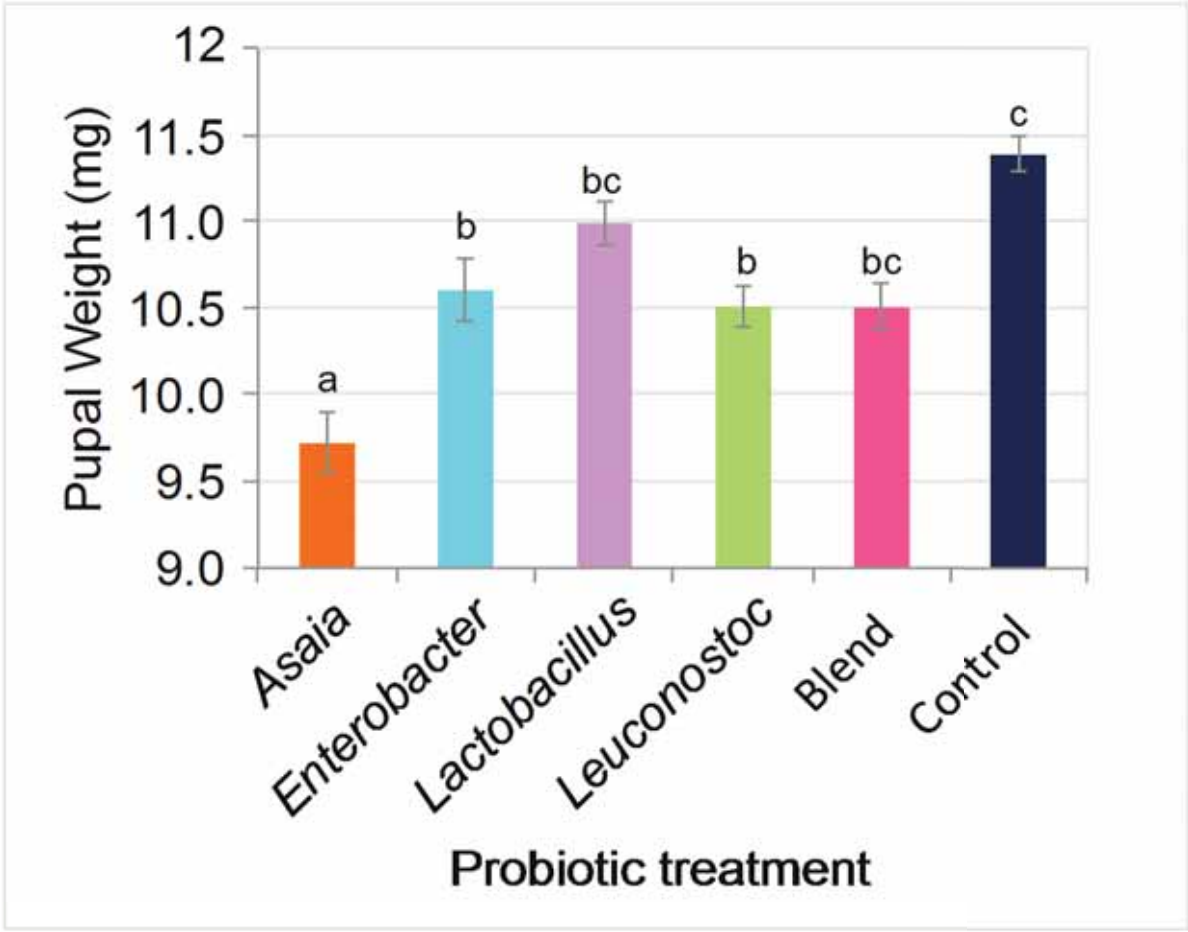
Larval development time

- *Enterobacter* and *Asaia* reduced LDT
- *Leuconostoc, Lactobacillus* and the blend increased LDT



Pupal weight

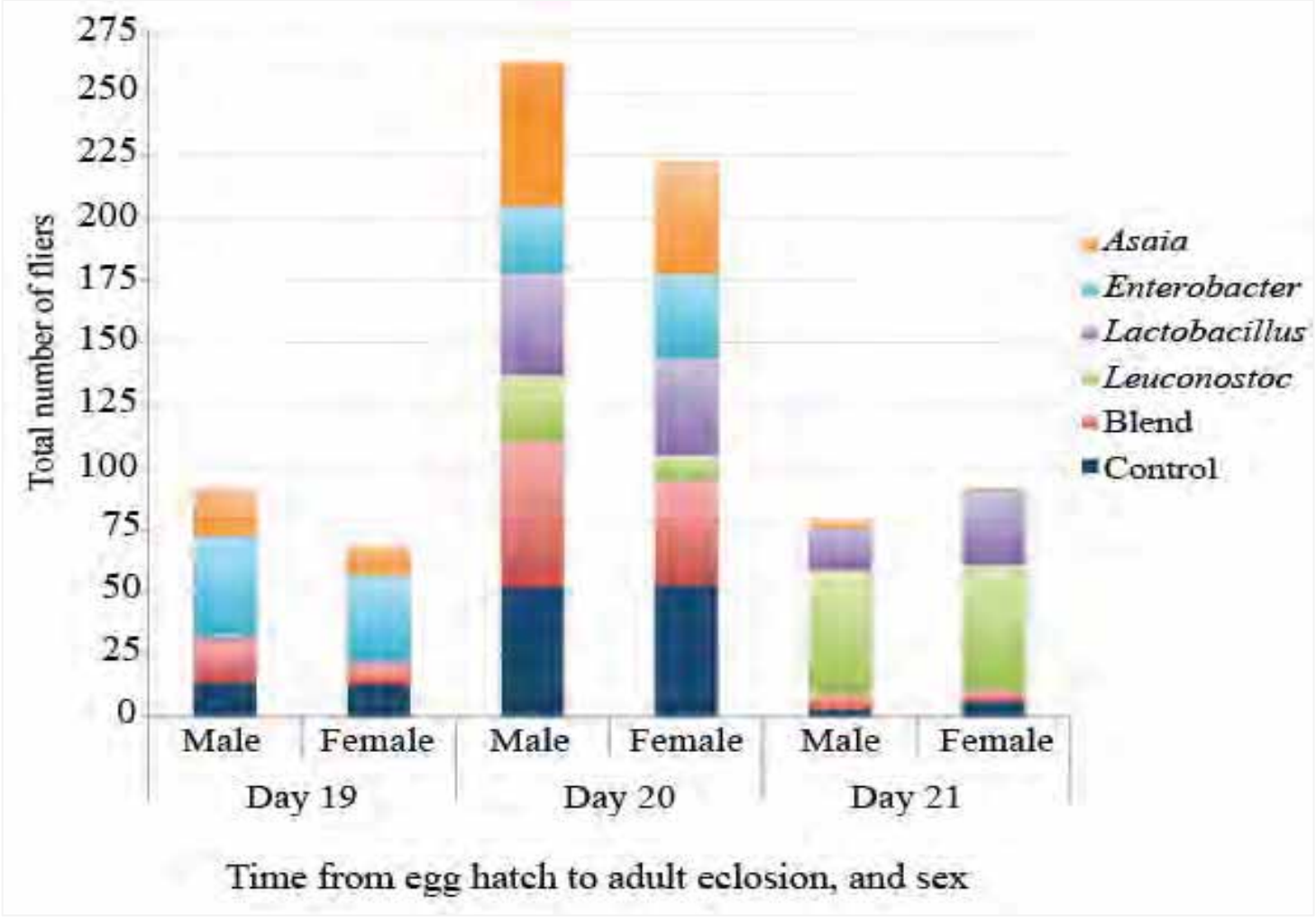
- *Asaia*, *Enterobacter* and *Leuconostoc* had lower pupal weight than the control ($p<0.001$)



Pupal weight seven days after pupation. n= 24 reps of 10 pupae per treatment

Adult eclosion and Flight

- Egg to adult eclosion was shorter with *Enterobacter* males (Day 19, df = 10, p=0.003)
- Both sexes of *Leuconostoc* and *Lactobacillus* had delayed egg-adult eclosion (Day 19, p=0.003,Day 21, p<0.001, both df 10)
- All treatments had numbers of fliers >90%, except *Lactobacillus* (87%)



Sex ratio

- The blend, *Asaia* and *Leuconostoc* resulted in more males than females *Lactobacillus* resulted in more females

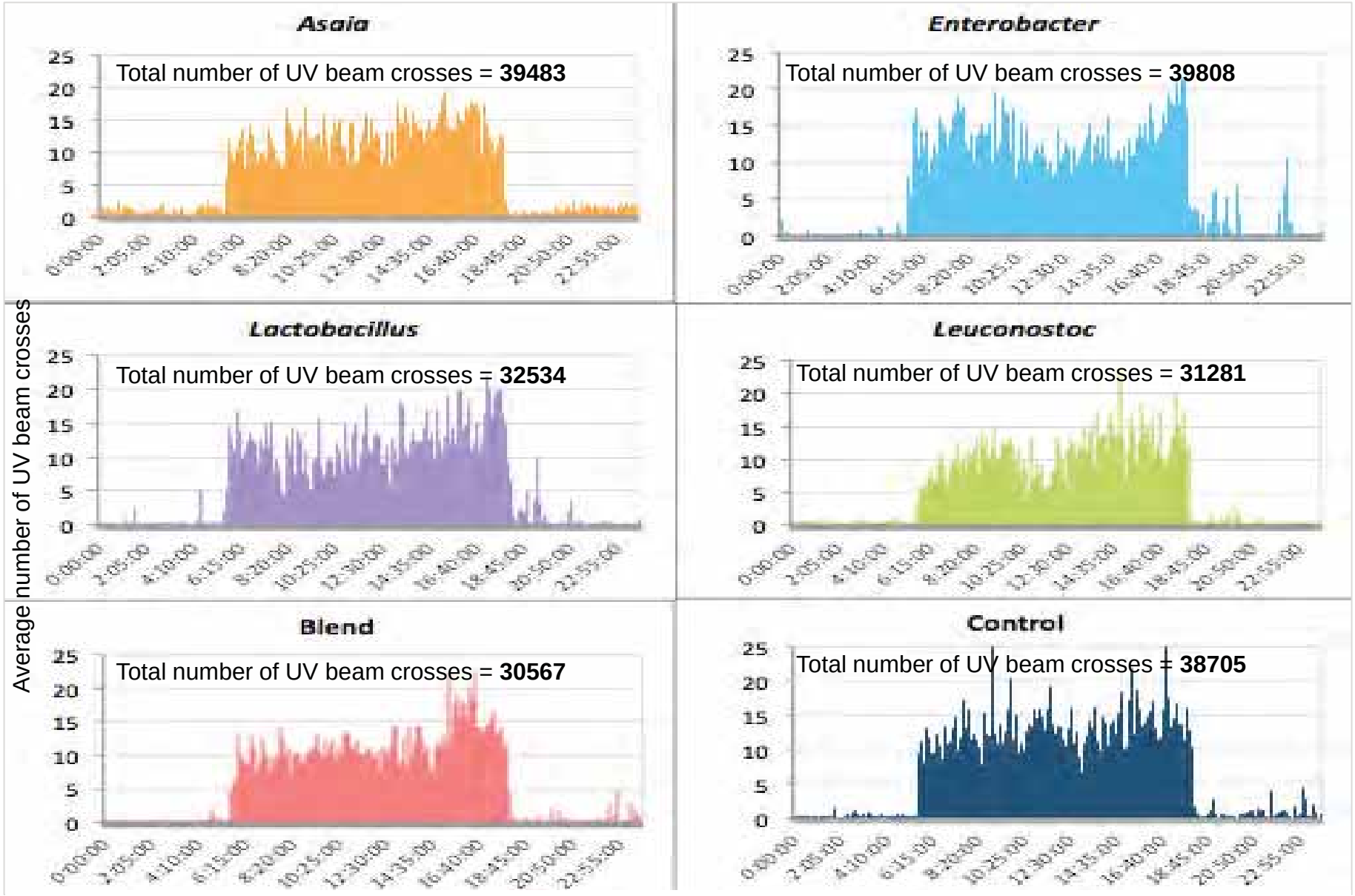
Sex ratio of probiotic fed larvae and the unfed control

Probiotic	Male	Female	M:F sex ratio
Asaia	79	57	1.4:1.0
Enterobacter	67	69	1.0:1.0
Lactobacillus	60	70	0.9:1.0
Leuconostoc	76	60	1.3:1.0
Blend	82	55	1.5:1.0
Control	69	72	1.0:1.0

Locomotor Activity

- LA was highest for *Enterobacter* and *Asaia* (p<0.0001)
- The blend, *Leuconostoc* and *Lactobacillus* had lower LA than the control (p<0.0001)

Locomotor activity monitor (Trikinetics)



Average daily locomotor activity measured as number of times flies cross a UV light beam within a glass tube. Measurements were taken at five minute intervals over four days. n= 4 reps per treatment, 2 m, 2 f.

Conclusions

- Sequencing of near full-length 16S rRNA gene sequences and comparing individuals facilitated analysis of the composition and diversity of *B. tryoni* larval gut bacteria at finer scale than previously possible
- *Asaia* likely to have an important role in larval *B. tryoni*
- Bacterial communities of wild larvae are more diverse
- First report of yeasts and yeast-like fungi in midgut of wild-collected *B. tryoni* larvae
- The probiotics tested influence a range of larval and adult quality traits
  - Shortened development time can reduce rearing costs
  - A male biased sex ratio would benefit SIT programs using bisex strains
  - The performance of sterile flies in the field may be improved by larval application of probiotics such as *Asaia* and *Enterobacter* that yield more active flies

Future work

- Consider both yeast and bacteria
- How does the microbial community change through all life stages
- Effects of probiotic fed larvae on post-irradiated (i.e. sterile) males

Acknowledgements

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Narit Thaochan
Paul Worden
Monjur Khan
Terry Osborne

Charles Sturt University
GRAHAM CENTRE for Agricultural Innovation
NSW GOVERNMENT Department of Primary Industries
Horticulture Innovation Australia
SITplus

THANKYOU

A Novel Molecular Sequencing Technique to Determine the Effect of Mass-rearing on the Queensland Fruit Fly Larval Gut Microbiome

Dr. Ania Deutscher

Dr. Olivia Reynolds and Dr. Toni Chapman (NSW DPI)

Dr. Catherine Burke and A/Prof. Aaron Darling (University of Technology Sydney)

A/Prof. Markus Riegler (Western Sydney University)

Research Interests

- Mycoplasmas
- Host-pathogen interactions (adherence mechanisms)
- Molecular diagnostic assay development
- Bacterial diagnostics
- Gut microbiota
- Probiotics
- Fruit flies

Current research project: Investigating the potential of beneficial microorganisms in the Queensland fruit fly (Qfly) larval diet

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Bactrocera tryoni (Froggatt) - Queensland Fruit Fly

Most serious insect pest of fruit and vegetable crops in Australia

Native to eastern Qld and north eastern NSW; however, spread to urban and horticultural areas in Qld, NSW, VIC and NT

With the phasing out of key organophosphate pesticides growers need alternatives

$$$ are being invested to improve Qfly SIT

NSW GOVERNMENT Department of Primary Industries

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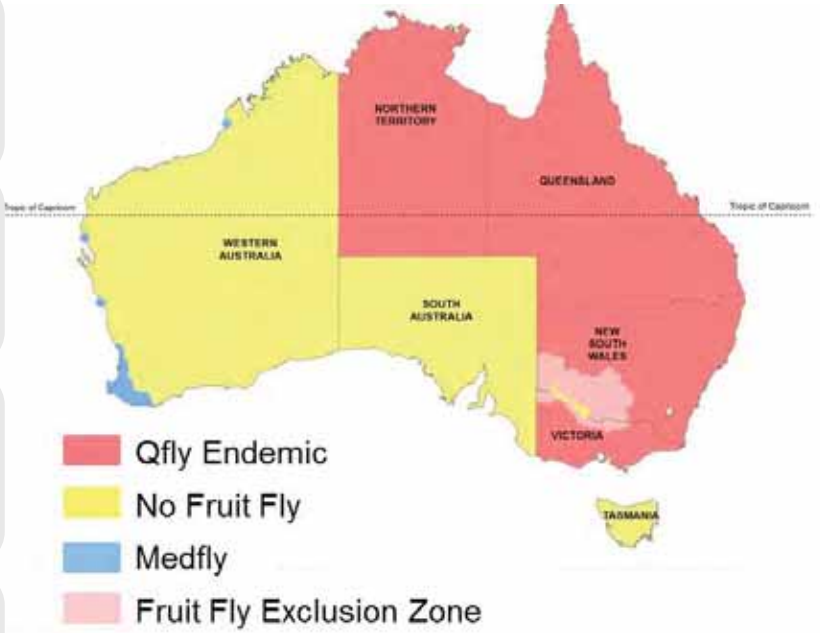

Picture courtesy of Peter Worsley, from Dominiak & Mapson (manuscript in preparation)

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Adult Qfly cages at the Elizabeth Macarthur Agricultural Institute (EMAI) Fruit Fly Production Facility (FFPF)

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Adult Qfly cages at the Elizabeth Macarthur Agricultural Institute (EMAI) Fruit Fly Production Facility (FFPF)

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Qfly SIT

- currently no genetic sexing strain
- mass-rearing reduces the quality of the released flies

What is needed:

- improved Qfly performance
- more efficient production

Does gut microbiota play a role?

