Hort Innovation

Final Report

SITplus: Raising Qfly sterile insect technique to world standard

Project leader:

Professor Phillip Taylor

Delivery partner:

Macquarie University

Project code:

HG14033

Project:

SITplus: Raising Qfly sterile insect technique to world standard (HG14033)

Disclaimer:

Funding statement:

SITplus: Raising Qfly sterile insect technique to world standard (HG14033) is funded by the Hort Frontiers Fruit Fly Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Macquarie University and contributions from the Australian Government.

Publishing details:

ISBN 978-0-7341-4759-2

Published and distributed by: Hort Innovation

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Content

Summary

Fruit flies are a major impediment to production and trade of fruit and vegetables globally. In Australia, the Queensland fruit fly (Qfly) is a serious endemic pest in Queensland, New South Wales, Australian Capital Territory, Northern Territory and Victoria. Further, outbreaks of Qfly occur regularly in South Australia, and have also occurred less frequently in Western Australia and Tasmania. For decades, broad spectrum insecticides provided high levels of protection against fruit flies at reasonable cost. But with increasing scrutiny and restrictions on usage patterns, there has been increasing demand for viable alternatives. The sterile insect technique is used widely to manage some of the most economically damaging fruit fly pests globally, and has been used intermittently to manage Qfly outbreaks in some regions. However, the technological foundations of the Qfly SIT program as of 2015 were weak; the historical program had been developed on a very modest budget in the early 1990s and there had been little investment in testing and improving the program since that time. Further, facilities were approaching end of serviceable life. At a time of increasing need for sustainable alternative approaches to the management of fruit flies, the available Qfly SIT program was rarely used and was of questionable efficacy.

Despite the uncertain state of Qfly SIT, the successful use of SIT overseas pointed to SIT as a potential pillar of future Qfly management in Australia in particular to protect and potentially regain area freedom. The availability of new molecular tools for the manipulation of gene expression and development of engineered genetic sexing strains showed great promise for the development of male-only releases in Qfly SIT, which would greatly increase efficacy and cost effectiveness. In 2014 the SITplus program was established with support from the Hort Frontiers Fruit Fly Fund to advance the development of Qfly SIT. As the SITplus program developed there was increasing awareness of just how little was known about the basic biology of Qfly in relation to traits of importance for SIT, and also of some basic aspects of SIT operations. Compared with overseas SIT programs, the technological state of Qfly SIT lagged years, or even decades, behind. HG14033 was proposed to address a wide diversity of knowledge and technology gaps, and to as quickly as possible close the gap between the research underpinnings of Qfly SIT and overseas programs of other species.

Given its broad remit, HG14033 is an unusually large and complex program, involving researchers and activity across the SITplus Program. HG14033 has provided deep foundations for Qfly SIT through robust R&D support in applied and strategic basic research. The structure of HG14033 recognised the diverse processes involved in an effective SIT program and provides support for each. Given the diversity of needs, HG14033 operated under 10 thematic areas and contained 26 distinct projects, each with its own leadership, team, collaborations and resources. HG14033 has supported very significant advances in Qfly SIT, not only closing the gap to overseas programs but in some project areas advancing Qfly SIT to a world-leading position. To ensure that the research findings are of high standard, and are available to future researchers, managers, and trade negotiators, the large majority of completed work is in, or is intended for, the international peer-reviewed scientific literature. The effectiveness of HG14033 is borne out in the remarkable success of Qfly suppression reported to date in FF17001, a project that translates the knowledge gained in HG14033 into trial operations. Further, the broad knowledge base provided in HG14033, and the advances made in understanding of Qfly ecology, behaviour and genetics provides a world-leading resource for the development of future innovations in SIT and other sustainable management tools. Finally, as an enduring legacy, HG14033 has made significant contributions to training and supporting the next generation of insect biosecurity researchers by integrating MRes and PhD students, and research fellows, as core participants in projects.

Keywords

Fruit Fly, Sterile Insect Technique, Area Wide Management, Queensland Fruit Fly

Introduction

The Queensland fruit fly (Qfly) presents the most difficult and costly biosecurity challenge to market access for many Australian fruit producers. For generations Australian growers have been able to rely on synthetic insecticides to control pest populations of these flies, and even to maintain substantial horticultural production of highly vulnerable crops in endemic areas. However, for almost as long as synthetic insecticides have existed there has been scrutiny of their non-target effects, especially environmental contamination and hazards to human health. As a consequence, horticulture industries have seen many cheap and effective insecticides lost for key use patterns and some banned altogether. Over the past 40 years, the organophosphate insecticides Dimethoate and Fenthion have provided a high degree of phytosanitary assurance for many crops, but these pillars of fruit fly control have been withdrawn from many use patterns, especially on fruit with edible peel that are near to harvest. That is, the type of fruit and the timing at which fruit are most vulnerable to fruit fly infestation. Alternative insecticides are either insufficiently effective or are also under scrutiny, and hence are unlikely to offer long-term solutions. There has been a growing need for new approaches to the control of Qfly, and other fruit flies, in Australia.

Area wide management (AWM) that incorporates the Sterile Insect Technique (SIT) has been very successfully implemented to control some major fruit fly pests overseas (e.g., Medfly, Melonfly, Oriental fruit fly Mexican fruit fly, Caribbean fruit fly, West Indian fruit fly), and has been used in Australia to manage outbreaks of both Medfly and Qfly. SIT programs involve the rearing of millions of flies each week under controlled factory conditions. Pupae are dyed for identification, rendered reproductively sterile by irradiation, then transported to areas of outbreak management or population suppression, reared to adulthood, and released. The released sterile male flies then mate with fertile wild flies, rendering their eggs inviable and thereby curtailing the reproduction of pest populations. SIT hence reduces crop damage by existing wild flies because eggs deposited by wild females mated by sterile males do not develop into larvae. SIT also reduces the number of fertile wild flies in the next generation because wild females mated by sterile males are unable to produce offspring. Ideally, SIT reduces wild populations to a level at which they are not sustainable, such that the pest populations are extinguished. Because outbreaks most often originate in urban areas, where broad-scale pesticide application is not an option, SIT provides a safe and sustainable tool to combat Qflies in the most effective target areas.

Medfly is a global pest and so Australia is able to draw heavily on the extensive findings of researchers working on this species around the world. For medfly, large SIT mass-rearing facilities exist in Mexico, Argentina, Peru, Portugal, Chile, Guatemala, Spain, Israel, South Africa and USA, and these operations are supported by substantial R&D programs in these regions. Many additional countries affected by Medfly contribute to R&D both for SIT and for compatible area wide management practices. In stark contrast to the global effort against Medfly, Qfly has a very limited distribution, being widespread in eastern and parts of northern Australia, also established in New Caledonia, French Polynesia and Pitcairn Island, and so control programs have a correspondingly limited research base to draw on. It is tempting to imagine that technology developed for SIT of one fruit fly species, such as Medfly, can be transferred directly to others. While some aspects of SIT are indeed readily transferable, many others are not. Each fruit fly species has its own distinct biological character, as does each management program and region. Accordingly, there is a need both to identify what technology can be adopted from programs of other fruit fly species and to establish key knowledge of each target species. Unlike insecticides, SIT is not a 'product' that is purchased and applied according to manufacturer instructions and for which the burden of product development falls on a manufacturer. Instead, the burden of product development for SIT falls on governments, growers and public research providers.

Although SIT has been part of the overall suite of potential control options for many years, SIT for Qfly has been insufficiently developed to be deployed with confidence as a mainstream control option. As historically deployed, Qfly SIT has been based on minimal understanding of the pest's biology and of SIT practises. A substantial R&D effort has long been needed to raise the standard of Qfly SIT, and to establish SIT as an effective mainstream Qfly control option for use in area wide management programs in Australia. The present project was developed by SITPlus in recognition of the need for significant research and investment to bring Qfly SIT up to the standards of overseas operations used to control other fruit fly species.

The substantial research program of HG14033 raises both the technology and scientific underpinnings of Qfly SIT to ensure that the SITPlus initiative, and the new Qfly factory (Port Augusta), succeeds in delivering viable and

sustainable solutions to Australian growers. The approach is integrative and collaborative, with broad participation from across the SITPlus partners to make best use of available expertise and facilities as a collective. Some projects emphasize immediate application, dealing with specific research needs (e.g., developing pre-release protocols), whereas other projects comprise strategic basic research that will equip the new SIT program with a robust knowledge base to ensure ongoing development and maintenance of high quality sterile flies and to enable further improvements in future.

Beyond the direct outcomes of addressing key knowledge gaps for effective implementation of Qfy SIT, HG14033 also makes a major contribution to training the next generation of insect biosecurity and entomology researchers for Australia. This project has supported the professional development of numerous PhD/MRes students and research fellows who have worked across institutional boundaries to carry out each element of their work at the most appropriate institution. Further, each research student and research fellow received supervision and / or mentorship from industry and from government research agencies. This is important as such an approach means that all of these early career researchers have strong grounding in both the university and governmental research sectors and has been broadly trained to tackle future biosecurity and trade access threats. All of the projects are cross-institutional, and through this approach the proposed research program has contributed to fostering a more coordinated and collaborative approach to fruit fly R&D in Australia. As much as possible, knowledge gained in HG14033 has been disseminated publicly in the primary peer-reviewed literature, in reports, in presentations, and in theses, thereby ensuring both high standards of outputs and availability of findings for future researchers and managers.

Methodology

SIT entails a series of sequential management practices and biological processes. Failure at any one step precludes movement to the next. To establish and run an SIT program, flies must be 1) collected and 2) domesticated, 3) mass-reared, 4) sterilized, 5) marked, 6) transported, 7) reared out and then 8) released. Following release, the flies must still 9) disperse, 10) survive, 11) mature, 12) join mating aggregations, 13) attract wild females, 14) court effectively, 15) mate, 16) inseminate and 17) induce sexual inhibition in their mates. The overall program was divided into 10 distinct Themes and 26 distinct Projects, each with its own set of local leadership, team, facilities and research training. Our Research Themes align with these sequential steps, and the projects under each theme address areas for which knowledge gaps exist at one or more of these steps.