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Gut Microbiota Matters

Fruit fly gut microbiota can have an effect on nutrition, pheromone production, communication, mating, defence against pathogens or host chemical defences and more…

Examples:

- The expression of bacterial *nif*H gene within the fruit fly larvae indicates that the bacteria are involved in nitrogen fixation (Behar *et al*., 2008);
- Bacteria may help in the reduction of sugar and triacylglycerides, when larvae are exposed to sugar rich diets (Huang & Douglas, 2015);
- Olive fly larvae require *Ca.* Erwinia dacicola to develop in unripe olives Ben-Yosef *et al.* (2015)

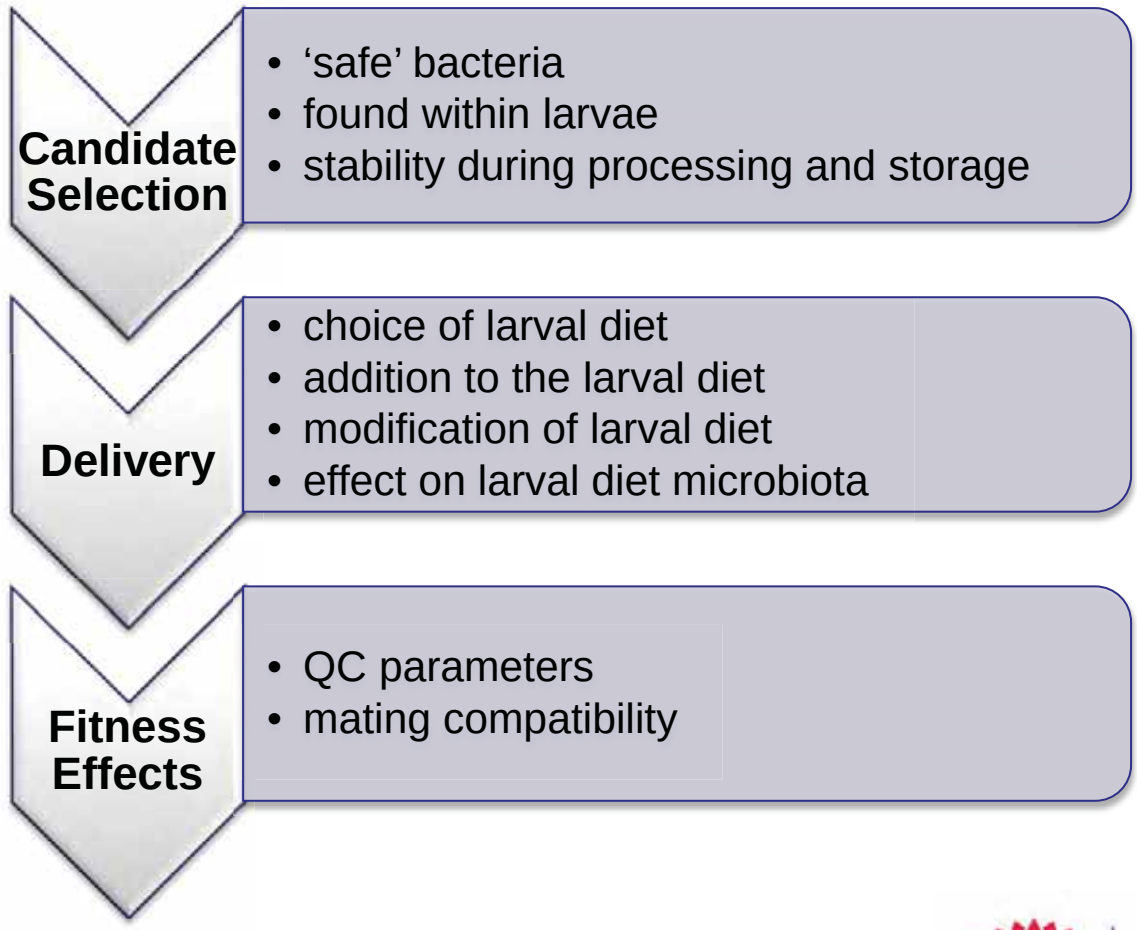
Progress in Fruit Fly Larval Diet Probiotic Supplements

Examples:

1. Medfly larval diet supplemented with *Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter* spp. (Hamden *et al*., 2013)
   - increase in pupal weight and adult size, in survival rate (10%), quantity of sperm stored;
   - enhanced sexual performance 5 days after emergence; and
   - increase in Enterobacteriacae and decrease in Pseudomonas in the gut – significant difference in Pseudomonas levels noticed at 5 day old males not at 1 day

2. Medfly larval diet supplemented with an *Enterobacter* sp. (Augustinos *et al.*, 2015)
   - significantly improved pupal and adult fly recovery rate; and
   - reduced egg to adult duration particularly for male flies

Developing a Qfly Larval Diet Probiotic Supplement



What is the microbial community of Qfly larvae?

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Determining the Microbial Community of Qfly Larvae

Aim:

- to identify bacteria present within the gut; and
- to understand microbiota variation

Methodology:

to compare gut bacteria from:

- wild & laboratory/facility larvae
- wild larvae from different hosts

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Determining the Microbial Community of Qfly Larvae

Sample Collection

- larvae from a range of Qfly infested fruits
- EMAI Fruit Fly Production Facility (FFPF) larvae
- larvae from several laboratory Qfly colonies

Sample Processing

- Midgut extracted and stored for future processing
- DNA extracted and quantified

Microbial Identity

- Targeted amplicon next-generation sequencing

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- several laboratory Qfly colonies

Sample Processing

- Midgut extracted and stored for future processing
- DNA extracted and quantified

Microbial Identity

- Targeted amplicon next-generation sequencing (NGS)

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Novel 16S rDNA Amplicon Next-Generation Sequencing Technique

designed by
Assoc. Prof. Aaron Darling and Dr. Catherine Burke
University of Technology Sydney (Australia)

See: Burke and Darling (2014) doi: http://dx.doi.org/10.1101/010967

- single molecule dual tagging scheme that helps with:
  - ✓ reassembly of full length sequences
    - ➢ better species resolution
  - ✓ offers large reductions in:
    - ➢ base calling errors
    - ➢ chimera (in vitro-recombination) detection
    - ➢ reduced amplification bias
- using Illumina MiSeq

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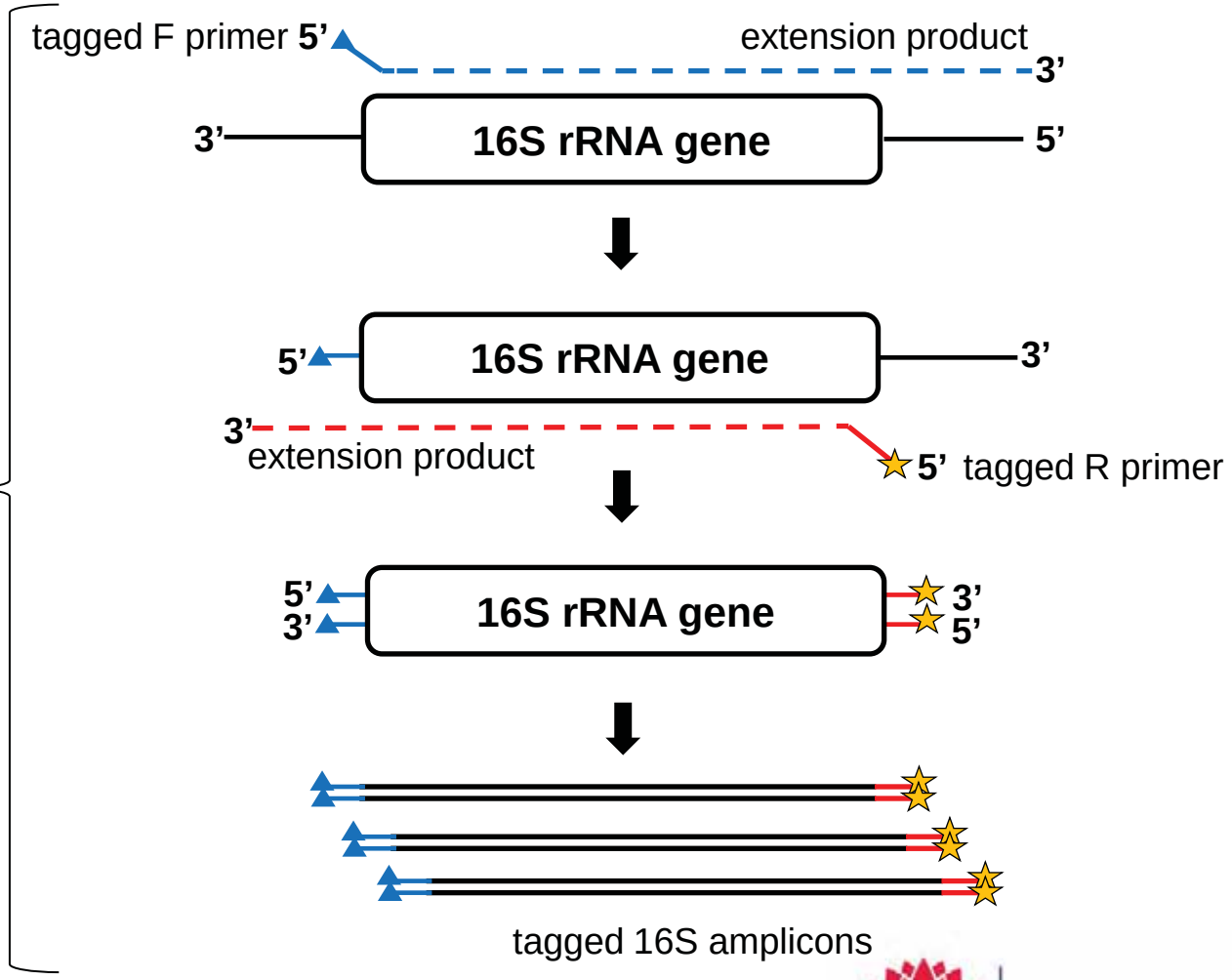
Novel 16S rDNA Amplicon NGS Method (Part 1)

Single molecule tagging and amplification



Burke and Darling (2014) doi: http://dx.doi.org/10.1101/010967

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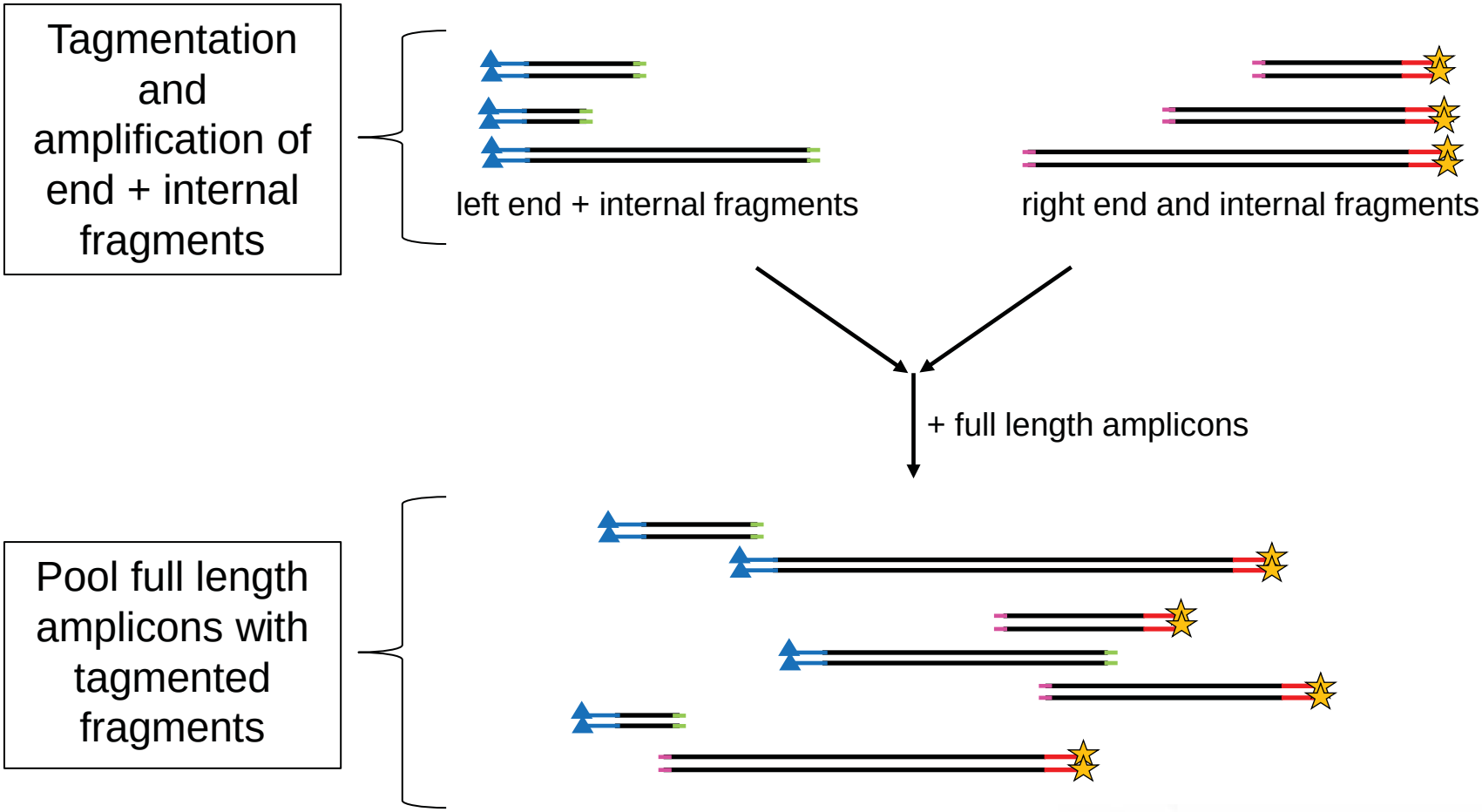
Novel 16S rDNA Amplicon NGS Method (Part 2)



Burke and Darling (2014) doi: http://dx.doi.org/10.1101/010967

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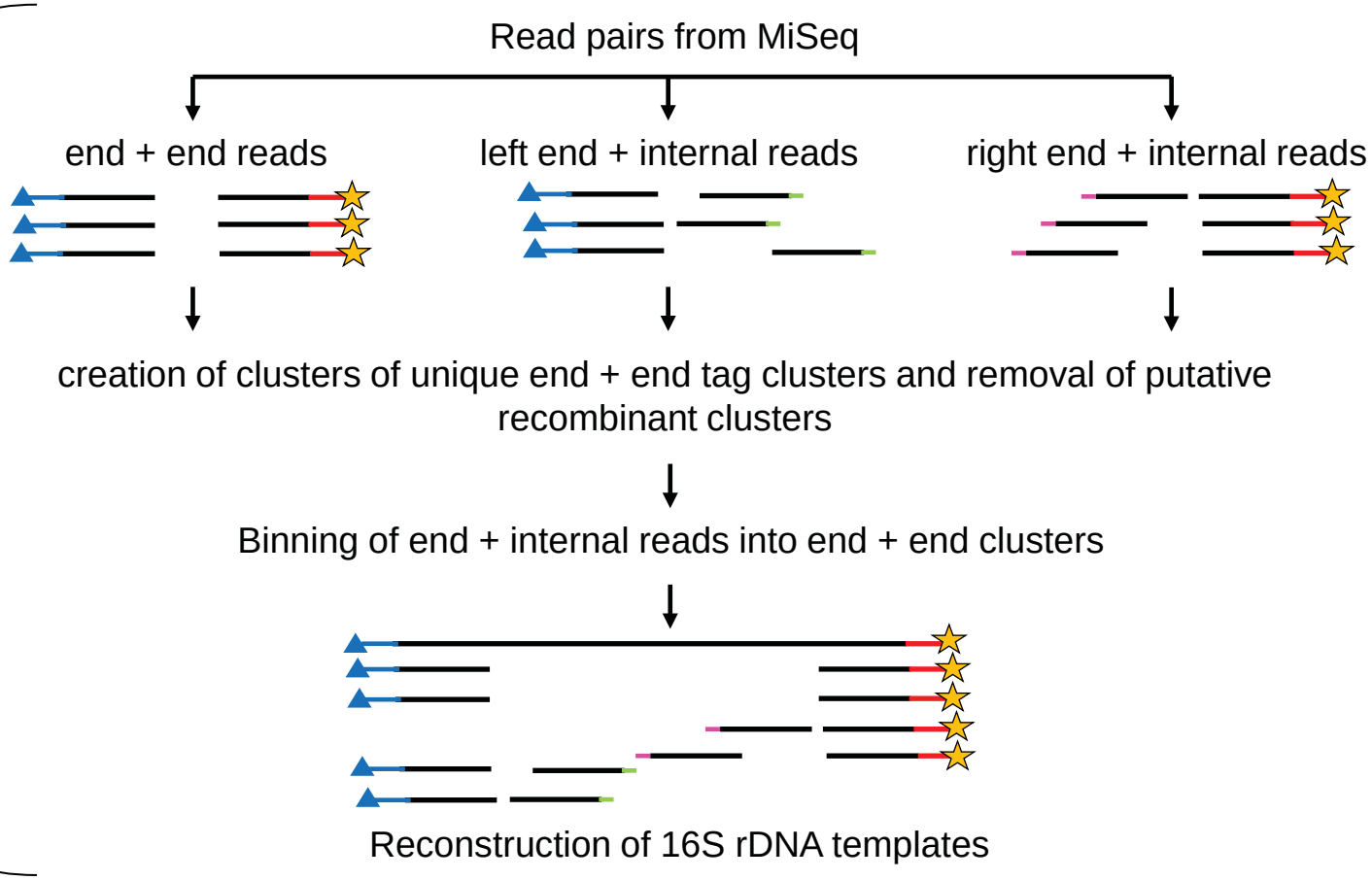
Novel 16S rDNA Amplicon NGS Method (Part 3)

Sequencing and assembly



Burke and Darling (2014) doi: http://dx.doi.org/10.1101/010967

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Full Length 16S rDNA Amplicon NGS Trial Run

- Samples = midguts from 3 larvae from:

Diet/Host	Source
White Flesh Peaches A & B	Backyard tree - Picton
White Flesh Peaches C & D	Backyard tree - Tumut
Carrot Larval Diet*	Laboratory Colony (NT DPIF)
Carrot Larval Diet	Laboratory Colony (NSW DPI)
Lucerne Chaff Larval Diet*	EMAI Fruit Fly Production Facility (Wk 34)
Lucerne Chaff Larval Diet^	EMAI Fruit Fly Production Facility (Wk 24)

*poor number of reads

^only 2 larvae included

- Difficulty amplifying 16S rRNA genes from some samples – related to bacterial load?

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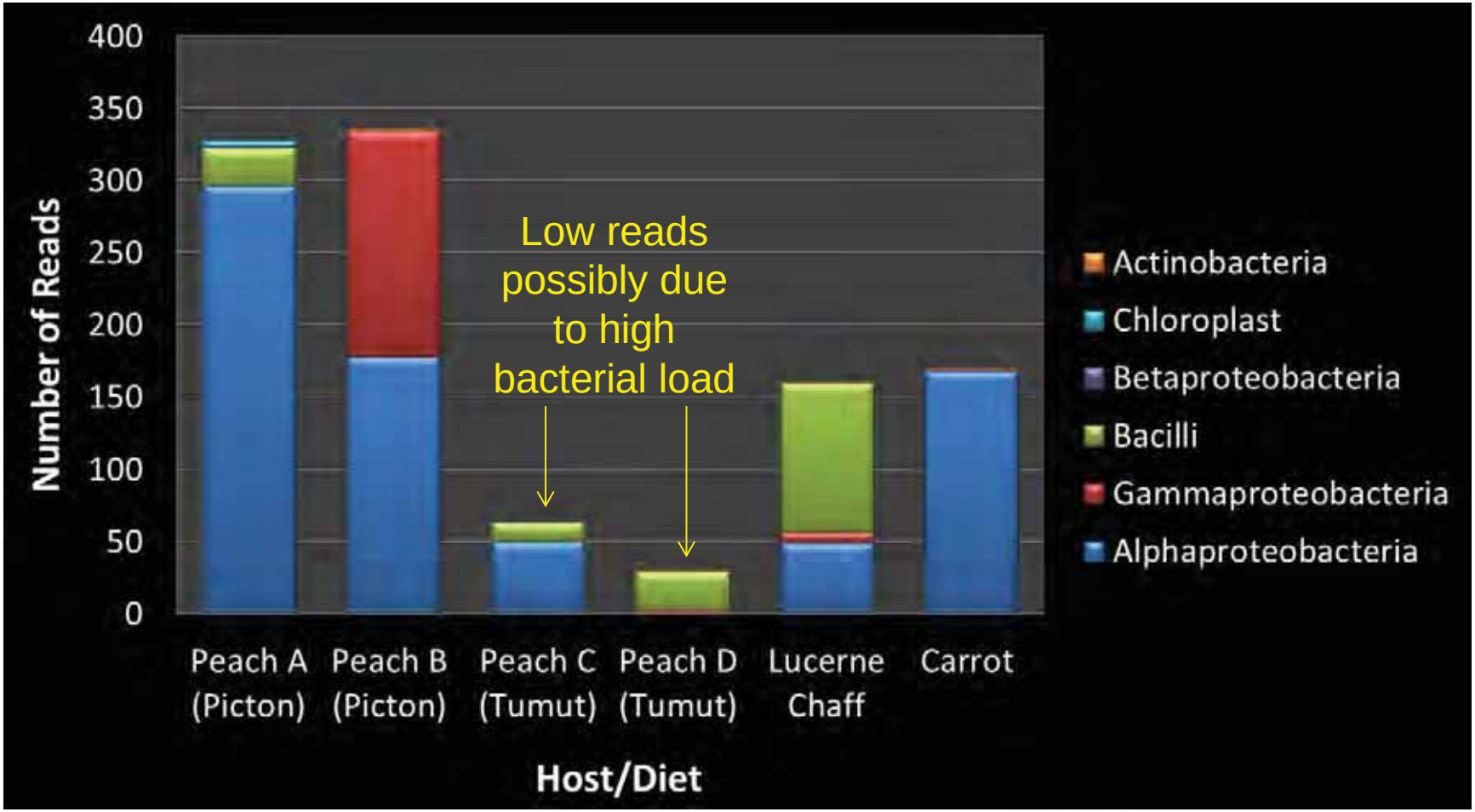
Full Length 16S rDNA Amplicon NGS Trial Run

- To check tagging → ran pool of normalised full length 16S rDNA amplicons on a MiSeq Nano v2 flow cell [2 x 250 bp paired end reads]
  - low sequence quality (to be expected with sequencing long-templates)
  - cluster density = 170 k/mm2 (next time load more)
  - 235k read pairs; 62.3k matched sample barcodes (why?)
  - a lot of unique tags observed in some wild larval midgut samples (high bacterial loads → fewer reads)
- Used ~150 bp end sequence of 16S rDNA amplicons (clustered reads to barcodes (>3 reads) & selected longest & best representative sequence)
- Reads classified taxonomically using Ribosomal Database Project (RDP) classifier program against RDP training set (50% bootstrap cut-off)

Taxonomic Classification of Reads from End ~150 bp of 16S rDNA Sequences



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Results & Conclusions

- Majority of reads belonged to Proteobacteria (Alphaproteobacteria) and Firmicutes (Bacilli)

- Low bacterial diversity in larval midguts in comparison to published deep sequencing results for adult fruit flies:
  - is this partly due to feeding on one diet?
  - only looking at the midgut?
  - and/or a smaller sample size?

Next Steps…

- Compare bacterial loads of larvae from laboratory fruit fly colonies to wild larvae;
- Optimise library preparation of samples from laboratory fruit fly colonies;
- Full flow cell run with the trial sample set on the MiSeq instrument → pick potential probiotic candidates to start trialing in Qfly carrot larval diet;
- Look at fruit fly larval midgut samples from a larger and more diverse set of samples using 16S rDNA amplicon NGS using the Illumina MiSeq

Acknowledgements

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NSW DPI EMAI Fruit Fly Team
Toni Chapman
Olivia Reynolds
Solomon Balagawi
FFPF Staff
Linda Falconer
Terry Osborne
Mohammad Shadmany
Deane Woruba

Collaborators
Catherine Burke (UTS)
Aaron Darling (UTS)
Fleur Ponton (MU)
Markus Riegler (UWS)
Phil Taylor (MU)
Narit Thaochan (PSU, Thailand)

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Bernie Dominiak (NSW DPI)
Peter Gillespie (NSW DPI)
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Jennifer Morrow (UWS)

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NT DPIF: Mary Finlay-Doney, Michael Neal

DAF: Brendan Missenden, Peter Nimmo, Lara Senior

MU: Narit Thaochan (also PSU, Thailand), Phil Taylor

UWS: Markus Riegler, Burhan Amiji, Deane Woruba (also NSW DPI)

Contact Details:

Ania Deutscher: ania.deutscher@dpi.nsw.gov.au

STERILE INSECT TECHNIQUE PROGRAMS FOR FRUIT FLIES ACROSS AUSTRALIA, MEXICO AND SOUTH AFRICA: A DETAILED COMPARATIVE

OLIVIA L. REYNOLDS[1], PABLO LIEDO[2] AND BRIAN BARNES[3]

[1]NSW Department of Primary Industries , Australia
[2] El Colegio de la Frontera Sur, Mexico
[3]Techical Consultant: FruitFly Africa (Pty) Ltd, South Africa

ECOSUR

NSW DEPARTMENT OF PRIMARY INDUSTRIES

STERIELE INSEK TEGNIEK
FRUITFLY AFRICA
STERILE INSECT TECHNIQUE

Goal

- A comparative approach to distinguish the consistencies and differences, of area-wide management across three continents
  - South Africa (Ceres, Western Cape); aerial
  - Mexico (Tapachula, Chiapas); aerial
  - Australia (Stanthorpe, Queensland); ground
- Aim to suppress respective pest fruit fly populations to very low levels

South Africa

- The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann)
- 2,200Ha of contiguous apple, pear and peach orchards
- Pilot program (2014/2015)

Mexico

- Mexican fruit fly, *Anastrepha ludens* (Loew)
- 30,000Ha of mango orchards; 95,000Ha
- Validation of AWIPM SIT for suppression in areas with high populations (2012-2014)

Australia

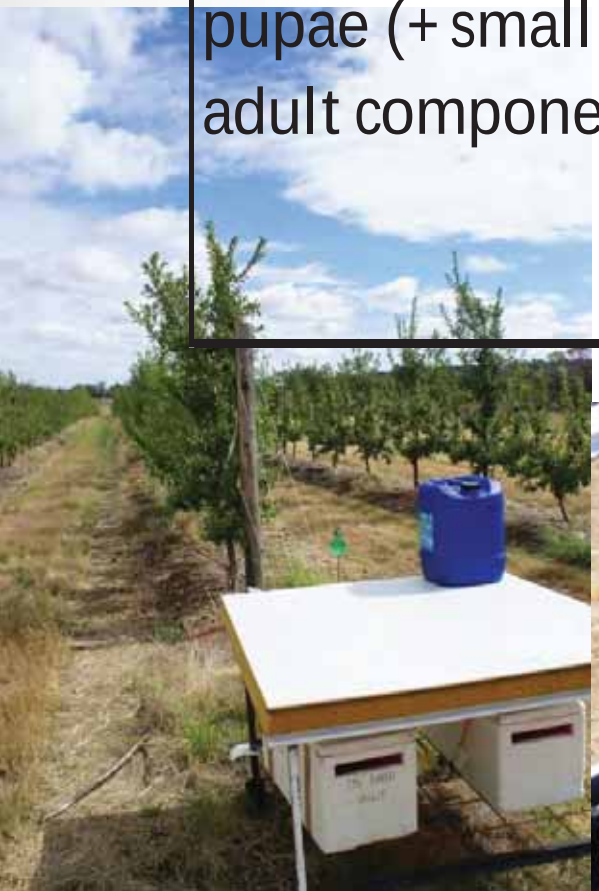
- Queensland fruit fly, *Bactrocera tryoni* (Froggatt)
- 120Ha of stone fruit; 60,000Ha ‘bush’
- Pilot study - model system for the roll-out of area-wide programs across the country

Flies

	Australia	South Africa	Mexico
Strain released	***Bisex***	***Vienna 8 GSS***	***Tap-7 (GSS)***
Number released/week	1.7 million males and females (50:50)	4.4 – 5.5 million male only	8.4 million male only plus 9.6 bisex
Release density	3,600 males/Ha	2000 – 2500 males/Ha	500 to 880 males/Ha
Cost per million flies	AU$2000/million	US$240)/million (=R3005)	GSS provided by Moscafrut program for free. US$265/million bisex strain

Release method

Australia	South Africa	Mexico
Ground release of pupae (+ small adult component)	Aerial: gyroplane; unchilled release machine; 5 million pre-chilled flies/flight	Aerial: Cessna 206 plane. 5 million chilled (maintained during flight) sterile flies/flight



Innovations

- SA: Pre-release diet for adults = agar cake with 15% sugar content
- Mex:
  - Pre-release diet for adults: 24:1 sugar: yeast dry diet
  - Augmentative biological control with *Diachasmimorpha longicaudata* 5 million per week
- Aus: Pre-release diet; *pupal release*: 3:1 sugar:yeast diet; *adult release*: 3:1 sugar:yeast diet + raspberry ketone