The knowledge gaps and advances addressed in HG14033 draw on a vast diversity of expertise, including genetics, bioinformatics, physiology, metabalomics, proteomics, transcriptomics, behaviour, physiology, climate modelling, logistic modelling, field ecology, developmental biology and more. This diversity of expertise was achieved by working fluidly across organisations, primarily including researchers at Macquarie University (Phil Taylor, Drew Allen, Marie Herberstein, Ian Jamie, Joanne Jamie, Shoba Ranganathan, Darrell Kemp, Linda Beaumont, Michelle Power), CSIRO (John Oakeshott, Owain Edwards, Gunjan Pandey, Rahul Rane, Matt Taylor, Mike Lacey, David Beale, Ros Mourant, David Midgley), NSW DPI (Toni Chapman, Bernie Dominiak, Solomon Balagawi, Bernice Sutcliffe), Plant & Food Research (Lloyd Stringer, Max Suckling, Ashrad El Sayad), SARDI (Peter Crisp, Lakshmi Nacey) together with significant contributions from researchers of organisations outside the SITplus program, including Boaz Yuval (The Hebrew University of Jerusalem, Israel), Diana Perez-Staples (Universidad Veracuzana, Mexico), Renee Catullo (University of Western Australia), Marianne Frommer (University of New South Wales), John Sved (University of New South Wales), Guy Westmore (Department of Primary Industries, Parks, Water and Environment, Tasmania), Jane Royer (Department of Agriculture, Fisheries and Food, Queensland), Chris Weldon (University of Pretoria, South Africa), and Juan Hurtdao (University of Buenos Aires, Argentina).

HG14033 also made extensive use of MRes and PhD research students (funded by Macquarie University as a component of co-investment) as an efficient and effective means to conduct substantial portions of the research program, supervised by expert researchers from all organisations. In taking this approach, HG14033 also provided a valuable platform for research training of the next generations of researchers and administrators in biosecurity and associated research and administration. Nineteen research students engaged in HG14033 have already submitted their theses (PhD (17): Humayra Akter, Maurizio Benelli, Saleh Adnan, Tahereh Moadeli, Darshana Rathnayake, Hue Dinh, Jess Inskeep, Rajib Majumder, Sabira Sultana, Angel Popa, Sally Noushini, Binh Nguyen, Jason Shadmany, Sushil Gaire, Jamil Biswas, Dean Southwood, Vivek Kempraj; MRes (2): Ahn Than, Asif Ahmed). A further four research students have been involved directly in delivering components of HG14033 and are due to submit their theses in 2021 (PhD: Cynthia Castro-Vargas, Asif Ahmed, Shirleen Prasad; MRes: Iffat Farhana), leading to the training of 21 PhD candidates.

HG14033 involved twenty four early career research fellows in many aspects of its operations, providing early career training and mentorship, including Soo Jean Park, Vivian Mendez, Donald Cameron, Jeanneth Perez, Renata Morelli, Fleur Ponton, Polychronis Remploulakis, Juliano Morimoto, Ania Deutscher, Tom White, Sabbir Siddiqui, Kate Lynch, Valentina Colombo, Bishwo Mainali, Maryam Yazdani, Heng Lin Yeap, Francisco Devescovi, Jack Horlick, Kate Mitchell, Lizzy Lowe, John Baumgartner, Shabnam Tabrizi, Sheemal Kumar, and Sui Fai (Ronald) Lee.

To ensure both high standards of outputs, and to ensure that findings are readily available to future researchers to make further advances and to trade partners to demonstrate Australian fruit fly biosecurity knowledge and capability, the large majority of activities in HG14033 are in, or intended for, the primary peer-reviewed literature. An online archive of all HG14033 outputs has been created on the SITplus Research Site of the Plant Health Australia Biosecurity Portal, including peer-reviewed publications (also available from primary publication sources, and ResearchGate), MRes and PhD theses (also available by free online access to Macquarie University thesis repository, https://www.researchonline.mq.edu.au/vital/access/manager/Index), oral and poster presentations delivered at external and internal meetings, and presentation abstracts.

Outputs

HG14033 was conducted as a series of distinct projects that were organised under ten themes. The detailed deliverables under each Theme and Project are archived in the SITplus Research site of the Plant Health Australia Biosecurity Portal. In accord with the intent to place as much work as possible in the public domain, most of the completed research is openly available in the peer-reviewed literature or in publicly available MRes and PhD theses.

The primary outputs of each Theme and Project are briefly summarised below:

Theme 1: Preserving genetic quality in domestication and mass rearing

Project 1: Characterize the 'domestication' process

Qfly SIT requires mass production of domesticated flies, but the consequences of domestication are poorly understood, as are potential approaches for maintaining quality. This project investigates aspects of Qfly biology that are affected by domestication, explores genetic underpinnings of such effects, and recommends approaches that may ameliorate negative impacts of domestication.

Theme 2: Production and delivery processes

Project 1: Defining Quality Control (QC) protocols

Factors affecting the quality of domesticated and irradiated Qfly are poorly understood. This project explores factors that can affect quality and provides a robust test of quality control protocols. This project also provided training on quality control procedures for staff in mass rearing and rear-out facilities under FF17001 and FF18003.

Project 2: Sterility induction and its consequences

Previous Qfly SIT programs have relied on gamma irradiation to sterilise the flies, but x-irradiation is emerging as a comparatively safe and economic alternative. This project assesses the dose response of pupal Qfly in terms of sterility and quality, and develops an assay to quantify absorbance of irradiation.

Project 3: Maintaining fly quality during transport

Pupae need to be transported long distances from the Qfly factory in Port Augusta to rear-out facilities in SA and in other states. This project investigates aspects of handling at the factory and during transportation that may impact on the quality of flies available for release.

Project 4: Logistics of production and delivery

In SIT programs, decisions need to be made about how best to transport pupae from a production facility to rear-out facilities that may be in diverse locations. This project models a range of scenarios to assess economic merits, and also produces a 'dashboard' that can be used to track changes in fly quality between batches both before and after transportation.

Project 5: Microbial gut symbionts

Microbes associated with some fruit flies have been found have massive impacts on performance, both as symbionts that aid in nutrition and as pathogens. This project explores the role of microbes in the biology of Qfly and considers potential applied significance in rearing and protection from immune challenges.

Theme 3: Pre-release treatments and release methods

Project 1: Pre-release treatments

Qfly have historically been released when just 2- 3 days of age and suffer high mortality such that few survive to maturity. This project seeks solutions to this serious constraint through pre-release treatments.

Project 2: Release methods

Aerial release is intended as a primary operational approach to Qfly SIT. This project trials and refined aerial release protocols for use in operations and pilot release programs (FF17001).

Theme 4: Post-release identification

Project 1: Visible markers

Sterile flies are marked with fluorescent dyes so that they can be distinguished from wild flies after release. This project assesses the available dyes, and reviews potential alternative marking methods.

Project 2: Identification through biomarkers

This project considers the possibility of using irradiation-induced molecular signatures as a means of distinguishing released sterile (irradiated) flies.

Project 3: Isotope ratio analysis

Fruit flies are what they eat. While Qflies in nature most have access to C3 sugars, mass-reared flies are fed C4 sugars and this leaves a signature in their body composition. This project investigates the viability of persistent isotopic signatures as a supplementary means of distinguishing sterile and wild flies.

Project 4: Genetic biomarkers

Natural hybridisation occurs in some fruit flies, and this project explores the genetic structure of such hybridisation with a view to potential creation of Qfly-compatible hybrids for SIT. The distinct genetics of such hybrids could be readily detected by routine molecular assays.

Theme 5: Ecological competence

Project 1: Genetics/Genomics of Qfly ecological fitness

Vulnerability to desiccation constrains Qfly populations and has major implications for SIT. Domestication increases vulnerability of Qflies to desiccation. To advance toward ameliorating the negative impacts of domestication, this project investigates genetics underpinning desiccation resistance.

Project 2: Regional variation in Qfly fitness genetics/genomics

One approach to addressing the negative impacts of domestication on desiccation resistance is to source mass rearing populations from regions that naturally express high levels of desiccation resistance. To identify populations that might serve as sources of desiccation resistant lines for SIT, this project assesses variation is desiccation resistance from across the range.

Theme 6: Applied landscape ecology

Project 1: Dispersal, maturation and survival

Following release, sterile flies need to disperse and mature, and survive long enough to participate in SIT. This project assesses factors affecting the movement and survival of released flies, and whether released sterile flies tend to co-locate with wild flies. This project also discovers a 6-8 fold increase in the field abundance of mature sterile males when released at 5 days of age rather than the usual 2-3 days pf age.

Project 2: Temperature tolerance and cool storage

Temperature tolerance is an important mediator of geographic distribution but in many insects and understanding of thermal tolerance can also be used to develop cool-storage protocols. This project assesses potential protocols for cool storage of Qfly eggs and pupae that might provide a means of regulating the flow from production to delivery.

Project 3: Adaptive potential of Q-fly to geographic distribution and climate change

Climate change is expected to modify regional suitability for fruit flies, including Qfly, which is in turn expected to result in changes in distribution, prevalence and risk. This project uses the latest modelling tools to predict likely changes in distribution of Qfly and other endemic fruit flies over the coming decades, as well as the potential geographic range in Australia of major exotic threats.

Project 4: Local drivers of fruit fly behaviour

To understand the distribution of flies in the landscape, and of the prospective co-location of sterile and wild flies, it is important to understand the drivers of fly behaviour on a local scale. This project explores the ways in which the movement and activity of sterile and wild flies is affected by local conditions.

Theme 7: Mating ability of sterile flies

Project 1: Variation and functions of pheromones

Pheromones are central in the mating biology of fruit flies, but the composition and function of Qfly pheromones is poorly understood. This project describes the pheromones produced, emitted and deposited by male and female Qfly, and assesses effects of domestication of pheromone emission and sexual calling behaviour.