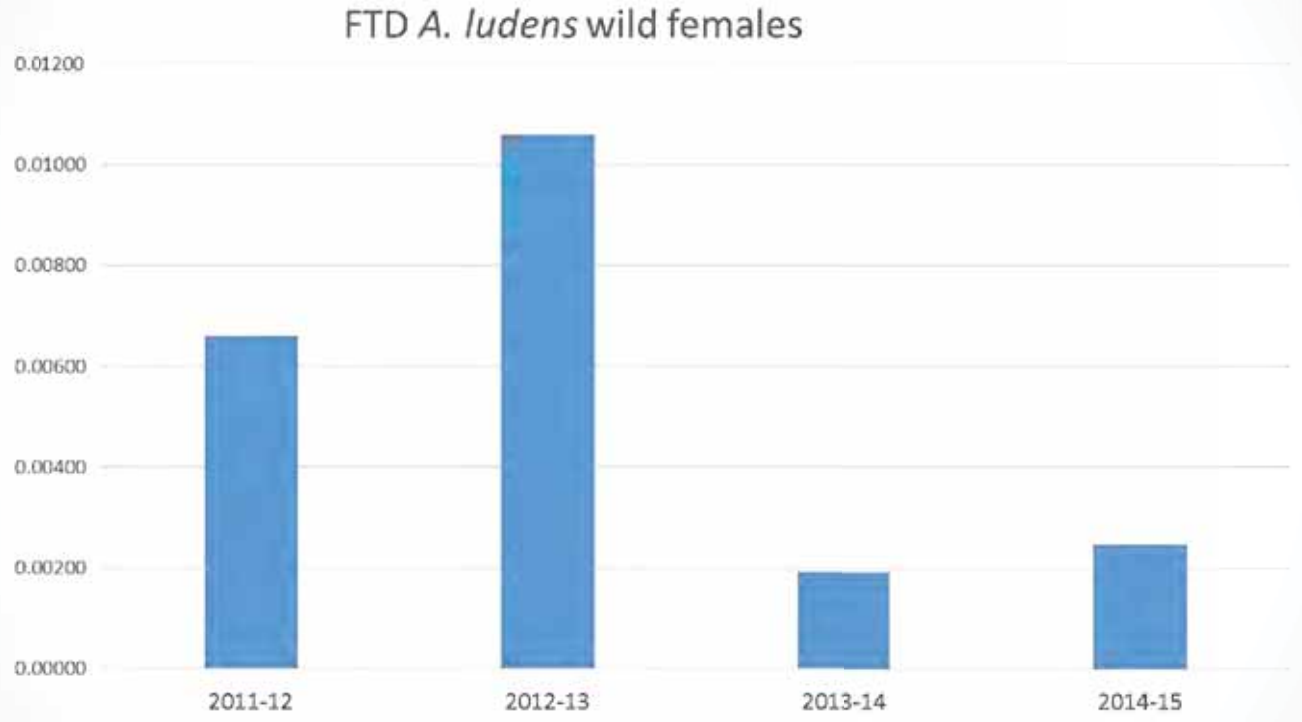
Overflooding ratio

- SA
  - Mean 42:1 (sterile: wild)
- Mex
  - 250 - 550 sterile: wild
- Aus
  - Mean 5:1?

SA: Wild fly reduction & market access

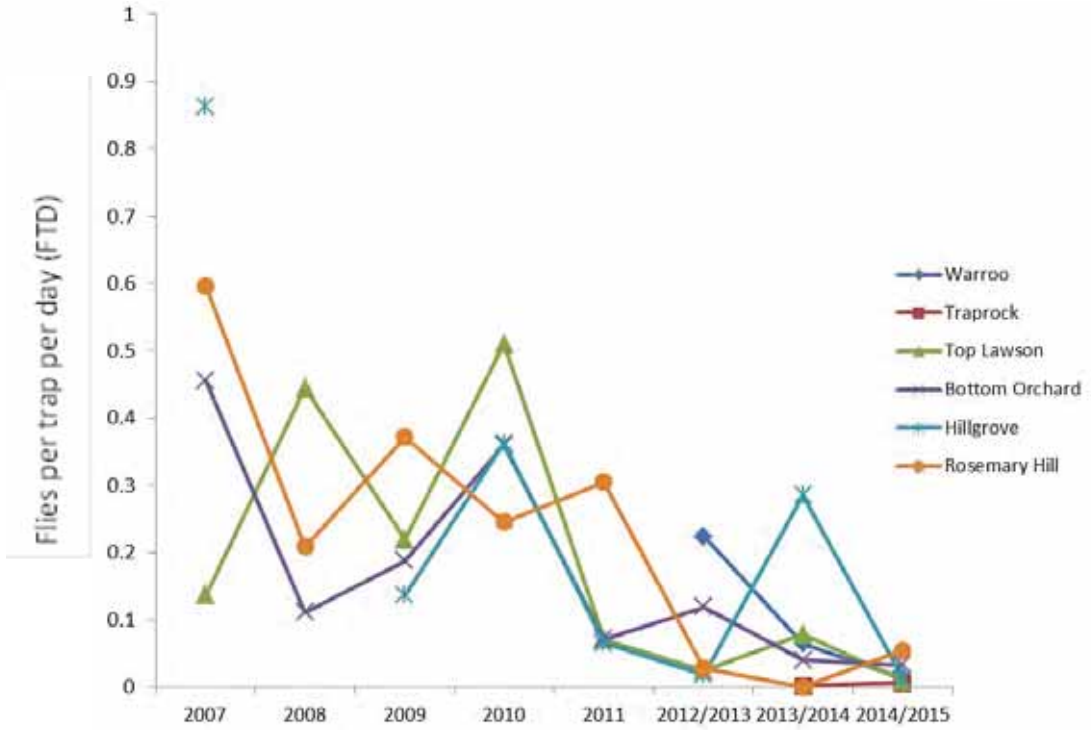
- 0.03 FTD (pre-trial 3y mean)→0.01 FTD (pilot project mean - lowest on record in area)
- Market: international

MEX: Wild fly reduction & market access



- Fruit infestation: estimated 0.6% (based on shipments rejected at the packing house due to ffly)
- Market: 25% international (mainly US), 75% domestic

AUS: Wild fly reduction & market access



- Fruit infestation; estimated <0.1%; no post-harvest treatment
- Market: domestic (90%) and international (10% to Singapore, Hong Kong, Malaysia, Indonesia and the United Kingdom (UK); nil market access issues

South Africa – Challenges & Successes

Constraints

- Inadequate funding; too few human resources
- Poor compliance with good on-farm fruit fly management practices
  - sanitation, host plant management, baiting.
- Limited AW effect of ground releases
- Logistics - geographical
- Some growers still sceptical of SIT

Successes

- After 17 years programme is still running
- A 50:50 funding partnership with national government
- A statutory levy on growers for medfly SIT
- Wider understanding of fruit flies, their behaviour, biology and control

Mexico – Challenges & Successes

Challenges

- Encourage the start of new similar programs in other fruit producing areas (likely to require another mass-rearing facility)
- Coordination of stakeholders, participants, resources etc
- To keep the current AWIPM-SIT program running!

Successes

- Feasible, even in tropical situations where fruit fly populations are large and increase quickly

Australia – Challenges & Successes

Challenges

- 'Bare bones' budget
- Labour cost in Australia
- Logistics and infrastructure

Successes

- Upto 25-fold reduction in wild fly numbers
- Pesticide usage down from 3-4 cover sprays/season to 1 or nil
- Reduced secondary pests & increased beneficials
- Grower uptake and dissemination

Lessons learned and conclusions

- AWIPM-SIT is feasible and cost effective, but
  - depends on each situation
- All programs cite similar issues
- Aus program is considerably more expensive than SA/MEX
- Persistence & longevity of programs are essential
- Large-scale operations: aerial essential
- Have the patience and perseverance

Future Directions

- AUS
  - approx. AU$60million investment in AWIPM SIT over 5 years
- SA
  - creation of low prevalence areas, leading to expanded international markets
  - expanding to aerial release over 58,000 ha – cost reduction to R1400/million
- MEX
  - continuation of the program


Thankyou

Characterisation of larval tephritid gut microbiota: towards understanding sterile Queensland fruit fly fitness and performance

Dr Olivia Reynolds
Senior Research Scientist, NSW Department of Primary Industries, Australia
Senior Adjunct Lecturer, Charles Sturt University, Australia
Jinshan Scholar, Fujian Agricultural and Forestry University, China

Queensland fruit fly

- *Bactrocera tryoni* Froggatt (Diptera: Tephritidae)
- Native to Australia
- Australia's most significant insect biosecurity threat to horticulture

Why is the Queensland fruit fly such a successful pest?

- Highly polyphagous
  - attacks almost all fruits and several fruiting vegetables; >240 plant species from 49 families
- Climatic adaptability
- Expansion of its cultivated host range

Sterile Insect Technique

- Case for the sterile insect technique (SIT) on an environmental, economic and biological basis is persuasive.
- What is SIT? SIT video
- Most effective under an Area Wide Management or geographically-isolated scenario
  - prevention, containment, suppression or eradication

Fly impacts

- *Mass-rearing* (e.g. long term inbreeding, fly adaptation to an enclosed environment, artificial diets, handling and shipping of the irradiated pupae), and *irradiation* affects fitness and performance (e.g. mating success of released sterile male flies)
  - changes in chemical, visual and physical cues

So, what can we do about this?

Microbial symbionts

- Gut microbiota can profoundly influence various aspects of insect health, fitness and behaviour
  - by impacting nutrition, physiology, metabolism, and immunity
- Feasible that changes in gut microbiota associated with fruit fly domestication and sterilization are also likely to influence the quality of the mass-reared fruit flies for SIT programs
- H: Addition of beneficial microorganisms may be a means of restoring or improving gut bacteria to positively influence *B. tryoni* production and performance

Probiotics

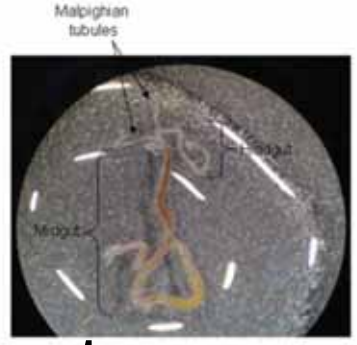
- Live microorganisms, which when administered in adequate amounts confer a health benefit on the host (FAO and WHO, 2006)

Aim

- To determine the difference in abundance and diversity of microbial symbionts of mass-reared and wild-collected larval *B. tryoni* as part of the SIT

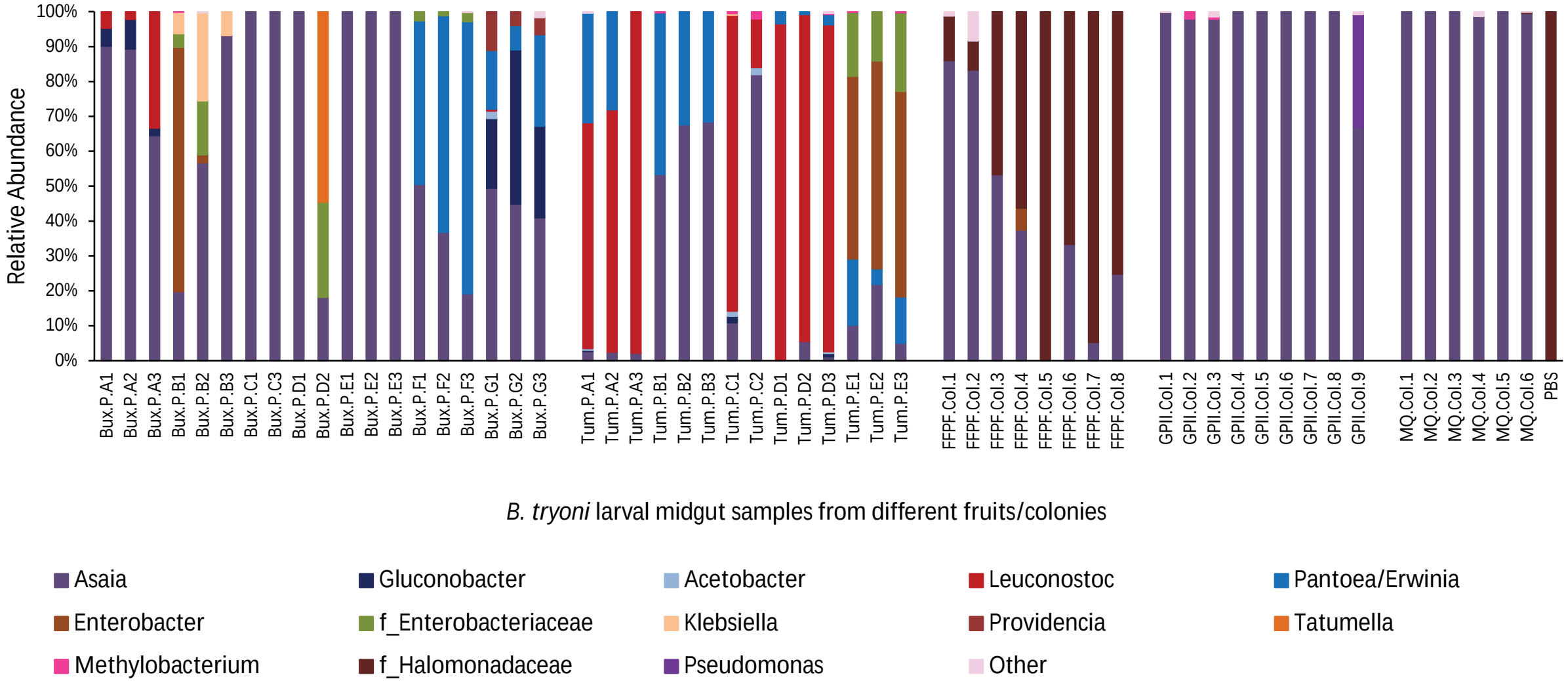
Method

- Near full length 16S rRNA Gene NGS (Burke and Darling, 2014); analyse the midgut bacterial community of wild (2 populations) and domesticated (3 colonies) *B. tryoni* larvae
  - genus level resolution (family level included when no clear match at genus/species in curated databases)
  - individual larval gut samples

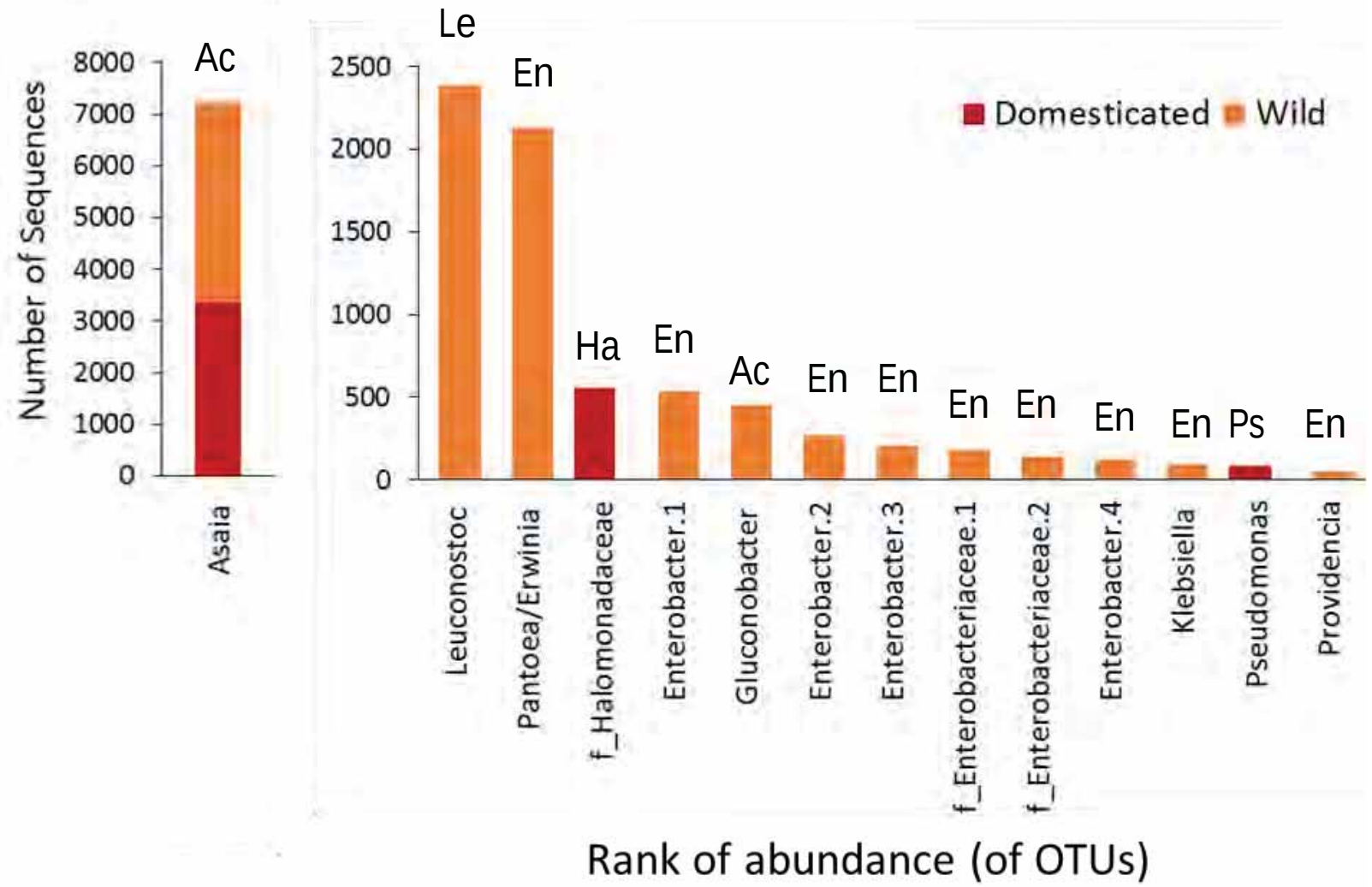


- Plating midgut microbiota of wild *B. tryoni* larvae to isolate potential probiotic candidates