Project 2: Genetics/Genomics of Qfly reproductive fitness

Given the central role of pheromones in Qfly mating biology, there is potential to improve strains by selecting for preferred pheromone profiles. This project ascertains the variation and heritability of pheromone profiles in Qfly.

Theme 8: Protecting sterile matings

Project 1: Prevalence and predictors of multiple mating

The number of times that females mate, and how they store and use the sperm of different males, can be a powerful influence on SIT success. If females do remate often, then it is important to know how the different mates contribute to paternity of offspring so that the risks of females remaining fertile despite mating with a sterile male can be understood. This project documents sperm storage and usage by Qfly, and also estimates the prevalence of multiple mating in field populations.

Project 2: Mechanisms of sexual inhibition

Ability of males to induce sexual inhibition in their mates is essential for SIT success. The more effectively that released males are able to prevent their mates from accepting a subsequent, potentially fertile, male, the more effective the sterile matings will be at inducing reproductive failure in mated females. This project explores in detail the molecular mechanisms that mediate induced sexual inhibition in Qfly.

Theme 9: Compatible control technologies

Project 1: Predators as control agents

With reduced pesticide use, natural enemies such as predators become a more effective means of regulating fruit fly populations. But predators also present a risk for the released sterile Qflies. This project explores the responses and vulnerability of Qfly to predators.

Project 2: The importance of crop hygiene

Untended fruit trees, or poor crop hygiene, present a substantial potential reproductive resource for Qfly populations. However, the extent of this impact is poorly understood. This project investigates the number of wild flies that can be produced by untended fruit trees, providing a pest reservoir.

Theme 10: Combining and implementing control technologies

Project 1: Enclosure testing of SIT and AWM practices

It is difficult and expensive to test efficacy of SIT in full scale trial operations such as in FF17001, but much can be learned from trials conducted in enclosures with wild and sterile flies. Such trials provide a far more realistic assessment than laboratory trials of survival, development and mating as they investigate the 'net' effect under conditions that are much closer to the field. This project tests the ability of sterile males to induce sterility in wild females in a replicated outdoor simulation.

Project 2: Field testing of SIT and AWM practices

Full field testing of SIT and AWM practices are underway in FF17001. This project identified those practices and procedures developed in the course of HG14033 that would provide the foundations for FF17001.

Outcomes

Findings for each identified Outcome in each Project are designed as a 'package' suitable for publishing in the peer-reviewed scientific literature, although in many cases findings are also available as chapters in publicly accessible MRes or PhD theses. Research training has been an important element of HG14033. Participation of MRes or PhD students funded by HG14033 in research outcomes is marked with ([†]) and participation of research fellows funded by HG14033 in research outcomes is marked with ([‡]).

Owing to the highly integrated nature of the research program of HG14033, there is natural overlap in some deliverables across Themes and Projects, as experiments commonly addressed multiple issues in concert. As such, the Outcomes are presented under the Theme and Project where they make the greatest contribution, but cross-referencing is also used to illustrate how each Outcome also contributes to other Themes and Projects.

Theme 1: Preserving genetic quality in domestication and mass rearing

Project 1: Characterize the 'domestication' process

Summary

Despite many years of domestication and mass rearing of fruit flies, remarkably little was known about the associated biological processes or how to manage them. It has been long known that there are significant changes in behaviour, physiology and morphology of fruit flies through domestication, and that there is a massive genetic 'bottleneck' in the first generations that severely restricts the genetic diversity of domesticated lines. One of the greatest concerns for the viability of SIT comes from the potential for the domestication process to impinge on the ecological or sexual competence of the flies, which would be evident in field releases as poor survivorship, poor distribution, and lower than expected levels of mating with wild type flies. This is a consistent theme for SIT programs around the world, as managers struggle to balance the competing demands of producing high numbers of flies and producing high quality flies. The easiest way to produce high numbers of flies is to drive them to high levels of factory adaptation, but this may mean that the flies produced have significantly diminished ability to survive and perform once released in the field.

Understanding the factors that influence ecological competence, particularly variation in abiotic stresses and stress resistance mechanisms and how they respond to domestication, can guide strategies to preserve or even promote such qualities in the fly production process. However, literature on abiotic stress resistance of Qfly has been patchy and has not sufficiently advanced the understanding of phenotypic variation in ecological fitness. This project monitored the effects of domestication on key abiotic stress resistance traits (heat, cold, starvation and desiccation), starting with a survey of regional variation in those traits and documenting how these traits change through domestication (see Theme 5 Project 2). Resistance to heat and desiccation decreased rapidly with domestication in some but not all populations. This suggests that genetic changes that reduce abiotic resistance could occur in a few generations of mass-rearing and that amelioration measures need to be implemented early in the mass-rearing process to minimise the rate of loss. Source populations for Qfly strain can vary substantially in ecologically important traits and how these traits respond to domestication. We therefore recommend evaluation of multiple source populations and rearing conditions to minimise deterioration of these wild type characters during domestication. Key genetic regions that are associated with desiccation resistance are identified (Outcome 1, see also Theme 5 Project 1) and biochemical mechanisms of resistance are elucidated elsewhere (Theme 5 Project 1), and this information can be used to identify changes in desiccation resistance in Qfly strains by molecular screening, to guide preservation of desiccation resistance, and potentially even to develop lines of Qfly with elevated desiccation resistance.

In addition to studies of desiccation resistance, which was anticipated to be the most critically under-studied, this project also found domestication-related chances in thermal preferences of Qfly (Outcome 2), with more domesticated lines preferring cooler locations which is in accord with findings of reduced environmental tolerance more broadly (Theme 5 Project 2). Beyond affecting survivorship in the field, such changes in thermal preference can affect dispersal and distribution of the flies following release, potentially resulting in some mis-match of

locations of sterile and wild flies at local scales (see Theme 6 Project 2). Domestication effects were also found in dietary needs of the flies, modifying the protein:carbohydrate ratios that maximise flight performance and mating propensity (Outcome 3).

Domestication had some positive effects on mating performance, likely as a consequence of the extreme selection on sexual performance in cages containing many thousands of flies, which is very different from the context of the field. Domesticated flies were found to have increased mating propensity and to transfer more sperm, as well as undiminished ability to mate with previously mated females and to induce sexual inhibition in their mates (Outcome 4). Further, in other project areas domesticated flies were found to release more pheromones owing to elevated calling performance (rapid wing beating that produces a calling 'song') (Theme 7 Project 1).

In addition to the studies presented under Theme 1 Project 1, very significant effects of domestication were found in other project areas and are reported under Theme 2 Project 1 (Quality control parameters), Theme 2 Project 5 (Microbiome and immunity), Theme 6 Project 4 (Microhabitat preferences & Sexual calling behaviour), and Theme 8 Project 2 (Mating propensity).

Conclusions & Recommendations

- Domestication reduces desiccation resistance, an important trait for field survival
- Desiccation resistance is variable between individual flies and is heritable
- Genes conferring desiccation resistance have been mapped
- Select source populations for SIT strains which have relatively high initial climatic stress resistance
- Develop rearing conditions that minimise loss of resistance during domestication in the SIT strain
- Use a genetic marker assisted breeding strategy to screen for and retain abiotic stress resistance
- Domestication reduces preferred temperature
- Domestication increases male mating propensity and insemination ability

Achievements

Outcome 1: Genomic regions contributing to changes in relevant fitness traits identified

Prasad S[†], Popa-Baez AD[†], Yeap HL[‡], Rane RV, Pandey G, Colombo V[‡], Lee SF[‡], Taylor PW & Oakeshott JG (manuscript) Genetic basis for desiccation resistance in the Queensland fruit fly.

Queensland fruit flies (*Bactrocera tryoni*) are prone to desiccation in field environments and their spatial and temporal abundances are heavily influenced by their resistance to this stress. Substantial variation for desiccation resistance in natural Qfly populations has been reported and that the resistance of most strains declines significantly within a few generations in the laboratory. The goal of this project was to elucidate the genetic basis of the variation.

The first resource developed for this work was a set of isofemale lines from different locations. Isofemale lines are widely used for insect genetic analyses because each such line is established from a single inseminated female so there is relatively little genetic variation within but large differences between them. Previous attempts to make such lines of Qflies have failed but we were able to maintain and screen 12 isofemale lines from four locations (Alice Springs, Mareeba, Narrabri and Sydney) by modifications to culture conditions which substantially improved the productivity of individually housed females. Bioassays of these lines revealed significant differences in desiccation resistance between them that were stable over generations, indicating a genetic (or epigenetic) basis for the differences.

The second resource needed for this work was a high-quality chromosome-level genome sequence assembly for Qfly. Earlier genomic work by Stuart Gilchrist, John Sved, Marianne Frommer and others had produced a draft assembly (GenBank GCA_000695345.1) but its coverage was incomplete and was insufficient for either formal gene annotation or chromosome-level assembly of component fragments. Using a combination of short- and long-read sequencing technologies and genome assembly platforms, this project has achieved a chromosome-level

assembly (GenBank GCA_016617805.2) and gene annotation and has made these resources publicly available at the National Center for Biotechnology Information database.

With these resources in hand, two genome-wide association studies (GWAS) were carried out to map the genes underlying genetic variation for desiccation resistance in Qfly. Crosses were established between two isofemale lines from opposite extremes of the phenotypic (desiccation resistance) distribution of resistances among all the isofemale lines. More than 500 progeny were then bioassayed for resistance individually at generations F4, F5 and F6. Groups of these individuals showing different levels of resistance were then subject to whole-genome sequencing. A new wild-caught strain from Sydney which earlier bioassays had suggested showed relatively high desiccation resistance, and was again bioassayed for resistance and sequenced the top and bottom 5% of the phenotypic distribution.

These two GWAS analyses, in combination with biochemical insights (Theme 5 Project 1), provide precise genetic markers for desiccation resistance that can be used in breeding programs for SIT strains to preserve Qfly desiccation resistance and hence ability to survive and impact pest populations once released into the field.

Outcome 2: Deleterious effects of domestication on temperature preferences

Lynch KE[‡], White TE & Kemp DJ (2018) The effect of captive breeding upon adult thermal preference in the Queensland fruit fly (*Bactrocera tryoni*). *Journal of Thermal Biology* 78: 290-297

The Queensland fruit fly (*Bactrocera tr*yoni) is a generalist pest that poses a significant threat to the Australian horticultural industry. This species has become broadly established across latitudes that encompass tropical to temperate climates, and hence populations occupy diverse thermal niches. Successful expansion across this range may have been brokered by evolutionarily labile features of breeding phenology, physiology and/or behaviour. We explored the potential role of behavioural flexibility by characterizing variation in adult thermal preference using a novel gradient apparatus. Flies oriented within this apparatus essentially at random in the absence of thermal variation, but sought and maintained precise positions when presented with an established gradient. Male and female flies from an 'old' colony (>300 generations) and a 'young' (F7) colony were compared. Whereas we found no difference between the sexes, flies from the young colony preferred higher temperatures (30.93 ± 7.30 °C) and had greater individual variation than their counterparts from the old colony (28.16 ± 5.63 °C). Given that *B. tryoni* are routinely maintained at 25 °C in the laboratory, a lower mean preference of the old colony is consistent with thermal adaptation. This is further supported by their reduced phenotypic variance, which follows as a logical consequence of stabilising selection given long-term environmental constancy. These results demonstrate that *B. tryoni* seek to thermoregulate via adult behaviour, and that individual temperature preference can be precisely measured using a gradient apparatus. The evidence for adaptive tuning of this behaviour has importance for both the design of captive rearing protocols as well as the prediction of invasive potential and species biogeography under future climatic variation.

Outcome 3: Determine effects of domestication on dietary needs

Majumder R[†], Adnan S[†], Ponton F[‡], Chapman T & Morimoto J[‡] (manuscript) Domestication affects how macronutrient balance modulates development and sexual performance of the Queensland fruit fly

Nutrition is a key modulator of insect fitness. For species considered pests and targeted by mass-rearing programs, here we are understanding how protein and carbohydrate (P:C) ratios of the larval diet along the domestication process influenced the polyphagous fly *Bactrocera tryoni* (aka 'Qfly') development in the laboratory facility. Our findings demonstrate that the number of generations of domestication (10, 31, >100 generations) affects how Qfly to development and sexual traits respond to Protein:Carbohydrate ratios (1.5:1, 1:1.6, 1:3.4) of larval. A balanced diet (P:C 1:1.6) increased the larval, pupal and adult fly weight to the intermediate domesticated colonies (generation 31) of Qfly. In addition, P:C ratio 1:1.6 in diet increased the flight ability performance of the Qfly colonies obtained from generation 31 demonstrated the significant influence of diet and domestication process on Qfly. Finally, we illustrated that mating propensity was significantly influenced by both domestication and larval diet. Our findings highlight that the domestication process of Qfly can modulate by the larval selective diet influenced on fitness-related traits.

Outcome 4: Determine effects of domestication on sexual performance

Pérez J‡, Mendez V‡, Yuval B & Taylor PW (in press) Domestication-related changes in sexual performance of Queensland fruit fly. *Insect Science*

In Sterile Insect Technique (SIT) programs, massive numbers of insects are reared, sterilized, and released in the field to impede reproduction of pest populations. The domestication and rearing processes used to produce insects for SIT programs may have significant evolutionary impacts on life history and reproductive biology. We assessed the effects of domestication on sexual performance of laboratory reared Queensland fruit fly, *Bactrocera tryoni*, by comparing an old (49 generations) and a young colony (5 generations). We evaluated mating propensity, mating latency, copula duration, sperm transfer, and ability to induce sexual inhibition in mates. Overall, both males and females from the old colony had greater mating propensity than those from the young colony. Copula duration was longer when females were from the old colony. There was no evidence of sexual isolation between the colonies as males and females from the two colonies had similar propensity to mate with flies from either colony. Males from the old colony transferred more sperm regardless of which colony their mate was from. Finally, males from both colonies were similarly able to induce sexual inhibition in their mates and were also similarly able to secure copulations with already-mated females. Positive effects of domestication on sperm transfer, coupled with maintained ability to induce sexual inhibition in mates and to secure copulations with previously mated females, highlights that domestication may have little effect, or even positive effects, on some aspects of sexual performance that may advantage mass-reared *B. tryoni* in SIT programs.

Theme 2: Production and delivery processes

Many aspects of the Qfly production process have been investigated in previous projects, and this enabled us to be quite targeted in the investigations of HG14033. For example, new larval diets were investigated in several HIA projects including the potential of alternative bulking agents and probiotic additives (MT13040) and high-productivity liquid larval diets (HG13045). Experience from historical operations of the decommissioned NSW DPI facility, and advice from fruit fly rearing facilities overseas, provide good guidance for the SITplus Qfly factory. However, limited information was available for some aspects of the production and delivery process, and Theme 2 addresses this shortfall.

Project 1: Defining Quality Control (QC) protocols

Summary

Implementation of SIT requires a cost-effective system for production and delivery of large numbers of high quality sterile male insects that can compete with their wild male for matings with wild females. However, mass produced Qfly often display poor quality arising from industrial processes – domestication, mass-rearing, irradiation, post-production handling, transportation, and release. It is important that the performance of mass-produced sterile insects is routinely assessed in the context of operational activities both for changes over time and in relation to standards. A series of quality control (QC) tests have been developed for use with tephritid fruit flies, mostly based on protocols that have been developed initially for use with medflies. These include routine tests such as percentage egg hatch, development time, emergence, longevity, and flight ability that are used in each production batch to monitor product quality and to identify production failures. For Qfly, there is a need to resolve what level of each parameter should be set as the accepted standard in a production setting and what production factors affect fly quality.

With an overall aim of refining production and delivery options and identifying QC tests that are most suited to Qfly biology, this project investigate in detail QC parameters in relation to variation in key production and post-production processes. Domestication effects are pervasive in fruit fly mass rearing programs, and this includes QC parameters in Qfly (Outcome 1). Domestication impacts QC parameters of some metrics in a repeatable manner, including decreased egg hatchability, shorter developmental time, higher fecundity, higher survival under stress and greater longevity. However, other QC parameters are more variable between colonies in ways that are not explained by domestication such that each colony has its own character. As such, colonies can be chosen on the

basis of preferred QC profile and it is important to assess colony performance against that colony's performance over time. Pupal size is one of the most basic QC parameters, and can be affected by both larval diet (Outcome 2) and larval rearing density (Outcome 3), with flow-on effects on other associated QC parameters such as adult emergence and sex ratio. The link between pupal size and sex ratio provides a crude tool for partial sex sorting of Q-fly pupae; the sex ratio of released flies can be somewhat male biased simply by eliminating the largest pupae. In addition to the routine QC assays that are conducted with each weekly production batch there are some additional assays that are advised as useful periodically. Locomotion assays are used to assay Qfly performance in Theme 2 Project 2 (Outcome 2), and such assays are very amenable to deployment as periodic QC assays. Field cage mating competitiveness and compatibility of sterile and wild flies is addressed as a periodic QC assays in Theme 3 Project 1 (Outcome 11).

Conclusions & Recommendations

- Some QC parameters change reliably with domestication, others are characteristic of colonies
- QC parameters of colonies from multiple sources should be screened for stable desirable performance metrics
- Larval diet and rearing density affect pupal size, which is closely linked to performance traits
- Pupal size can be included as an easily measured and informative routine QC metric
- Field cage mating competitiveness and compatibility assays, should be conducted at least annually
- Consider the potential practical application of partial sex sorting on the basis of pupal size
- QC manual procedures be strictly adhered to at all factory and rear-out facilities
- QC operations should be regularly audited to ascertain compliance
- Retraining be implemented when non-compliance is identified

Achievements

Outcome 1: Establish effect of domestication on quality control parameters as a baseline for standards

Gaire S[†], Pokhrel S, Biswas MJH[†], Mainali BP[‡], Mendez V[‡], Pérez J[‡], Taylor PW & Rempoulakis P[‡] (manuscript) Effect of domestication on quality control parameters of the Queensland fruit fly *Bactrocera tryoni*.

Quality of domesticated insects for SIT is measured in terms of productivity and performance metrics that indicate physiological or behavioural changes and may affect ability of reared insects to survive, disperse and mate with field populations after release. The measurement comprises a detailed suite of quality control (QC) assays has been developed for tephritid fruit flies (FAO/IAEA/USDA 2014). Different SIT programs vary somewhat in which standard QC measures are prioritised, as well as in some details of how they are deployed. Also, in experimental settings, it is commonplace to also include additional non-standard measures that probe finer details of performance that may yield a more biological understanding of mechanisms that impact fly quality. The study investigated effects of domestication on QC parameters of old and young Q-fly colonies originating from three distant geographical locations in Australia; Sydney (Generation >100 and 7), Brisbane (Generation 59 and 14) and Cairns (Generation 29 and 9). Standard QC parameters including development time, pupal recovery, pupal weight, adult emergence, sex ratio, flight ability, fecundity, egg hatchability, and longevity were assessed. Regardless of their origin, older Q-fly colonies had lower egg hatchability, shorter developmental time, higher fecundity, higher survival under stress and greater longevity. The study highlights the importance of understanding the effects of continued domestication on quality control measures when setting standards for assessment of fruit fly strains used in SIT programs, particularly when using strains that are regularly replaced or infused with wild stock. In addition, findings of this study support the need for a careful choice of Qfly strains for SIT application because there is substantial variation between colonies that are not universally explained by domestication such that it is not possible to answer the basic question of 'how do QC parameters change through domestication' in a simple or universal manner. Colonies need to be assessed on a case-by-case basis. Some metrics such as hatching percentage, developmental period, egg to pupae recovery and fecundity do change reliably with domestication. However, others do not and instead appear to reflect other factors such as source material region or rearing conditions.