Relative bacteria abundance



Rank Abundance of OTU's



> 50 near full-length sequences

- *Asaia sp.* (Acetobacteriaceae) dominant in both domesticated and wild larvae
- Enterobacteriaceae most prevalent bacteria
- Long tail indicates striking disparities in abundance; majority in wild larvae
- Greater diversity in wild than domesticated larvae

Diversity and relative abundance of bacterial taxa in *B. tryoni* larval midguts

- Overall, low bacterial diversity
- Low variation within a population (artificial diet or within a single peach), but high population variation between peaches (inc. from the same tree and different geographical locations)
- Greater diversity in wild-collected larvae (1-13 OTUs, median 4), than domesticated larvae (1-4 OTUs, median 2) (Mann–Whitney U = 168, n1 = 33 , n2 = 23, P < 0.01 one-tailed)
- Enterobacteriaceae, Leuconostocaceae and other acetic acid bacteria (Gluconobacter and Acetobacter)
  - present in wild larvae; generally absent from domesticated larvae

Deutscher et al. under review. Genus *Asaia is* a Prevalent Larval Midgut Bacterium of Wild and Domesticated *Bactrocera tryoni*. *Microbiome*.

Asaia

- Prevalent in both wild and domesticated larvae
  - detected in majority of gut samples (55/56)
  - important role in *B. tryoni* larvae
- Detected in adult *B. tryoni*, but at relatively low levels and not prevalent in all samples (Morrow *et al*., 2015)
- Acetic acid bacteria is common in gut symbionts of insects with a high-sugar diet, e.g. honeybees, mosquitoes, leafhoppers
- Important for larval development in *Anopholes gambiae;* involved in expression of host genes in cuticle formation (Mitraka *et al*., 2013), role in nitrogen fixation

Fungi - Larval Midguts

- Diverse range of yeasts and yeast-like fungi cultured from wild *B. tryoni* larval midguts (genera: *Aureobasidium, Candida, Cryptococcus, Hanseniaspora*, *Pichia*, *Saccharyomcyes* and *Starmerella*)
  - Cultured yeasts from 15 out 17 wild larvae
  - Yeasts were amongst the dominant colony types
  - Yeasts present in midguts from larvae on diverse diets: apples, oranges, peaches, cherry guavas, plums
- Negligible cultivable yeasts in domesticated compared to wild larvae (preliminary results)

Deutscher, AT, Reynolds, OL & Chapman, TA. In press. Yeast: an Overlooked Component of *Bactrocera tryoni* (Froggatt) Larval Gut Microbiota. *Journal of Economic Entomology*

Significance of Yeasts in *B. tryoni* Larval Midguts

- First report of yeasts and yeast-like fungi in midgut of wild-collected *B. tryoni* larvae
- Single previous report of yeasts in alimentary canal of tephritid larvae (Darby and Kapp, 1934)
- Yeasts identified are frequently found in fruits
  - yeasts likely obtained from the diet
- Yeasts
  - antimicrobial properties – can influence type of bacteria present in the gut
  - nitrogen rich
  - possess extracellular enzymes that can increase nutrients otherwise unavailable to larvae
  - differ in biochemical and functional properties

  → improved artificial diets or protein baits for traps

Next Steps & Future Directions

- Determine the function of fungi and bacteria
- Introduce isolated probiotic candidates to larval diet
- Fitness testing of candidates as potential probiotics
- Several questions including:
  - How does bacteria change through all life stages
  - Are yeasts active, vegetative, or in spore state?
  - Are yeasts lysed in the gut?
  - Characterise fruit fly-yeast interaction – would live yeast in the artificial larval diet improve fruit fly fitness and perhaps reduce diet contamination (reduce the use of antimicrobials)?

THANKYOU

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My co-authors: A. Deutscher, C. Burke, T. Chapman, A. Darling & M. Riegler

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SITplus

Appendix H.

The sterile Queensland fruit fly mass production process: where it starts and finishes

Balagawi. S[1], Osborne. T[1], Liang. W[1], Aiken. D[1], Edwards. D[1]
1. Elizabeth Macarthur Agricultural Institute, Woodbridge road, Menangle, NSW 2568

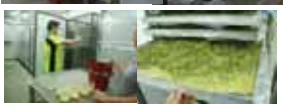
SITplus

1. Colony establishment & maintenance

Adult stock colony is established using pupae collected from wild flies

Once the wild colony is successfully established in the lab, it is continually maintained using sugar, water, and yeast protein as part of the adult fly diet

2. Egg collection & larval diet mixing



Egging cups are placed in cages on Mondays and eggs are collected from these cups on Tuesdays of each week

Larval diets are made using lucerne chaff, sugar, yeast protein and preservatives on Mondays and / or Tuesdays

Eggs are placed on the diet trays on Tuesdays

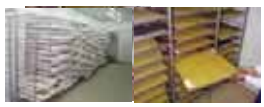
3. Egg hatching & larval collection

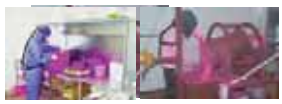
Eggs are incubated inside the larval towers in a 26°C & 80% rH room up until Friday

Egg start hatching on Thursday/ Friday

Larvae start jumping out of the diet trays and into the larval collection trays on the following Monday

4. Fly Pupation, Dying and Packaging



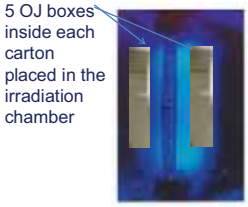


Larvae from each day (Monday-Thursday) are stored in trays to allow pupation

Pupae are dyed the following Monday

The dyed Pupae are placed in plastic bags and into OJ boxes and are ready for transportation to ANSTO for irradiation

5. Pupae irradiation & shipment



Pupae are irradiated at the Australian Nuclear Science and Technology Organisation (ANSTO) on Tuesday at 60-65Gy using a colbat-60 irradiator

Pupae are then repacked and express-posted to various stakeholders within Australia on Tuesday afternoon

6. Quality Assurance/ Control tests

Various key quality control (QC) tests using samples of the dispatched pupae are weekly undertaken in the laboratory

NSW GOVERNMENT Department of Primary Industries

Government of South Australia

Horticulture Innovation Australia

Simultaneous test on the effect of irradiation, transportation and fly generation on some key quality parameters of Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae)

Balagawi. S[1], Osborne. T[1], Bloomfield. C[2], Dominiak. B[3], Reynolds. O[1]

1. Elizabeth Macarthur Agricultural Institute, Woodbridge road, Menangle, NSW 2568
2. Orange Agricultural Institute, 1447 Forest road, Orange, NSW 2800
3. NSWDPI Head Office, 161 Kite street, Orange, NSW 2800

SITplus

Introduction

Queensland fruit fly (Qfly), *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) is a major quarantine pest fruit fly species in Australia and infests a wide range of economically important horticultural commodities, hence restricting both domestic and international markets of these hosts. As a consequence, various pre- and post- harvest pest management strategies and rigorous market access protocols have been established and implemented market access of these host commodities. Recent banning/ restriction of the key chemicals (e.g., dimethoate and fenthion) has meant that alternative environmentally friendly and safer management strategies such as the use of sterile insect technology (SIT) are required. Sterile insect technique (SIT) has been used to manage this pest in the field, and the success of sterile male mating with wild female depend on its ability to emerge from pupae, survive, fly to locate female and mate. However, the sterile male's ability to successfully achieve all these behaviours can be affected by factors such as irradiation, transportation conditions and the age of the fly colony. This study aimed to simultaneously assess the effect of irradiation, transportation and age of fly colony on quality of the mass-reared Qflies.

Methodology

1. Egg collection & larval diet mixing

Qfly eggs were collected from adult fly cages each week and 3.5ml of eggs were placed on 5kg larval diet in a larval rearing tower holding 28 trays. Larval diets were made using lucerne chaff, sugar, yeast protein and preservatives



2. Egg incubation & larval collection

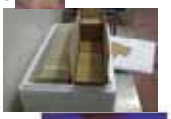
Eggs were incubated inside the larval towers in a 26°C & 80% rH room up until Friday were the top and bottom lids of the tower were removed to allow ventilation

First set of larvae that jumped out of the towers were collected, weighted and set out for pupation and process was repeated for three consecutive days



3. Pupae dying, irradiation & dispatch

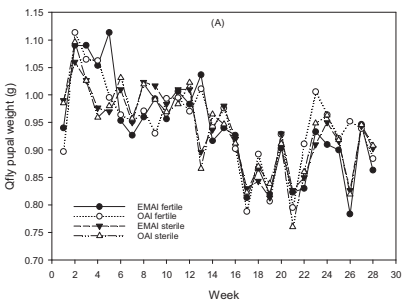
Three days before fly emergence, the pupae for the sterile flies used in the test were weighed, dyed, and irradiated using a cobalt 60 gama irradiator at ANSTO at 70-75 Gy, while the pupae for fertile flies were not dyed or irradiated. 100g of fertile and sterile test pupae were kept at EMAI while the same quantity of fertile and sterile pupae were expressed-posted to OAI to undertake the key quality control tests.

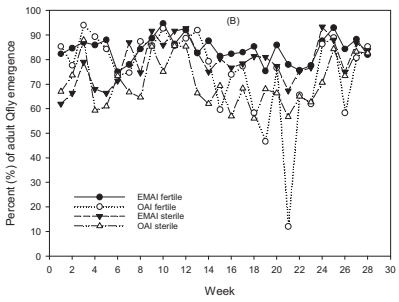



4. Qfly QC measure at EMAI & OAI

Quality control tests were done as soon as the pupae were received at both sites. The three QC tests discussed here included pupal weight, % fly emergence and % flight ability of Qfly


Results





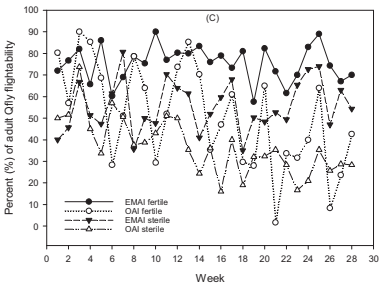


Fig 1: Effect of irradiation, transportation and colony age on pupal weight (A), fly emergence (B) and flight-ability (C) of mass-reared Qfly

Synthesis

The results from this study showed that as the age of fly colony increases, pupal weight decreases (Fig 1A). Both irradiation and transportation had strong interaction and significantly decreased fly emergence, and this observation was prominent as the fly colony got older (Fig 1B). The similar effect that irradiation and transportation had on fly emergence was also observed for the flight ability of Qfly (Fig 1C). This study demonstrates that older fly colony, irradiation and transportation do negatively affect quality of sterile flies and that considerable effort has to be taken to minimize these negative consequences.

NSW GOVERNMENT Department of Primary Industries

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Gut Bacterial Diversity of Wild and Domesticated *Bactrocera tryoni* Larvae

A.T. Deutscher[1,2], C.M. Burke[3], A.E. Darling[3], T.A. Chapman[1], O.L. Reynolds[1,2], M. Riegler[4]

[1]Biosecurity and Food Safety, NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia; [2]Graham Centre for Agricultural Innovation (an alliance between NSW Department of Primary Industries and Charles Sturt University), Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia; [3]The ithree institute, University of Technology Sydney, Sydney, NSW, Australia; [4]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW, Australia

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Background

- Gut microbiota can influence fruit fly development, nutrition, fitness, physiology, behaviour and mating performance (Hamden *et al*., 2013, Sharon *et al*., 2010, Yuval *et al*., 2013, Broderick *et al.,* 2014);
- Relatively little is known about the influence of artificial fruit fly rearing on the larval gut microbiome; and no study has investigated what bacteria are present in the gut of *B. tryoni* larvae;
- A better understanding of *B. tryoni* larval gut microbial ecology could lead to improved larval diets and mass-rearing processes resulting in the production of better quality mass-reared *B. tryoni* for the sterile insect technique (SIT).

Aims

To identify what bacteria are present in the guts of *B. tryoni* larvae, and how the gut bacterial community of wild larvae compares to domesticated larvae.

Methods

B. tryoni Larvae Collection

- 3 domesticated populations reared on carrot diet that originated from the same colony: EMAI Fruit Fly Production Facility (FFPF_Col); Macquarie University (MQ_Col); Gosford Primary Industries Institute (GPII_Col);
- 2 wild populations from white-fleshed peaches in NSW: Buxton (Bux_P); Tumut (Tum_P)

Sample Processing & Sequencing

- Each larva was dissected and midgut collected;
- DNA extracted from individual larval midguts;
- 16S rDNA sequenced on an Illumina MiSeq using the method by Burke and Darling (2016) resulting in sequence reads larger than 1300 bp

Microbial Identification

- Sequences were clustered at 99% similarity to identify operational taxonomic units (OTUs; a proxy for species) and further analysed using tools implemented in the Quantitative Insights Into Microbial Ecology (QIIME) pipeline

Results and Discussion

- 14,710 16S rRNA gene sequences (>1300 bp) were analysed; however, the number of sequences differed per sample;
- Gut bacterial diversity is low: max. 13 OTUs (a proxy for species) per larva;
- Very few OTUs were common to both wild and domesticated larvae (Fig. 1);
- Gut bacterial diversity is significantly reduced in domesticated larvae compared to wild larvae ($p<0.0005$);

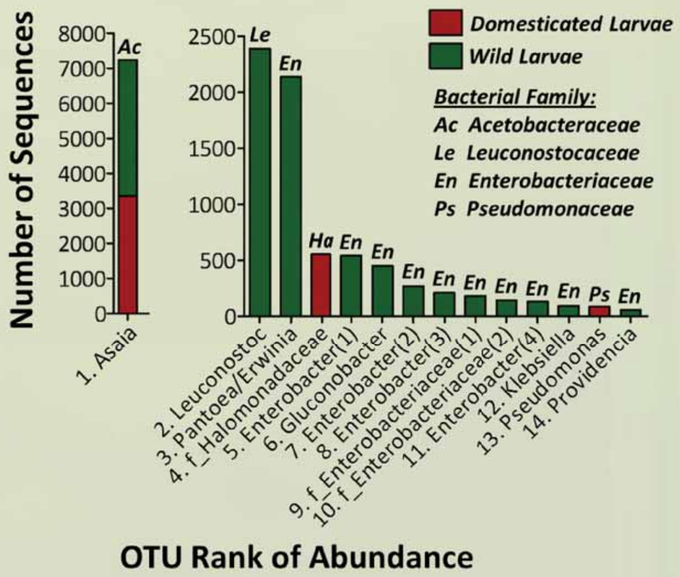

Fig. 1. Rank abundance plot showing the composition of OTUs (bacterial species) with >50 sequences from *B. tryoni* larvae sampled. The OTU rank is followed by the OTU name. A number following the OTU name indicates it is a different OTU. Where possible, taxonomic assignment is at the genus level, otherwise the family level is provided and indicated with an 'f'.