Outcome 2: Establish effect of larval diets on pupal size quality control parameters

Gaire S[†], Pokhrel S, Allen AP, Mainali BP[‡], Taylor PW & Rempoulakis P[‡] (manuscript) The effect of larval diets on pupal size distribution and quality parameters of the Queensland fruit fly.

Pupal size is one of the most important quality control metrics, and can be assessed as pupal diameter. Compared with batch weighing, a common alternative, sorting pupae individually by diameter provides better information on pupal size distribution and variance. The relationship of production system, quality parameters and sex ratio based on pupal size needs to be evaluated to obtain a large number of quality males released in the wild to suppress the population. This study assessed the size distribution of pupae that had been reared on gel, carrot and lucerne chaff-based larval diets. A pupal size sorter was used to separate the pupae into 13 diameter categories which were then pooled into five general categories for quality control analysis (very small, small, medium, large, very large). We found a stronger correlation between pupal size and pupal weight inferring that the either pupal size or pupal weight can be included as one of the QC parameters. Gel diets produced more pupae, and produced pupae of more uniform size that were of quality as good as or better than those from carrot and lucerne chaff diet. Wing length of adult flies was positively associated with pupal size. Across larval diets, very small pupae had low adult emergence and flight ability rate, and the larger pupal sizes had a heavily female-biased sex ratio. Higher productivity and homogeneous pupal size distribution, with higher percentage of males on gel than on carrot and lucerne chaff diets would have an advantage over the other diets for SIT application. This study demonstrates that adult size depends on larval diet, stressing the importance of conditions in insect mass rearing. Very small size pupae perform poorly in terms of emergence and flight ability. Inclusion of pupal size as Q-fly quality is worth considering. The link between pupal size and sex suggests that the size parameter could be used (to a limited extend) for sexing purposes to obtain a somewhat male-biased release program in bisexual releases. For sorting purposes, a larger pupal sorter could be manufactured to handle the large production volumes with acceptable precision to rapidly sort size groups.

Outcome 3: Establish effect of egg seeding density and pupal size distribution on quality control

Gaire S[†], Pokhrel S, Mainali BP[‡] & Rempoulakis P[‡] (manuscript) The effect of egg seeding density on pupal size distribution and quality control parameters of the Queensland fruit fly.

In SIT, millions of sterile insects are released into the field to suppress reproduction of pest populations. Accordingly, SIT requires efficient and economical mass production and sterilization of vast numbers of high-quality flies. Dietary modifications have all made valuable advances in the performance and economy of gel larval diets for rearing of Q-fly, and additional advances might be made by manipulating egg loading density. Increased egg density in larval diets to increase production may result in a trade-off with fly quality. There is value in identifying the nature of this trade-off in order to maximise production of flies that meet required quality control standards. In this study, fly larvae at egg-seeding densities of 10, 15, 23.33, 25, 30 eggs/gram 39 of gel diet were reared to pupae. A pupal size sorter was used to separate the pupae into 13 diameter categories. The pupae were then broadly grouped into small, medium and large, and underwent QC evaluations. Lower egg seeding densities resulted in larger pupae. The egg loading density influenced pupal productivity and size but had little impact on adult QC parameters. The pupal weight of early hopping larvae was higher than that of late hopping larvae and pupal weight was inversely proportional to the egg seeding density; pupal weight decreased as egg seeding density increased. Higher pupal weight with low egg seeding density is probably due to lower competition for nutrition and space. Effects of larval density on growth and development of flies have been ascribed to several mechanisms including competition for food among larvae, increased energetic expenditure owing to a greater frequency of conspecific interactions, exposure to potentially toxic waste products from nearby larvae, or by variation metabolic heat generation. In addition, significant effects of pupal size were found on adult emergence, wing length and male percentage, thereby vindicating pupal size as a general quality control measure. Considering the size of pupae and economy of mass production, 25 eggs/g of gel diet is recommended for production of Qfly for SIT programs. The findings suggest that the main aim of production facility should be to produce bigger but uniform pupae as they perform better than the smaller pupae.

Outcome 4: Translate learnings from QC R&D into manuals and training

The learnings from Theme 2 Project 1, together with the use of QC protocols in other projects across HG14033, culminated in the development of a Quality Control manual for use in the SITplus sterile Qfly factory and at each of the rear-out facilities. The manual was prepared in consultation with the SITplus Technical Advisory Committee and stakeholders at each location including Terril Marais (SITplus factory manager, Port Augusta), Ami-Louise Cochrane (SITplus factory, Port Augusta), Feroz Khan (SITplus factory, Port Augusta), Joanne Dawson (Agriculture Victoria, Tatura), Glen Warren (NSW DPI, Yanco), and Emma O'Connell (NSW DPI, Yanco). The QC protocol was reviewed and approved by the SITplus Technical Advisory Committee, and was distributed to all users.

Through the course of HG14033, and especially as FF17001 came into operation, there was need for regular review of procedures, both to ensure that procedures were effective and also to ensure that procedures were being implemented in a consistent manner across all locations. Discord amongst facilities was sometimes found, and required active management. For example, it was discovered during the SIT Plus Technical Advisory Committee meeting in December 2019 that the factory, because of the large scale of QC tests, had adopted non-compliant systems and methodologies for QC tests such that there were operational differences in QC protocols between the factory and the rear-out facilities. As a consequence, while data collected at each site could be used to monitor changes within that site over time it would not be possible to reliably assess effects of transportation (which requires comparisons between sites and hence consistent protocols). To resolve the differences in QC practices between the factory and the rear-out facilities, a training workshop was arranged at the Netley facility in Adelaide with participants from the factory and the rear-out facilities. Dr Bishwo Mainali (MU), SITplus director Dan Ryan and Industry Liaison officer Chris O'Connor (MU) visited the SITplus factory to audit QC practices before the workshop.

Project 2: Sterility induction and its consequences

Summary

Historical Qfly SIT programs relied on gamma irradiation for sterility induction, using doses and protocols developed under HG06040 and subsequently confirmed as effective under operational conditions by staff of NSW DPI. But gamma radiation will not be available for the new SA facility, and this means that alternative methods for sterility induction will need to be available.

Recent developments suggest x-rays as a alternative source of sterilizing radiation for SIT. Accordingly there is a need for development of protocols. Pathological effects of gamma irradiation used for sterility induction have been reported in many fruit flies, and should be anticipated for x-rays as well. In Qflies, for example, gamma irradiation can cause reduced activity levels (Dominiak et al. 2014 Pest Management Science), increased mortality when under stress (Collins et al. 2009 Journal of Economic Entomology), and greatly diminished sperm production (Radhakrishnan et al. 2009 Animal Behaviour). While the source of ionizing radiation is different, the overall effects of gamma radiation and x-radiation should be similar. Nonetheless, as Qfly SIT moves to an x-ray based irradiation system there is a need to understand the pathological pitfalls and how to ameliorate these.

This project conducted detailed investigation of dose for sterility induction and pathology induced by x-rays. Irradiation dose needs to balance sterility induction and quality preservation. Accurate measurement of H2AX is a promising option to ensure correct calibration and the dose received by insect tissues (see also Theme 4 Project 2 for assay development). This project confirms appropriate dosage of x-irradiation to sterilize Q-flies for SIT, and assesses somatic damage caused by irradiation that could potentially reduce fly quality.

Conclusions & Recommendations

- Sterilization by x-rays is comparable in dose response to sterilization by gamma rays
- Doses of 60 – 70 Gy provide a high level of sterility and retain high quality
- γH2AX assays provide a reliable marker for biodosimetry
- γH2AX signal is persistent, and can serve as a molecular marker to identify sterile flies post-release

- Irradiation reduces motility of flies and ability to tolerate starvation, and these changes should be taken into account when considering dispersal and survival of flies especially in challenging environments.

Achievements

Outcome 1: Develop an assay to confirm irradiation dose absorption in insect tissues

Siddiqui S[‡], Nacey LC, Taylor PW & Crisp P (manuscript) A monoclonal γH2AvB antibody to detect prior irradiation in Queensland fruit fly; a new tool for biodosimetry and identification of irradiated insects.

H2AX, a member of the histone H2A family, becomes phosphorylated to form γH2AX In response to double-strand breaks (DSBs) in chromosomal DNA. Although polyclonal rabbit γH2AvB antibody shows great promise as a molecular marker of ionising radiation (IR) for *Bactrocera tryoni* (Froggatt) - Queensland fruit fly (Q-fly) - the use of monoclonal γH2AvB antibody for detection of IR-induced DNA damage in Q-fly remains unexplored. We here report on the development of the first monoclonal γH2AvB antibody to the phospho-specific form of Q-fly γH2AvB and, by immunofluorescence assays, characterise the sensitivity and specificity of this antibody to DSBs resulting from exposure to IR in the nuclei of Q-fly cells. Compared with non-irradiated pupae, γH2AvB level was significantly elevated in nuclei of flies emerging from pupae that had been irradiated at 40, 50, 60 and 70 Gy. Overall, there was a significant positive relationship between IR dose and γH2AvB level. Receiver-operating characteristic curves were carried out for visually scored γH2AvB parameters; percentage of cells containing γH2AvB resulted in a significant area under the curve value of 0.9997 with 98% specificity and 100% sensitivity for discrimination of adult flies irradiated at 70 Gy as pupae from unirradiated controls. Visual scoring results showed an elevated percentage of nuclei containing γH2AvB up to 30 days following exposure to 40, 50, 60 and 70 Gy of IR, compared with the nuclei of non-irradiated control flies. There was a significant positive correlation between γH2AvB signal and the percentage of non-viable egg across the irradiation treatments. Pupae irradiated at 7 days after pupation (3 - 4 days before emergence) exhibited lower levels of γH2AvB than pupae irradiated 9 days after pupation (1 - 2 days before emergence). The monoclonal antibody derived in the present study is effective to confirm the dose received during IR of flies released during Sterile Insect Technique (SIT) eradication programs, and hence provides a valuable new tool for the development of IR procedures and quality assurance in SIT programs, as well as for identifying sterile flies that are recaptured in monitoring traps.

Outcome 2: Assess effects of irradiation on environmental tolerance

Inskeep J[†], Adnan S[†] & Taylor PW (manuscript) Irradiation and hydric stress affect the survival and activity of Queensland fruit flies.

Applications of the sterile insect technique (SIT) for management of *Bactrocera tryoni* (Froggatt) may be improved by identifying, and ameliorating, the impact of stressors that constrain performance of released sterile insects. Irradiation used to sterilize insects induces somatic tissue damage that can directly reduce performance and can increase susceptibility to other sources of injury or stress. SIT is commonly deployed in hot, dry regions of Australia and desiccation can be a major source of stress and mortality for sterile *B. tryoni*; domestication tends to reduce desiccation tolerance in *B. tryoni* and this effect may be exacerbated by irradiation. The present study investigates the separate and combined effects of irradiation and desiccation stress on survival and locomotor activity of male and female *B. tryoni*. In survival under stress assays, males died faster than females and there was a significant interaction between effects of stress treatment (starvation vs. starvation and desiccation) and irradiation. Fertile flies exposed to starvation alone lived longer that those also exposed to desiccation, but for sterile flies there was no difference between stress treatments with flies exposed to both treatments having short survival that was similar to the fertile flies exposed to desiccation stress. Locomotor activity was higher in male flies, and this may contribute to their lower survivorship under stress; however locomotor activity does not provide a general explanation, as fertile flies were more active than sterile flies but had similar or higher survivorship, depending on stress. Sterile males depleted more lipid stores than fertile males before death when exposed to starvation alone, but the pattern was reversed when flies were also exposed to desiccation. For females, there were no differences between stress treatments in lipid depletion before death. Our results indicate that irradiation of *B. tryoni* to induce sterility reduces activity levels and also ability to tolerate starvation, but does not reduce ability to tolerate the combined effects of starvation and desiccation.

Project 3: Maintaining fly quality during transport

Summary

In SIT programs, flies are produced in a mass-rearing facility under controlled environmental conditions, and are then irradiated, and transported as pupae to distant locations for emergence, pre-release holding, and release. Insects are reared on artificial diets, packed and sterilized, usually by irradiation under hypoxia. Hypoxia, irradiation and vibration are important stressors experienced by fruit fly pupae during production, packing and transportation. Fruit fly pupae are usually packed into air-tight containers before irradiation and held until hypoxic in order to protect the pupae from oxidative damage during irradiation. Hypoxia is also useful for delaying adult emergence, increasing the time available for transporting pupae to the rear-out facilities. However, prolonged exposure of pupae to hypoxia can reduce quality of sterile fruit flies, even when at the lowest reliable sterilizing dose. Irradiation is used to induce sterility in fruit flies for most SIT programs, but somatic damage caused by irradiation can reduce the quality of sterile flies. Vibration generated when handling pupae during production operations before transport, such as jarring and tumbling to coat in dye at early pupal ages, can increase mortality and reduce fly quality. Further, vibration of pupae during transport can damage fruit fly pupae, resulting in reduced emergence and reduced quality of emerged adult flies. Exposure to stressors during these operations may reduce fly quality at the point of delivery contributing to deficiencies in the overall SIT program. Since the rear out centres are distantly located, it is important to identify what aspects of the transportation process might reduce fly quality (e.g., heat stress, chilling, hypoxia, vibrations). Understanding the impact of these stressors on quality control parameters, such as adult emergence and flight ability, can enable improvement of practices.

The project identifies and describes factors affecting quality during transportation and their contribution to the SIT application, and proposes methods for mitigating them by (1) investigating and identifying the key aspects of transportation that contribute to negative effects on Qfly quality at the point of delivery and (2) investigating transportation systems that maintain the highest possible fly quality at point of delivery. Information from this project provided key inputs to development of a transportation logistics model for SIT operations (Theme 2 Project 4). The work under this Project are operationally linked to activities under Theme 2 Project 1, providing additional insights to factors affecting QC parameters.

Pupae are held in hypoxia for irradiation and during transportation, and initial studies focused on detrimental impacts of hypoxia on QC parameters (Outcome 1), identifying significant declines in quality when pupae are held in hypoxia for four days or longer (as can be common in transportation from a remote production facility in Port Augusta to a remote rear-out facility in Tatura or Yanco under FF17001). These effects can be partly ameliorated by ensuring that the pupae are not exposed to high temperatures (Outcome 2, 3). In addition to hypoxia, pupae are often exposed vibration both during handling in the factory and during transportation and this can significantly impact QC parameters, especially after the sixth day of pupation (including the age at which pupae are usually transported) (Outcome 4). It is hence very important that transportation protocols minimise vibration as much as is practically possible.

Conclusions & Recommendations

- Hypoxia is required for irradiation and transportation, but more than two days is detrimental
- Periods of hypoxia required for irradiation and transportation should be minimised to preserve fly quality
- Where longer periods of hypoxia are required, quality is better retained at 18^{o}C than higher temperatures
- Consideration should be given to use of insulated boxes and chilling for transporting pupae
- Pupae are very vulnerable to damage from handling and vibrations, especially after the sixth day of pupation
- Exposure of pupae to vibrations should be minimised wherever practical

Achievements

Outcome 1: Determine quality control effects of pupal hypoxia during irradiation and transportation

Gaire S[†], Rempoulakis P[‡], Taylor PW & Mainali BP[‡] (manuscript) Trade-off between duration of pupal hypoxia for irradiation and transportation and quality control parameters of sterile Queensland fruit fly *Bactrocera tryoni*.

For SIT, fruit fly pupae are usually held under hypoxic conditions to mitigate the harmful effect of irradiation. Pupae are typically held in plastic bags where oxygen is depleted through the metabolic activity of the pupae. Hypoxia also delays adult emergence, increasing the time available for transporting pupae to rear-out facilities. Queensland fruit fly (Qfly) pupae are usually sealed in plastic bags or canisters to create hypoxic conditions, before exposure to ionising radiation to induce sterility. Post-irradiation. the sterile insects are transported to distant locations still under hypoxia which though required may diminish fly quality. To date, studies investigating the effect of hypoxia on fly quality have focused on emergence rates and have indicated modest reductions. A different view emerges in this study that includes a complete analysis of fly quality. This study investigated the effect of 0, 1, 2, 4, 6 42 and 8 days of storage of irradiated pupae under hypoxia at 18°C or 25°C. In addition to assessing standard quality control parameters, a locomotor activity monitor device was used to assess supplementary parameters including pre-emergence period and activity under nutritional stress. Adult emergence was not affected up to 2 days of hypoxia and pupae stored at 18°C had a higher emergence rate than those stored at 25°C. Flight ability remained at acceptable levels when pupae were stored for up to 2 days at either temperature but was substantially reduced when pupae were stored for 4 days or longer. Procedures that enable longer time frames for irradiation and transportation of pupae to rear-out facilities while maintaining fly quality would be of significant operational value in Qfly SIT programs. Of note, the study provides valuable guidance on the use of hypoxia during irradiation and transportation of Qfly pupae from factory to rearing-out facilities. Excessive exposure of Q-fly pupae to hypoxia has significant impact on their emergence and flight ability. It is recommended that Qfly pupae can safely be exposed to hypoxia for up to two days. Longer periods of hypoxia should be avoided, but if required should be at 18°C rather than 25°C. Further investigation of the mechanisms responsible for reduced emergence and flight ability of flies held under hypoxia is recommended, together with further investigation of protocols that might ameliorate effects. Given that nutritional status may underpin effects of hypoxia on flight ability, the possibility that flight ability is at least partly recovered during pre-release adult holding, when ample food is available, should be investigated.

Outcome 2: Determine effect of prolonged hypoxia at different temperature on Qfly performance

Mainali B[‡], Benelli M[†], Taylor PW & Rempoulakis P[‡] (manuscript) Effect of temperature and packing of pupae of Queensland fruit fly on quality control parameters.

Effects of hypoxia and temperature, and exposure period on the emergence and flight ability of Q flies were assessed. Pupae raised on gel-based diet from laboratory colony were placed in sealed (hypoxia) and unsealed bags and stored at five different temperatures for a period of 30 and 60 hours. High temperatures and long exposure duration caused detrimental effects on the emergence and flight ability of the pupae in sealed bags. Those stored at high temperature under hypoxic condition for long duration had higher number of partially emerged, deformed and non-emerged flies and had a smaller number of fliers. Though emergence of the pupae in the sealed bags was less affected at lower temperature regime, number of fliers was significantly reduced when the duration of hypoxia was increased. Furthermore, we measured the time needed for flies to enter hypoxic conditions, and this happens rapidly within 20 mins approximately.

Outcome 3: Determine effect of fluctuating thermal regime and hypoxia on sterile flies

Benelli M[†], Yu H, Taylor P, Rempoulakis P[‡] & Mainali B[‡] (manuscript) Effect of fluctuating thermal regime and hypoxia on quality control parameters of Queensland fruit fly

We assessed effect of intermittent or continuous exposure of Queensland fruit fly pupae to 10°C in combination with other stressors such as hypoxia and irradiation on their fitness. We found no effect of either intermittent or continuous exposure of pupae to 10 °C up to 30 h on the tested QC parameters such as pupal weight, emergence rate, deformed and or partial emergence, flight ability and male proportion. As storing the pupae at lower

temperature has been proven beneficial in terms of fly quality, this information is vital to maintain quality of flies during transportation from the factory to the rear-out centers. Data loggers to monitor temperature and RH have been incorporated with the Qfly shipments that are being sent to Yanco and Tatura. Also, accelerometers are placed with the shipments to monitor vibrations during the transportation of pupae to the rear-outs. This approach will provide essential guidance for delivery to rear-out centres.