Results and Discussion (cont.)

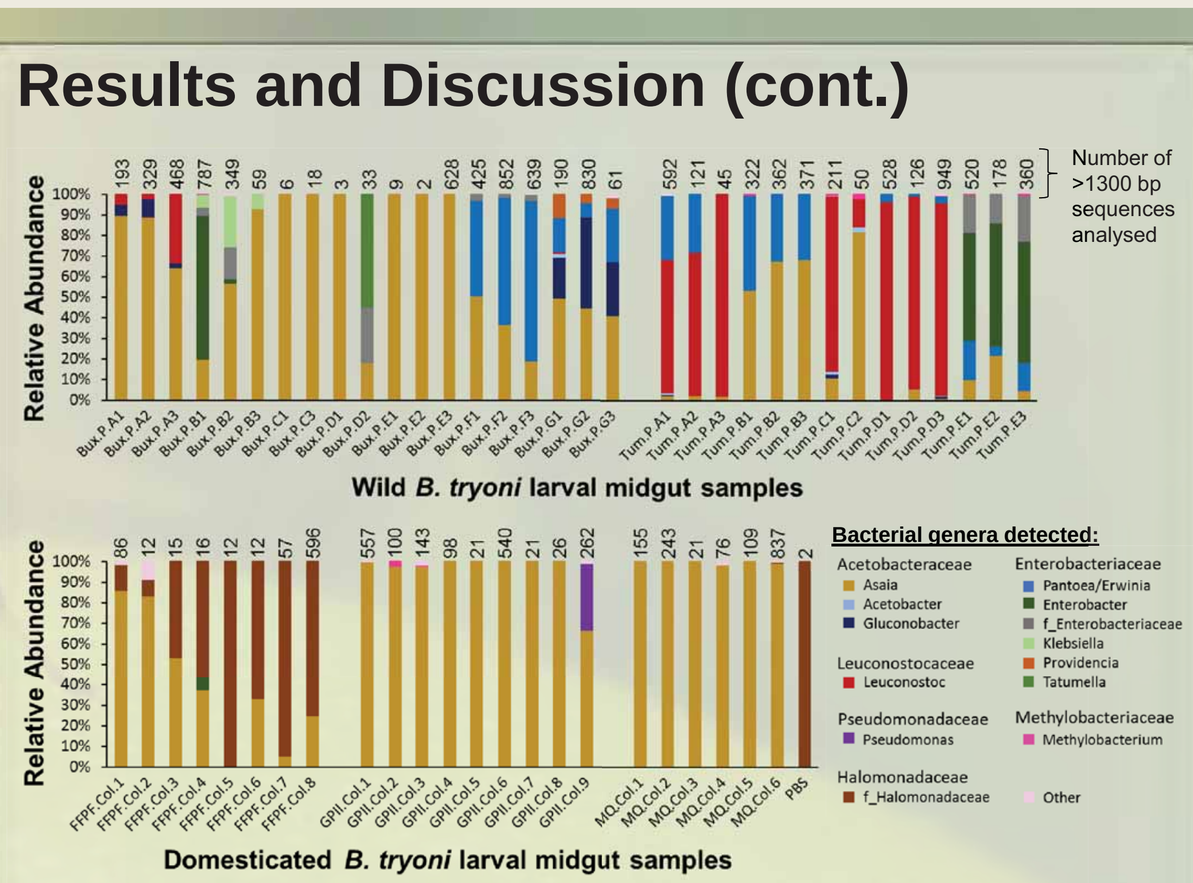

Fig. 2 Relative abundance of bacterial taxa in *B. tryoni* larval midguts. Sequences belonging to OTUs from the same genus or family, when genus could not be determined, were pooled. The group 'Other' includes OTUs with ≤ 5 sequences, and do not belong to the other families listed. Bux, Tum, FFPF, GPII and MQ refer to the source of samples (see methods), P and Col indicate whether the larva was from a peach or a domesticated colony, respectively. Larvae from the same peach have the same letter before the larval number, e.g. Bux.P.A1, Bux.P.A2, Bux.P.A3 were different larvae from the same single peach.

- Larvae feeding within the same peach or on the same batch of artificial carrot diet share similar gut bacteria;
- *B. tryoni* larval gut bacterial community is predominantly composed of bacterial families often found in fruit: Acetobacteraceae, Enterobacteriaceae, and/or Leuconostocaceae (Fig. 2);
- *Asaia* detected in 55 out of 56 *B. tryoni* larvae; *Asaia* increases mosquito *Anopheles gambiae* larval developmental rate (Chouaia *et al*., 2012; Mitraka *et al*., 2013);
- Adult female *B. tryoni* introduce Enterobacteriaceae into the fruit during egg oviposition (Fitt and O'Brien, 1985), while *Leuconostoc* is typically associated with the early stages of fruit fermentation;
- The f_Halomondaceae OTU was detected in the negative control (PBS); therefore, it may be a reagent contaminant, which is not unusual when sequencing samples with a low bacterial mass.

Conclusions

- Domesticated *B. tryoni* larvae have a reduced gut bacterial diversity compared to wild larvae - Enterobacteriaceae and Leuconostocaceae are largely absent from domesticated *B. tryoni* larvae;
- *Asaia* could be an important bacterium for *B. tryoni* larvae;
- Diet plays a role in shaping the *B. tryoni* larval gut bacterial community;
- High bacterial diversity between wild *B. tryoni* larvae from different peaches from the same tree may indicate that different bacteria can perform the same functional role(s).

Acknowledgements

We thank the following people for the *B. tryoni* larval samples: B. Dominiak, A. Gleeson, P. Treloar, A. Jessup, J. Perez, and S. Balagawi; N.Thaochan for dissecting the MQ larvae; M. Liu and P. Worden for technical assistance; N. Deutscher for helping with writing the scripts to sort the assembled scaffolds; and C. Jenkins for useful discussions.

This project has been funded by Horticulture Innovation Australia using the summerfruit industry levy with co-investment NSW Department of Primary Industries and funds from the Australian Government.

A. E. Darling and C. M. Burke receive research funding from Longas Technolgies Pty Ltd, which has licensed related technology for long amplicon sequencing from UTS.

For more information contact Ania Deutscher at ania.deutscher@dpi.nsw.gov.au or on (02) 4640 6212.

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Near full-length 16S rRNA gene NGS revealed *Asaia* as a common larval midgut bacterium of *Bactrocera tryoni*

A.T. Deutscher[1,2], C.M. Burke[3], A.E. Darling[3], M. Riegler[4], O.L. Reynolds[1,2*] and T.A. Chapman[1*] *joint last author

[1]Biosecurity and Food Safety, NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia; [2]Graham Centre for Agricultural Innovation (an alliance between NSW Department of Primary Industries and Charles Sturt University), Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia; [3]The ithree institute, University of Technology Sydney, Sydney, NSW, Australia; [4]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW, Australia **Email: ania.deutscher@dpi.nsw.gov.au**

Background

- The **Queensland fruit fly, *Bactrocera tryoni***, is an economically significant horticultural pest in Australia;
- The Sterile Insect Technique (SIT) is a pest management tool that can be used to control and eliminate *B. tryoni*;
- The sterile mass-reared male *B. tryoni* released need to be competitive against the wild male *B. tryoni* to mate with the wild females, resulting in infertile eggs and a suppression of the population;
- Gut microbiota can influence fruit fly development, quality, mating preference and mating performance (Hamden *et al*., 2013, Sharon *et al*., 2010, Yuval *et al*., 2013);
- The influence of artificial fruit fly rearing on the larval gut microbiome, and the bacteria present in the gut of *B. tryoni* larvae are poorly known;
- A better understanding of *B. tryoni* larval gut microbial ecology could lead to improved larval diets and mass-rearing processes resulting in the production of better quality mass-reared *B. tryoni* for SIT.

Aims

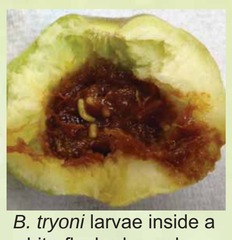
To identify *B. tryoni* larval gut bacteria and compare the gut microbiome of wild and domesticated larvae.

Methods

1. *B. tryoni* larval collections

Suffix used in this Study	Wild or Domesticated (Generation)	Substrate	Source	Collection Date	Number of Larvae Analysed
Bux_P	Wild	Peach	Residential property, Buxton, NSW	Jan 2015	20 (from 7 fruit)
Tum_P	Wild	Peach	Residential property, Tumut, NSW	Feb 2015	15 (from 5 fruit)
GPII_Col*	Domesticated (F24)	Artificial carrot diet	Gosford Primary Industries Institute, Ourimbah, NSW	Dec 2014	9
FFPF_Col*^	Domesticated (F78-81)	Artificial carrot diet	Fruit Fly Production Facility, Menangle, NSW	July 2015	8
MQ_Col*	Domesticated (unknown)	Artificial carrot diet	Macquarie University, North Ryde, NSW	June 2015	6

*originated from the same colony
^reared on lucerne chaff larval diet for at least 40 generations, but reared on carrot diet when collected



B. tryoni larvae inside a white-fleshed peach

B. tryoni larvae feeding on carrot diet

2. Sample processing and sequencing

- Each larva was surface sterilised and dissected → midgut collected → DNA extracted;
- 16S rDNA libraries were constructed and sequenced on an Illumina MiSeq following the method by Burke and Darling (2016) (Fig. 1);
- Only sequences (scaffolds) >1300 bp were analysed.

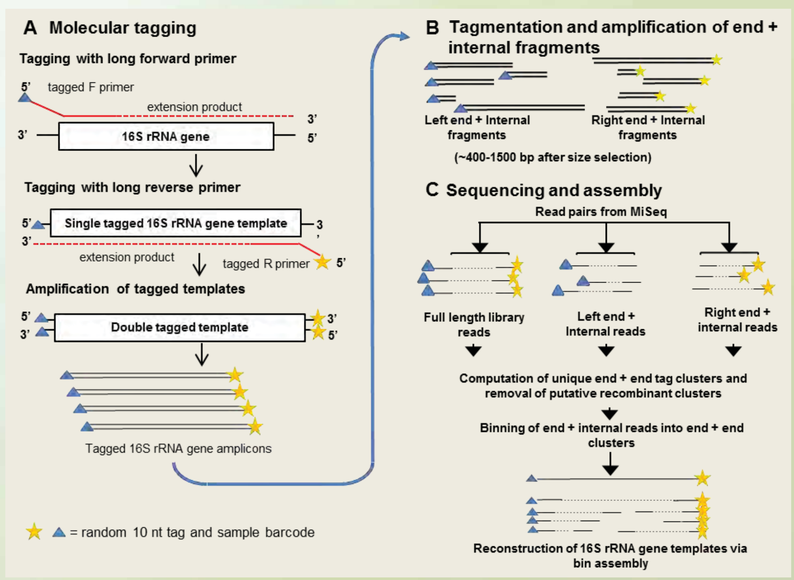


Figure modified from Burke and Darling (2016).

Figure 1. Overview of the near full-length 16S rRNA amplicon sequencing method (Burke and Darling, 2016).

(A) 16S rRNA gene template molecules were tagged with unique tags via two single rounds of annealing and extension. Tagged templates were amplified via PCR using primers complementary to the adapter sequences. Sample libraries were pooled.

(B) Full-length 16S rRNA gene amplicon Illumina libraries were tagmented using the standard Nextera method, and two pools of products were amplified which contain either the left end of the tagged amplicons and an internal region, or the right end of the amplicon and an internal region. This procedure adds Nextera adapters for sequencing at the internal end of the fragments.

(C) Full-length and tagmented libraries were pooled and paired end sequenced, and the unique molecular tags were used to computationally group sequences from the same progenitor 16S rRNA gene molecule for assembly of near full-length sequences.

3. Microbial identification

- Sequences were analysed using QIIME; OTUs were picked using open-reference picking method, UCLUST algorithm, 99% similarity, against the Greengenes 16S rRNA reference database, min. of 2 sequences per OTU;
- Taxonomic assignment of representative OTU sequences were checked against RDP 16S rRNA, NCBI RefSeq_genomic and NCBI nr/nt databases.

Results and Discussion

- 32,337 sequences assembled based on the molecular tags from 5,160,368 Illumina read pairs;
- 14,710 16S rRNA gene sequences (>1300 bp) were analysed; the number of sequences differed per sample;
- Rarefaction analysis showed that we captured the bacterial diversity for the majority of samples (data not shown);
- Low gut bacterial diversity: max. 13 OTUs per larva;
- Very few OTUs were common to both wild and domesticated larvae (Fig. 2);
- Gut bacterial diversity significantly reduced in domesticated larvae (median OTUs = 2) compared to wild larvae (median OTUs = 4) ($p<0.0005$).
- Larvae feeding within the same peach or on the same batch of artificial carrot diet share similar gut bacteria (Fig. 3);

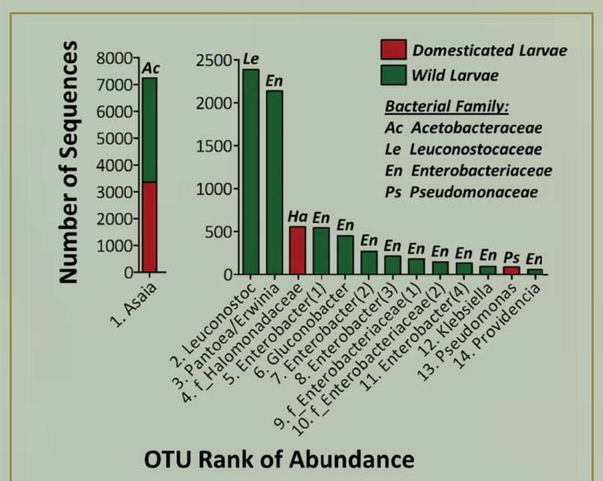


Figure 2. Rank abundance plot showing the composition of OTUs with >50 sequences from *B. tryoni* larvae sampled. The OTU rank is followed by the OTU name. A number following the OTU name indicates it is a different OTU. Where possible, taxonomic assignment is at the genus level, otherwise the family level is provided and indicated with an 'f'.

- High bacterial diversity between wild larvae from different peaches from the same tree may indicate that different bacteria can perform the same functional role(s);
- Bacterial genera commonly found in fruit were detected in wild larvae, but mostly absent from domesticated larvae (Figs. 2 & 3);
- Adult female *B. tryoni* introduce Enterobacteriaceae into the fruit during egg oviposition (Fitt and O'Brien, 1985), while *Leuconostoc* is typically associated with the early stages of fruit fermentation;
- *Asaia* detected in 55 out of 56 larvae (Fig. 3); *Asaia* increases mosquito *Anopheles gambiae* larval developmental rate (Chouaia *et al*., 2012; Mitraka *et al*., 2013) → clustering of *Asaia* sequences at 100% similarity revealed *Asaia* sequence diversity in wild larvae, but similarly between larvae feeding on same diet (data not shown).

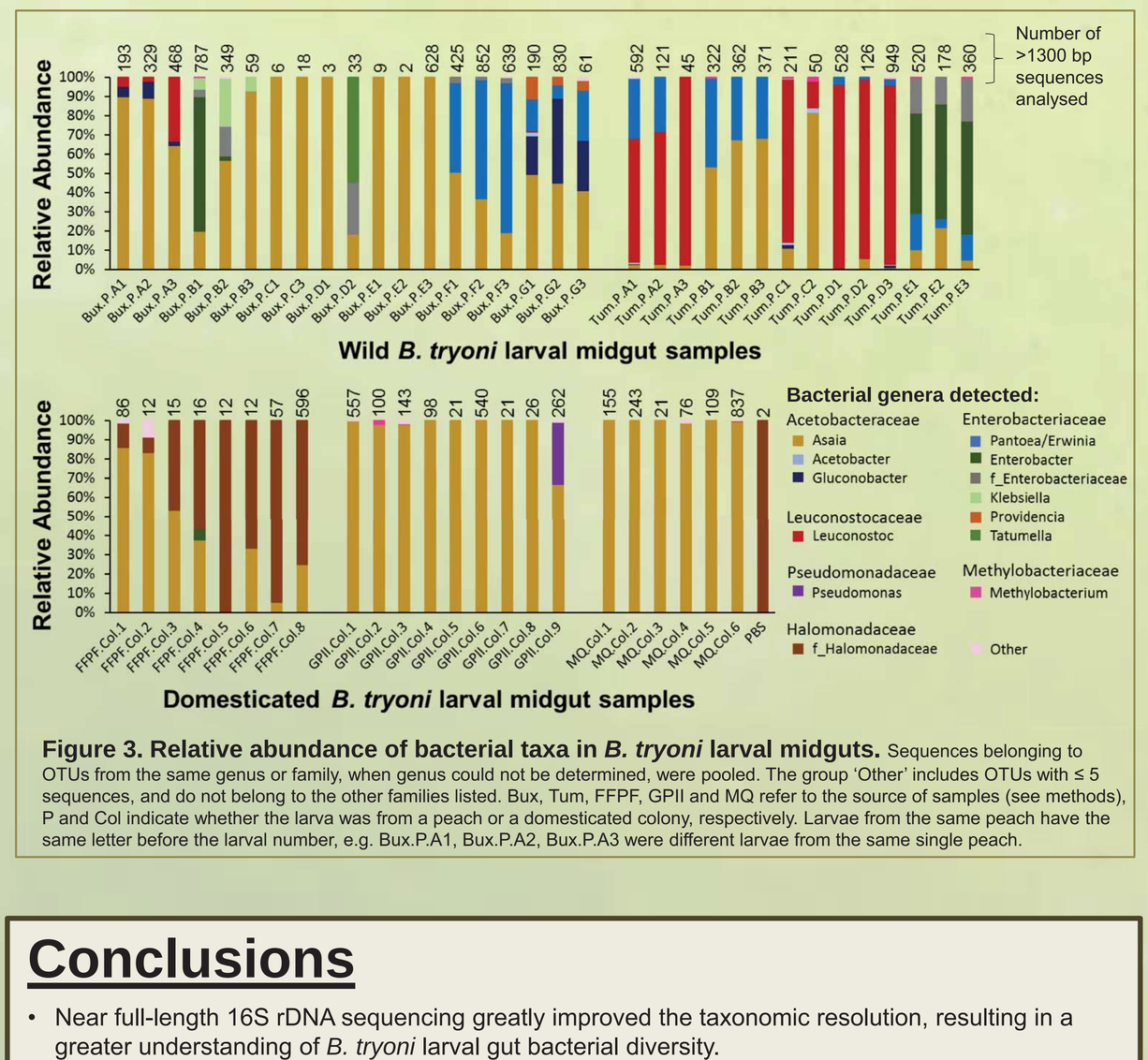


Figure 3. Relative abundance of bacterial taxa in *B. tryoni* larval midguts. Sequences belonging to OTUs from the same genus or family, when genus could not be determined, were pooled. The group 'Other' includes OTUs with ≤ 5 sequences, and do not belong to the other families listed. Bux, Tum, FFPF, GPII and MQ refer to the source of samples (see methods), P and Col indicate whether the larva was from a peach or a domesticated colony, respectively. Larvae from the same peach have the same letter before the larval number, e.g. Bux.P.A1, Bux.P.A2, Bux.P.A3 were different larvae from the same single peach.

Conclusions

- Near full-length 16S rDNA sequencing greatly improved the taxonomic resolution, resulting in a greater understanding of *B. tryoni* larval gut bacterial diversity.
- *Asaia* could be important for *B. tryoni* larvae.
- Diet shapes the *B. tryoni* larval gut microbiome; therefore, artificial diets used in mass-rearing are likely to be largely responsible for the reduced larval gut bacterial diversity in domesticated larvae.
- The quality of flies produced, and therefore the effectiveness of SIT, could be improved through a greater understanding of the functional role(s) of gut microbiota.

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Acknowledgements

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This project has been funded by Horticulture Innovation Australia using the summerfruit industry levy with co-investment NSW Department of Primary Industries and funds from the Australian Government.

A. E. Darling and C. M. Burke receive research funding from Longas Technolgies Pty Ltd, which has licensed related technology for long amplicon sequencing from UTS.

Effective Area-Wide Management of the Queensland fruit fly

Olivia Reynolds[1,2], Terry Osborne[2], Solomon Balagawi[2] & Peter Worsley[3]

[1] Graham Centre for Agricultural Innovation (an alliance between NSW Department of Primary Industries and Charles Sturt University), Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2567, Australia, [2] NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2567, Australia, [3] NSW Department of Primary Industries, Head Office, Orange, NSW 2800 Australia

$7billion Australian Horticulture Industry

Queensland fruit fly 'Qfly' is the most significant insect biosecurity threat
Polyphagous; attacks almost all fruit crops and several fruiting vegetable crops
Key chemicals for Qfly control are limited

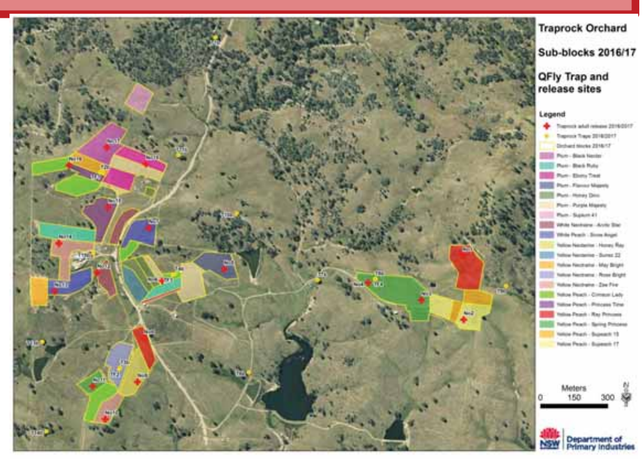
Sterile Insect Technique

Uses mass-reared insects, irradiated to render them sterile, to flood the wild male fruit fly population with released sterile male flies thereby minimising the possibility of wild insects mating to produce viable eggs
Most effective under an Area Wide Management or geographically-isolated scenario

Goal
Establish an effective model Area Wide Management Program incorporating the Sterile Insect Technique Program, to provide control of Qfly in an *endemic* area, that will inform the development of similar future campaigns.

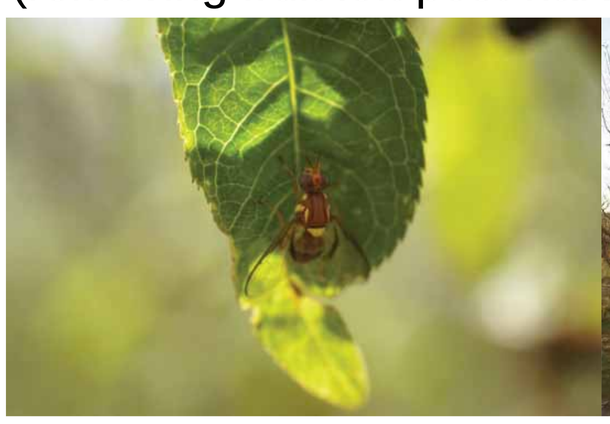
AWM Site

- Four orchards totalling approx. 100Ha; control (no sterile flies) and trial (sterile fly releases) orchards
- Medium chill stone fruit; AU$6-7million/annum
- Geographically isolated from urban centres; largely sheep country
- Low-medium endemic *B. tryoni* population



Sustainable Management

The program uses a combination of techniques to suppress fly numbers including sterile 'bisex strain' Qfly releases (approx. 4000 sterile males/ha), bait sprays, male annihilation technique (MAT), sanitation (removing fruit left post-harvest and managing non-commercial hosts) and fruit monitoring.





Outcomes After Two Consecutive Seasons

- Decreases in wild Qfly populations as high as *22 fold*
- Wild Qfly remain suppressed to extremely low levels; <0.025 flies/trap/day
- Very low levels of fruit damage; e.g. <60 fruit infested in 60Ha
- Decreases in chemical from upto 4 cover sprays/season to 1/nil
- Numerous beneficial insects: "*I didn't have to spray for anything that bites, sucks or chews*", Andrew Finlay, Chair, Summerfruit Australia
- Fruit sold domestically (mostly Brisbane, Sydney & Melbourne) and internationally (Singapore, Hong Kong, Malaysia, Indonesia and the United Kingdom)
- No market access issues


Acknowledgements
Fruit Fly Production Facility (FFPF) at the Elizabeth Macarthur Agricultural Institute, Menangle, New South Wales (NSW), Australia for supply of sterile Qfly
This project has been funded by Horticulture Innovation Australia using the summerfruit industry levy with co-investment from the Traprock Growers and NSW Department of Primary Industries and funds from the Australian Government as part of the SITPlus Initiative.

The effects of bacterial probiotics fed to larvae of Queensland fruit fly (*Bactrocera tryoni)*. Do they improve fitness and performance under the Sterile Insect Technique?

Shuttleworth, L.A. (1), Osborne T (1), Collins D. (1), Reynolds, O.L. (1,2)

(1) NSW Department of Primary Industries, Biosecurity and Food Safety, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia; (2) Graham Centre for Agricultural Innovation, Charles Sturt University, Orange, NSW, Australia.

Email: olivia.reynolds@dpi.nsw.gov.au

INTRODUCTION

Queensland fruit fly (Q-fly), *Bactrocera tryoni* (Diptera, Tephritidae; Fig. 1) is native to Australia, and is a pest and biosecurity threat to its $9 billion horticultural industries. Q-fly is controlled using a range of tools including the Sterile Insect Technique (SIT). Application of bacterial probiotics to larvae may influence health, fitness and behaviour of flies.

Figure 1. *Bactrocera tryoni* adult

METHODS

Individual and a blend of live bacteria from the genera *Asaia, Enterobacter, Lactobacillus,* and *Leuconostoc* were isolated from wild larvae and provided as probiotic supplements to mass-reared larvae.

Fitness and performance traits were tested including time to adult eclosion, flight ability, sex-ratio, and locomotor activity. The sample number for adult eclosion, flight ability, and sex-ratio were 3 reps of 50 pupae per treatment. Samples for locomotor activity were taken every 5 mins over the 24 hour period with 4 reps (2 male and 2 female flies per rep).

A Quasi-Poisson Generalised Linear Model was used in R to determine significant differences ($P<0.05$) between probiotic fed treatments and the control (without probiotics) for effective fliers and sex on each day of adult eclosion. Linear regression analysis was used to determine differences in locomotor activity.

RESULTS

Adult eclosion and flight

- On day 19, *Enterobacter* males had significantly higher eclosion and flight ($p=0.003$), while *Leuconostoc* males and females were significantly lower ($p=0.013$). On day 20, *Leuconostoc* females had significantly lower eclosion and flight ($p=0.011$). On day 21, eclosion and flight of *Lactobacillus* and *Leuconostoc* males and females were significantly higher ($p<0.001$) (Fig. 2). Degrees of freedom for these analyses was 10.

- Most treatments had percentage of fliers >90% except *Lactobaillus* which had 87%.

Sex-ratio

- The blend, *Asaia* and *Leuconostoc* resulted in more males than females (Table 1). *Lactobacillus* resulted in more females.

Locomotor activity

- All probiotic treatments had locomotor activity significantly different to the control. *Enterobacter* and *Asaia* had significantly higher activity (*Asaia* $p<0.00008$, Fig. 3 shows *Asaia* and the control as examples).

- The blend, *Lactobacillus* and *Leuconostoc* all had lower activity.

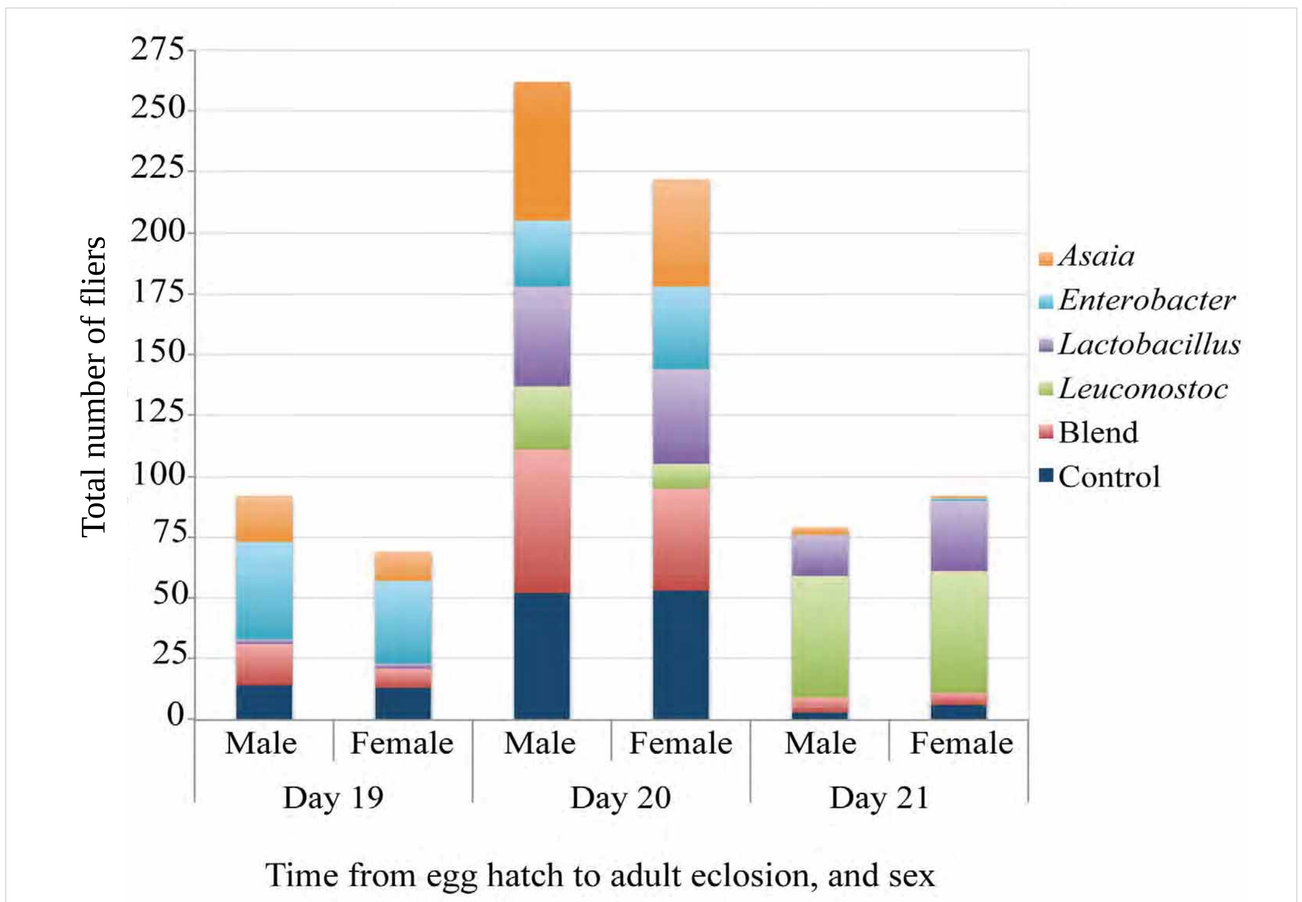


Figure. 2 Time to adult eclosion, and total number of fliers emerging on each day from egg hatch.

Table 1. Sex ratio of probiotic fed larvae and the unfed control.