Outcome 4: Effect of hypoxia, irradiation and vibration on Qfly established

Benelli M[†], Mainali B[‡], Taylor PW & Rempoulakis P[‡] (2021) Reduced quality of sterile Queensland fruit fly following post-production stress from hypoxia, irradiation and vibration. *Journal of Pest Science* 94: 473–485.

Hypoxia, irradiation and vibration are important stressors that are experienced by pupae during production, packing and transportation. However, little is known about the impacts of such stressors on Queensland fruit fly (Q-fly quality. Such stressors can significantly reduce the number and quality of the released insects, with serious consequences for the ultimate performance of SIT programs. Two laboratory experiments were conducted to investigate the response of Qfly to such post-production stressors, with the aim of then developing guidelines that minimize quality reductions for SIT programs. Experiment 1 evaluated survival and flight ability after 3-, 6- and 9-day-old Q-fly pupae experienced vibration of 5, 30, 60 and 300 s duration (exposure time). These ages are particularly relevant to processes in the rearing and irradiation facility, as pupae may be extracted from the pupation media early in development at 3 or 6 days and packed for irradiation at 9 days. Similarly, the selected exposure times to vibration represent a range that pupae may encounter during the rearing process. Experiment 2 evaluated the effect of hypoxia, irradiation and vibration, in isolation or in combination, on 9-day-old Q-fly pupae. This age is most relevant to transportation as, after packing and irradiation, the pupae are transported under hypoxia over distances of many hundreds of kilometers by road, during which they can be exposed to vibration from the vehicle or from handling when transferred between vehicles. Based on our results, we offer recommendations regarding pupal handling to minimize reductions in the quality of flies released in Q-fly SIT. Knowledge of the factors that play a role in susceptibility to stress is needed to establish guidelines for minimizing the consequences for fly quality. From a practical point of view, the relationship between pupal age and tolerance to vibrations in Q-flies suggests that handling of pupae should be minimized after 6 days from pupation. Exposure time to vibrations should also be minimized. Considering that transport of pupae to rear-out centers occurs at the pupal age that is the most sensitive to this type of stress (close to adult emergence) and that pupae are irradiated beforehand; effective procedures are needed during transportation. This includes the use of appropriate packaging to reduce the magnitude of vibrations experienced by pupae during transport. Irradiation and vibration exhibited a multiplicative negative effect on flight ability parameters. For the purpose of minimizing quality reductions throughout SIT operations, it is not recommended to subject pupae to intense and extensive vibrations, especially during late pupal ages. Logistics for long-distance shipment of pupae, from factory to rear out facilities, should also be carefully evaluated to avoid unnecessary prolonged periods of hypoxia. The findings of the present study are also relevant to development of procedures for other fruit flies, and for other insects, in which handling and transportation are required for SIT.

Project 4: Logistics of production and delivery

Summary

Logistics planning of SIT releases involves strategic and operational decision-making. This project investigates cost-effective options for delivering sterile Qfly pupae from the SITplus production facility in Port Augusta to rear-out facilities for subsequent release in target locations. Conceptually, SITplus delivery logistics are affected by three interrelated factors: (i) the required release volumes across target locations, (ii) the cost and logistics of shipping these volumes to rear-out centres, and subsequently to target locations, and (iii) the losses in fly quality due to shipping.

Release volumes. Operational decisions on priority target locations for SIT releases, the volume of released flies (overflooding ration), and the frequency of releases are influenced by a host of factors, including biological (e.g. suitable climatic conditions, wild population size, effective overflooding ratios and release frequencies) and regulatory/socio-political (permissible locations of release, fruit fly management (SIT and other) in the broader

landscape). However, in the initial phase of the SITplus program, release priorities and volumes are likely pre-determined based solely on socio-political and pragmatic considerations (funding, political and community/landholder support), as biological information on optimal Qfly releases is currently limited. In addition, release priorities may rapidly shift in response to new or emerging incursions. This project will develop tools and methodologies to inform decisions about release prioritization and fly allocation based on fixed assumptions about release parameters. As more biological information becomes available during the piloting phase of the SITplus program, it may be integrated into these methodologies.

Shipping costs and logistics. Tools and methodologies for supporting decisions on the shipping of fruit fly pupae from the production facility to all available or planned (stationary or mobile) rear-out centres will be developed. These will initially focus on the timing and costs of delivery, as specific trade-offs between shipping options and conditions and losses in fly quality are not yet known (see below). Delivery logistics are constrained by a range of factors related to pupae production (production volume, irradiation and dispatch schedule), shipping (type of transport (road, air), reliability of transport, distance, accessibility, cost, budget), adult rear-out (location, capacity, rear-out schedule) and release (target location, release volume/frequency).

Shipping effects on fly quality. Little is known about the effects of shipping (e.g. dependent variables such as time in hypoxia, temperature, vibration) on the quality of sterile fruit fly pupae, and ultimately the volume of viable adult fliers available for release in target locations. As improved information on this important decision factor will become available as part of Theme 2 Project 3 above, it may be integrated into delivery logistics planning.

Conclusions & Recommendations

- A model for logistics planning is developed
- Continually update data that can be integrated with the available logistics model to improve value
- Periodically review available services and costs in order to adopt faster, cheaper and more quality-preserving options when they become available

Achievements

Outcome 1: Develop a decision making tool to support transportation decisions

The objectives of this project involved providing operational guidance for the SITplus program while at the same time developing insights and tools that are applicable to changing circumstances and priorities. Hence, a tiered approach was adopted: (a) a generic conceptual model of factors affecting shipping cost effectiveness was developed, (b) the model's elements were incorporated into operational decision-support tools, and (c) these tools were populated with data (where available) or assumptions for specific release scenarios and other supply chain variables as specified by SITplus management.

Five release scenarios for the 2018/19 fruit fly season were investigated in this project. These included planned releases for the purpose of (1) pest suppression in residential areas in Cobram (Victoria, with adult rear-out in Tatura), pest suppression in (2) residential and (3) horticultural areas in Hillston (New South Wales, with adult rear-out in Yanco) and (4) pest prevention in the fruit fly free Riverland (South Australia, with adult rear-out in Netley). A scenario for (5) unplanned releases in the event of pest outbreaks in Adelaide (with adult rear-out in Netley) and northern Tasmania (with adult rear-out on Flinders Island or in Devonport) was also considered.

Decision-support tools included a spreadsheet tool for informing decisions about release prioritisation and fly allocation across multiple target locations and a spreadsheet tool for planning and coordinating weekly delivery of pupae to rear-out centres. While assumptions were often uncertain and specific to release scenarios, our tools are sufficiently flexible to allow for integration of new information and different assumptions.

Analyses of tool outputs indicated that, for the 2018/19 fruit fly season, expected production targets at the National Sterile Insect Facility were sufficient to meet the requirements of all planned releases, while large or recurring unplanned outbreaks were likely to result in significant supply gaps to meet the additional demand. For each rear-out centre, there were only few practical shipping options. Delivery times were generally shorter when

shipments are picked up at 5:00PM and shipped overnight and faster delivery often traded off with higher costs. Minimum shipping costs (for 7 million pupae) ranged from $500 to $2000 depending on rear-out location.

This research highlighted the need for better data on the effects of shipping and other supply chain variables on Qfly quality, which ultimately determines the volume of sterile pupae that need to be mass-produced to achieve effective population control in the field. As a first step in this endeavour, a draft evaluation dashboard was developed that provided, for the first time, an integrated and interactive visualisation of SITplus mass-production and fly quality trends.

Project 5: Microbial symbionts

Summary

Microbial symbionts are important insect allies, contributing to nutrition, health and reproductive success. There has been a long, but haphazard, history of research into Qfly symbionts and it is generally accepted that symbiotic bacteria are an important cornerstone of their biology. MT13040 and MT13045 both compared and developed new larval diets for Qfly and each of these projects included some exploration of the potential importance of microbial symbionts as components of nutrition, as aids in digestion, and as pathogens. However, the main focus of these projects was testing new bulking agents (MT13040) and developing new diet formulations (MT13045) such that each of these projects was very limited in its ability to advance understanding of the Qfly microbiome. Much more work was needed in this area. Internationally, there is a very significant effort underway to develop probiotics for fruit fly larval diets. To reach and maintain technological parity with SIT programs for other fruit fly species overseas there has been a need for much more focused research that specifically targets the potential value of gut symbionts as probiotics that can improve larval development and adult performance. A molecular approach to assess the gut microflora at the different life stages of both the domesticated and the wild fly has been required. This enables accurate determination of the microbial symbionts that are missing from the gut microflora of mass produced flies, identification of gut microorganisms which are vertically transmitted (via eggs or larvae), enabling an understanding about how the mass-rearing process affects the fruit fly gut microflora at each life stage. These results allow for a targeted selection of prospective probiotic candidates.

This project commenced with describing the composition and variety of the bacterial (Outcome 1) and fungal (Outcome 2) microbiome associated with larval Qfly in diverse fruits in nature. While it is valuable to have a baseline in the field, for SIT Qflies must be domesticated and mass-reared, and the consequences this for the microbiome are explored (Outcome 3). Through the domestication process the larval diet may affect which elements of the natural microbiome persist and which are lost such that colonies domesticated using different diets may have quite distinct microbiome profile (Outcome 4). Each developmental stage - larvae, pupae, and adults – has a distinctive microbiome profile, indicating that the functional significance of each element of the microbiome may change through development (Outcome 5). Parental microbiome is found to be a strong driver of offspring health through transgenerational effects (Outcome 6), and so preservation of a healthy microbiome in mass rearing facilities is important to maintain health and productivity of rearing colonies as well as production standards for release. Given the importance of the microbiome for nutrition and general health, it may be expected that the microbiome of flies in the field is particularly well suited to the local conditions. However, after release in the field where they are exposed to diverse bacteria and fungi, the sterile flies do not acquire a wild-type microbiome but instead maintain a characteristic microbiome profile (Outcome 7). Pre-release probiotics of wild-type microbiota have sometimes been suggested as a means of preparing sterile flies for life after release but the findings of this project indicate that such probiotics would not persist. Nutrition is important for development and health in mass rearing facilities, and so to generate background information required to understand effects of the microbiome and pathogens, this project conducted as a series of studies of nutritional balance in Qfly including effects of diet heterogeneity on larval aggregation and net nutrition (Outcome 8, 9), the consequences of increased larval density (e.g., to upscale production beyond normal operating capacity) for adult traits (Outcome 10), and the viability of alternative sources of carbohydrates (Outcome 11). The impact of larval density and nutrition are then investigated together with microbial management (Outcome 12). The microbiome is demonstrated to be very important for larval development, highlighting the need to ensure proper management of the microbial environment from early stages of development (Outcome 13). The microbiome is also found to be very important in adults as a mediator of nutritional state (Outcome 14).