Probiotic	Male	Female	M:F sex ratio
Asaia	79	57	1.4:1.0
Enterobacter	67	69	1.0:1.0
Lactobacillus	60	70	0.9:1.0
Leuconostoc	76	60	1.3:1.0
Blend	82	55	1.5:1.0
Control	69	72	1.0:1.0

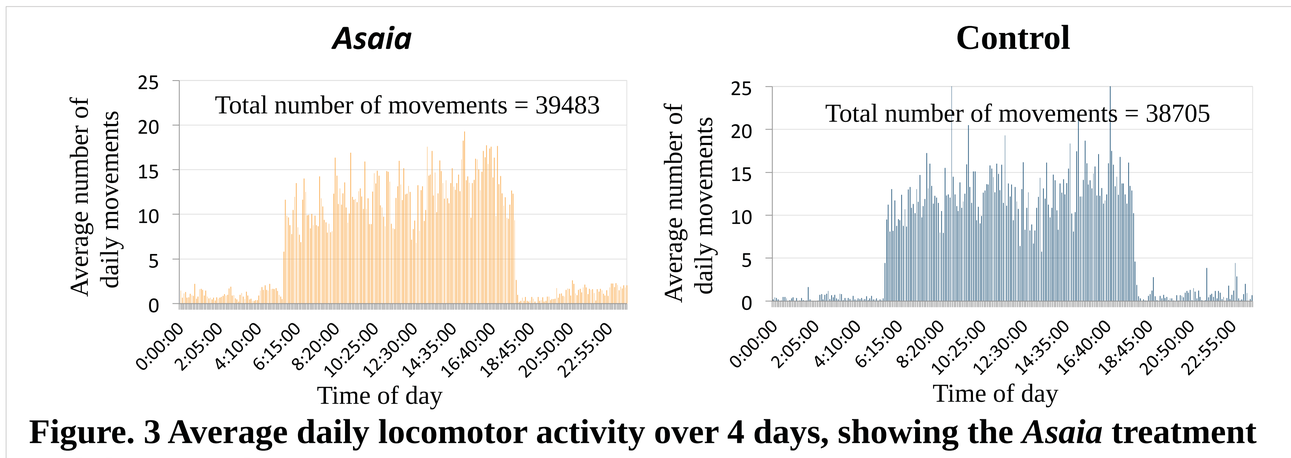


Figure. 3 Average daily locomotor activity over 4 days, showing the *Asaia* treatment and the control

CONCLUSIONS

Our findings suggest bacterial probiotics fed to larvae influence a range of larval and adult quality traits. Shortened development time can reduce rearing costs, and a male biased sex ratio would benefit SIT programs using bisex strains. The performance of sterile flies in the field may be improved by larval application of probiotics such as *Asaia* and *Enterobacter* that yield more active flies. While these studies have implications for fertile *B. tryoni*, future studies need to test the effects of probiotic fed larvae on post-irradiated sterile males under SIT programs.

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Appendix I.

NSW GOVERNMENT Department of Primary Industries

Media Release

27 February 2014

Innovative birth control program to protect Fruit Crops

The Department of Primary Industries is embarking on a new research program to help address one of the most significant biosecurity threats to Australian horticulture.

An innovative control initiative targeting Queensland fruit fly or Qfly has been developed by Dr Olivia Reynolds and her team at the NSW Department of Primary Industries' Elizabeth Macarthur Agricultural Institute.

"We have established an Area Wide-Integrated Pest Management (AW-IPM) program that incorporates the sterile insect technique (SIT), to specifically target the breeding cycle of this major pest." Dr Reynolds said.

"SIT is a method of biological control, where we release large numbers of sterile insects that compete with fertile insects to mate, which effectively reduces the overall population.

"It is an environmentally benign and cost-effective control option for fruit flies of major economic importance such as Qfly."

Queensland fruit fly feeds and breeds on a variety of important fruit and vegetable crops and is recognised as one of the key biosecurity pests threatening horticulture in Australia.

"This new AW-IPM SIT program is not only a preventative control option but is intended to have a positive impact on society by improving the quality of horticultural products at a lower cost, while protecting the environment and human health.

"Chemical controls are increasingly coming under scrutiny due to environmental and health concerns and we have responded to the need to find alternate 'softer' in-field control options for Qfly by incorporating the SIT in an AW-IPM program.

Dr Reynolds said programs such as this across the world have successfully incorporated the SIT to control fruit flies and include prevention, containment, eradication and suppression of these pests

"This program will operate on several properties, growing mostly Summerfruit, in a uniquely geographically isolated area away from urban centres in south-eastern Queensland near the New South Wales border," Dr Reynolds said.

"AW-IPM has a strong emphasis on treating all habitats of the pest population preventing migrants re-establishing significant infestations.

"In contrast, conventional control methods have a narrow focus protecting crops from direct attack by pests," said Dr Reynolds.

Dr Reynolds said it's hoped the program will deliver a reduction in the fruit fly population as well as a reduction in pesticide use.

"Other benefits of this project may include protection of the health of farm workers, reduced environmental costs through reduced insecticide residues in fruit, water reservoirs and soil and strengthening research and development support of the stone fruit industry."

This project forms part of the SITPlus initiative led by Commonwealth Scientific and Industrial Research Organisation, Horticulture Australia Limited, Plant and Food Research, DPI and Regions South Australia. This project has been funded by Horticulture Australia Ltd using voluntary contributions from the 'Trap Rock' growers, and funds from the Australian Government.

Media Contact: Rachel Buchanan on 6391 6386 or 0477 361 732

Appendix J.

MACLURA POMIFERA (RAF.) SCHNEID.: A NEW HOST RECORD FOR *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE) and *DELIA PLATURA* (MEIGEN) (DIPTERA: ANTHOMYIIDAE)

Olivia L. Reynolds[1], T. Osborne[2] and A. Finlay[3]

[1] *Graham Centre for Agricultural Innovation (New South Wales Department of Industry and Charles Sturt University), Private Bag 4008, Narellan, NSW 2567, Australia.*
[2] *New South Wales Department of Industry, Private Bag 4008, Narellan, NSW 2567, Australia.*
[3] *Pikes Creek Orchard, 3895 Texas Rd, Stanthorpe Qld 4380, Australia.*

Summary

Three dipteran flies, *Bactrocera tryoni* (Froggatt), *Delia platura* (Meigen) and an unidentified species, family Muscidae, have been reared from fruit collected from Osage orange, *Maclura pomifera* (Raf.) Schneid. in Stanthorpe, Queensland, Australia. This is the first record of *B. tryoni* and *D. platura* recorded in *M. pomifera* fruit and has management implications for this tree species, particularly in and surrounding horticultural production areas.

Keywords: Queensland fruit fly, Seedcorn maggot, biosecurity, Osage orange, horticulture, pupae, adult flies

INTRODUCTION

The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) is Australia's most significant biosecurity threat to horticulture, infesting nearly all commercial fruit crops (White and Elson-Harris 1992) and fruiting vegetables (Hancock et al. 2000). This polyphagous pest has been recorded on over 240 host species from 48 families (Hancock et al. 2000) including 60 wild hosts from 25 families (Drew 1989, Hancock et al. 2000). The Australian native is endemic throughout much of its range in south-eastern Australia (Drew, 1989; Mathuthantri 2010) and also occurs in some South Pacific Island nations including New Caledonia and French Polynesia (Drew et al. 1978) and the Torres Strait Islands (Hancock et al. 2000). The adult fly is 6-8mm in length (NSWDPI 2012) orange to brown, with distinctive yellow markings (White and Elson-Harris 1992), and can be morphologically distinguished from related species using a taxonomic key (Drew, 1989). Banana-shaped eggs (<1mm length) are laid into the flesh of mature and ripe fruit where they hatch and the creamy-white to pale yellow larvae feed on fruit pulp and associated bacteria until they reach approximately 8-11mm in length, before they leave the fruit, burrow into the soil and pupate. Adults emerge from the soil, before locating food, shelter and a mate (White and Elson-Harris 1992). The females are capable of mating within a week to 10 days after eclosion and can produce several hundred eggs during their lifetime.

The seedcorn maggot (also known as the onion maggot or bean seed fly), *Delia platura* (Meigen) (Diptera: Anthomyiidae) is, as its common name suggests, a reported pest of germinating corn and soybeans ((Funderburk et al. 1983, Gessell 2000). It also attacks other species such as cabbage, cucumber, green beans, melon, turnips, lettuce, onion, seed potatoes and other cruciferous vegetables (Kessing and Mau 1991). It is often considered a secondary pest as it is associated with plants that have been damaged by insects or disease (Brooks 1951). *Delia platura* is a native of Europe but now occurs on all continents except Antarctica (Griffiths 1991). In Australia, *D. platura* has been verified from all states and territories, except the Northern Territory (http://www.ces.csiro.au/aicn/system/c_1114.htm; accessed 22 June 2015). The grey-brown adult flies are 5-6mm long with three stripes down their scutum. The white, elongated (0.16 cm length) eggs are deposited in clusters among plant debris and/or on seeds or around plant stems near the soil surface (Bennet et al. 2011). Most studies report that the greyish/yellow larvae, which grow to about 0.50-0.63 cm long (Kessing and Mau 1991), complete their entire development within the soil by burrowing into seeds or feeding on cotyledons emerging from seeds (Bennett et al. 2011). Larvae pupate in the soil before adults emerge (Gesell 2000). Unlike *B. tryoni* that overwinter as adults (CAB International 2015), *D. platura* survives the winter in the pupal stage in soil, and adults emerge in early spring (Higley and Pedigo 1984). The flies mate within two to three days after emerging, and each female lays an average 270 eggs (Bennett et al. 2011).

The Osage orange, *Maclura pomifera* belongs to the Moraceae (Order: Rosales), the mulberry family. This family includes some important temperate and tropical fruit species, particularly across parts of Asia, the Pacific and the Middle East. Some of these species are also reported hosts of *B. tryoni*, including *Artocarpus heterophyllus* (Lamk (1979)) (jakfruit)

and *Morus nigra* (L.) (mulberry) and also species from the *Ficus* genus including *F. carica* (L.) (edible fig) and *F. macrophylla* Desf. Ex Pers. (Moreton Bay fig). Several genera in the mulberry family are valuable sources of timber (http://www.britannica.com/plant/Moraceae; accessed 22 June 2015), with *M. pomifera* a favoured timber by wood turners in Australia. The plant is dioecious, i.e. there are separate male and female plants (Burton 1990). *Maclura pomifera* is native to Oklahoma, Texas and Arkansas in the USA (Little 1979), and has been planted in greater numbers than almost any other tree species in North America. Branches may bear short, stout spines which led directly to the invention of barbed wire. Although the fruit of *M. pomifera* is not considered edible, the seeds may be eaten by humans (http://www.eattheweeds.com/maclura-pomifera-the-edible-inedible-2/; accessed 22 June 2015). In New South Wales, Australia *M. pomifera* is considered an environmental weed.

MATERIALS AND METHODS

Five Osage Orange fruit, *Maclura pomifera*, were collected by Andrew Finlay, on the 15 February 2015 from two separate trees located within 300m, at Pikes Creek Orchard, 3895 Texas Rd, Stanthorpe Qld 4380 (28º40'40.45"S 151º34'46.08"E and 28º40'30.8"S 151º34'40.6"E).

The fruit was packaged and sent to the Elizabeth Macarthur Agricultural Institute (EMAI), New South Wales (NSW) where it was received on 18 February 2015. Whole fruit were placed individually over moistened vermiculite (4:1; vermiculite: water), in enclosed clear buckets with mesh-covered ventilation holes in a controlled environment room at 26ºC ± 1ºC, 65%RH ± 10%RH and 14:10 hour light: dark cycle. Fruit was held above the vermiculite on a container covered with fine mesh, allowing the passage of juice into the container but excluding larvae entering the container. Emerged adult flies were identified by the Agricultural Scientific Collections of NSW Department of Primary Industries, Orange, NSW. Voucher specimans of the flies reported were deposited in the Collections Unit.

On 15 April 2015, a cue-lure baited Lynfield trap was placed in a single *M. pomifera* tree (the last fruit had fallen from the tree several weeks prior to trapping commenced) located at EMAI (S30°06'51.2", E150°43' 50.5") and checked weekly for six consecutive weeks.

RESULTS

New host record for *Bactrocera tryoni* and *Delia platura*

On the 3 March 2015, *D. platura* and another unidentified dipteran (Muscidae) commenced adult eclosion from three of the five fruit containers. Three days later adult *B. tryoni* were observed (Table 1). *Bactrocera tryoni* only emerged from fruit from which either *D. platura* or the unidentified muscid sp. had emerged, however the anthomyiid and muscid species were not found to occur together in the same fruit (Table 1). Flies only emerged from fruit which had blackened areas (Fig. 1). Adult male *B. tryoni* were trapped in the Lynfield trap located in a single *M pomifera* tree for two consecutive weeks, before trap catches fell to zero (Table 2).

Table 1. The total number of dipteran flies recorded from five whole Osage orange, *Maclura pomifera* fruit collected from two trees located near Stanthorpe, Queensland and a note on whether the fruit was damaged (blackened).

Whole fruit	*Bactrocera tryoni* (Diptera: Tephritidae) adults	*Delia platura* (Diptera: Anthomyiidae) adults	Unknown sp. (Diptera: Muscidae) adults	Fruit blackened (Yes/No)
1	31	0	54	Yes
2	0	0	0	No
3	8	0	11	Yes
4	0	0	0	No
5	1	23	0	Yes
Total	40	23	65	

Fig. 1. Osage orange, *Maclura pomifera*, leaves and fruit; the latter showing a blackened area caused by the secretion of the milky fruit juice drying after bruising. Image taken by A. Finlay.

Table 2. The total number of *Bactrocera tryoni* trapped in a cue-lure baited Lynfield trap located in a fruiting *Maclura pomifera* tree at Elizabeth Macarthur Agricultural Institute, New South Wales over a six week period.

Date	*Bactrocera tryoni*	
	Male	Female
22/04/2015	5	0
29/04/2015	4	0
6/05/2015	0	0
13/05/2015	0	0
20/05/2015	0	0
27/05/2015	0	0

DISCUSSION

Bactrocera tryoni has an extensive host range and distribution, including records on related species of *M. pomifera*, including ten species of Moraceae (Hancock et al., 2000). However, this finding is of importance, as *M. pomifera* is distributed throughout much of eastern Australia, and is therefore likely to act as a successive, or at least, an occasional host, facilitating the spread of this major insect pest. The fruit of *M. pomifera* is unlike that of the fleshy fruits which *B. tryoni* typically infests, and is more similar to fruit of *Clivia miniata* (Lindley) Regel reported as a larval host of *B. tryoni* by May & Drew (2003). Evidence of male *B. tryoni* trapped in a *M. pomifera* tree in NSW, together with infested fruit in Queensland, suggests the possibility that there is some level of *B. tryoni* activity linked with this tree species.

Delia platura is sometimes considered a secondary pest, attacking plant tissue that is diseased through bacterial or fungal infestation (Bailey 2007). The larvae are typically found underground and therefore not thought to be susceptible to much predation, with few reported natural enemies (Reid, 1940). This first report of the larvae of *D. platura* found feeding in the fruit of *M. pomifera* may have management implications. Although *O. platura* is no considered not a major pest this crop is widely grown in the Americas, and could be an alternate host for this pest in other countries where it is grows and *O. platura* occurrs.

The three *M. pomifera* fruit which were recorded as infested with dipterans in this study were all observed to have blackened areas. When bruised, the fruit exudes a bitter milky juice which will blacken the fruit on drying (Burton 1990). Further studies are required to determine whether the fruit was infested with a single species, diseased or damaged before other species were able to oviposit or whether one, or all, species were able to oviposit regardless of prior infestation. Further, it is unclear if populations of *B. tryoni* could be sustained by this host in the absence of other, more favourable hosts.

The implications for management of *M. pomifera*, particularly in and surrounding horticultural production areas are important, given its newly recorded larval host status of *B. tryoni*, and to a lesser extent, *D. platura*.

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