While some elements of the microbiome are beneficial or neutral with respect to fly health, others are pathogenic and this project also investigates the Qfly immune capability. Larval diet is found to have a substantial impact on immune competence of adults (Outcome 15), and there is hence scope to improve the ability of released sterile flies to stave off infection through choice of larval diet composition. However, the flies are not without active means of addressing immune challenges, exhibiting nutritional self-medication by increasing macronutrient intake (Outcome 16). Increasing complexity, nutrition can even have transgenerational effects on immunity (Outcome 17). Within generations, the possibility of immune priming, challenging insects with particular pathogens before release so that they might be protected from those pathogens after release, could increase survivorship of released sterile insects. However, this project found no evidence of functional immune priming that could be used in this way (Outcome 18).

Conclusions & Recommendations

- Qfly have a rich bacterial and fungal microbiome, which varies with host or larval diet
- Elements of bacterial microbiome are passed from mother to offspring
- The microbiome changes very substantially during domestication and through development
- The microbiome is important for development and for offspring performance
- Production processes should facilitate the exposure of early larval stages to appropriate microbiota
- The microbiome is important for adult nutrition
- The microbiome remains stable after release; pre-release probiotics hold little promise of improving fly quality
- Some heterogeneity in larval diets is acceptable, as the larvae are very capable of selective foraging and of tolerating variable diet
- Larval crowding has some negative impacts on adult quality; care must be taken when considering expansion of production above the usual levels
- Immune function is linked to microbiome and diet
- Immune priming is not supported as a means of enhancing post-release protection from pathogens

Achievements

Outcome 1: Identify the bacterial microbiome

Majumder R[†], Sutcliffe B, Taylor PW & Chapman TA (2019) Next-Generation Sequencing reveals relationship between the larval microbiome and food substrate in the polyphagous Queensland fruit fly. *Scientific Reports* 9: 14292

Insects typically host substantial microbial communities (the 'microbiome') that can serve as a vital source of nutrients and also acts as a modulator of immune function. While recent studies have shown that diet is an important influence on the gut microbiome, very little is known about the dynamics underpinning microbial acquisition from natural food sources. Here, we addressed this gap by comparing the microbiome of larvae of the polyphagous fruit fly *Bactrocera tryoni* ('Queensland fruit fly') that were collected from five different fruit types (sapodilla *Manilkara zapota* [from two different localities], hog plum *Spondias mombin*, pomegranate *Punica granatum*, green apple *Malus pumila*, and quince *Cydonia oblonga*) from North-east to South-east Australia. Using Next-Generation Sequencing on the Illumina MiSeq platform, we addressed two questions: (1) what bacterial communities are available to *B. tryoni* larvae from different host fruit; and (2) how does the microbiome vary between *B. tryoni* larvae and its host fruit? the abundant bacterial taxa were similar for *B. tryoni* larvae from different fruit despite significant differences in the overall microbial community compositions. Our study suggests that the bacterial community structure *of B. tryoni* larvae is related less to the host fruit (diet) microbiome and more to vertical transfer of the microbiome during egg laying. Our findings also suggest that geographic location may play a quite limited role in structuring of larval microbiomes. This is the first study to use Next-Generation Sequencing to analyze the microbiome of *B. tryoni* larvae together with the host fruit, an approach that has enabled greatly increased resolution of relationships between the insect's microbiome and that of the surrounding host tissues.

Outcome 2: Identify the fungal microbiome

Majumder R[†], Sutcliffe B, Midgley DJ, Taylor PW & Chapman TA (2020) Fruit host-dependent fungal communities in the microbiome of wild Queensland fruit fly larvae. *Scientific Reports* 10: 16550

Bactrocera tryoni (Froggatt), the Queensland fruit fly (Qfly), is a highly polyphagous tephritid fly that is widespread in Eastern Australia. Qfly physiology is closely linked with its fungal associates, with particular relationship between Qfly nutrition and yeast or yeast-like fungi. Despite animal-associated fungi typically occurring in multi-species communities, Qfly studies have predominately involved the culture and characterisation of single fungal isolates. Further, only two studies have investigated the fungal communities associated with Qfly, and both have used culture-dependent techniques that overlook non-culturable fungi and hence under-represent, and provide a biased interpretation of, the overall fungal community. In order to explore a potentially hidden fungal diversity and complexity within the Qfly mycobiome, we used culture-independent, high-throughput Illumina sequencing techniques to comprehensively, and holistically characterized the fungal community of Qfly larvae and overcome the culture bias. We collected larvae from a range of fruit hosts along the east coast of Australia, and all had a mycobiome dominated by ascomycetes. The most abundant fungal taxa belonged to the genera *Pichia* (43%), *Candida* (20%), *Hanseniaspora* (10%), *Zygosaccharomyces* (11%) and *Penicillium* (7%). We also characterized the fungal communities of fruit hosts, and found a strong degree of overlap between larvae and fruit host communities, suggesting that these communities are intimately inter-connected. Our data suggests that larval fungal communities are acquired from surrounding fruit flesh. It is likely that the physiological benefits of Qfly exposure to fungal communities is primarily due to consumption of these fungi, not through syntrophy/symbiosis between fungi and insect 'host'.

Outcome 3: Establish effects of domestication on the microbiome

Majumder R[†], Taylor PW & Chapman TA (manuscript) Dynamics of the Queensland Fruit Fly microbiome under changes in host environment

Domestication in an artificial rearing facility can have a significant evolutionary impact on life history and biology of the insect. The Queensland fruit fly (aka 'Qfly'), *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), is a threatening economic pest and one of the major challenges for horticulture industries in Australia. The sterile insect technique (SIT) is being developed to manage outbreaks in regions that remain free of Qfly and to reduce populations in regions where this species is endemic. The biology of the Qfly is intimately connected to its microbiome. While there are numerous studies of the microbiome in Qfly larvae and adults, the transition of the microbiome through domestication process across different generations remains unknown. In this study, high-throughput Illumina sequencing was used to assess the Qfly microbiome in colonies reared for five generations (generation 1 to generation 5) from nature on recently developed artificial gel-based diet. Beta diversity analysis showed that the bacterial communities from generation 5 (G5) were found separately clustered from other generations. At the genus level, bacterial communities were significantly different between the generations and mostly altered at G5. However, communities converged at Phyla to family taxonomic levels. We observed high abundance of the *Morganella* and *Burkholderia* at the genus level in the larval and pupal stages respectively at G5 but completely absent in other generations. Overall, our findings demonstrate that the domestication process strongly influences the microbiome of the Qfly. In addition to elucidating changes in the microbiome between generations, this study characterizes the Qfly microbiome present at the establishment of the domesticated colonies prior to SIT.

Outcome 4: Establish effects of artificial diet on the microbiome

Majumder R[†], Sutcliffe B, Adnan SM[†], Mainali B[‡], Dominiak BC, Taylor PW & Chapman TA (2020) Artificial larval diet mediates the microbiome of Queensland fruit fly. *Frontiers in Microbiology* 11: 576156

Larval diets used for artificial rearing can have a significant effect on insect biology. The Queensland fruit fly (aka "Qfly"), *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), is one of the greatest challenges for fruit growers in Australia. The sterile insect technique (SIT) is being developed to manage outbreaks in regions that remain free of Qfly and to reduce populations in regions where this species is endemic. Factory scale rearing is essential for SIT;

however, artificial larval diets are known to affect the microbiome of Qfly, which may then affect fly performance. In this study, high-throughput Illumina sequencing was used to assess the Qfly microbiome in colonies reared, for five generations from nature, on two common artificial diets (carrot and gel). At generation five (G5), the microbiome was assessed in larvae, pupae, adult males and adult females and standard fly quality control parameters were assessed together with additional performance measures of mating propensity and survival under nutritional stress. At the genus level, bacterial communities were significantly different between the colonies reared on the two larval diets. However, communities converged at Phyla to family taxonomic levels. Bacterial genera of *Morganella, Citrobacter, Providencia,* and *Burkholderia* were highly abundant in all developmental stages of Qfly reared on the gel diet, when compared to the carrot diet. Despite abundance of these genera, a greater percentage of egg hatching, heavier pupal weight and a higher percentage of fliers were found in the Qfly reared on the gel diet. Mating propensity and survival under nutritional stress was similar for adult Qfly that had been reared on the two larval diets. Overall, our findings demonstrate that the artificial larval diet strongly influences the microbiome and quality control measures of Qfly, with likely downstream effects on performance of flies released in SIT programs.

Outcome 5: Identify changes in the microbiome through development

Majumder R[†], Sutcliffe B, Chapman TA & Taylor PW (2020) Microbiome of the Queensland fruit fly through metamorphosis. *Microorganisms* 8: 795.

Bactrocera tryoni (Froggatt) (Queensland fruit fly, or "Qfly") is a highly polyphagous tephritid fruit fly and a serious economic pest in Australia. Qfly biology is intimately linked to the bacteria and fungi of its microbiome. While there are numerous studies of the microbiome in larvae and adults, the transition of the microbiome through the pupal stage remains unknown. To address this knowledge gap, we used high-throughput Next-Generation Sequencing (NGS) to examine microbial communities at each developmental stage in the Qfly life cycle, targeting the bacterial 16S rRNA and fungal ITS regions. We found that microbial communities were similar at the larval and pupal stage and were also similar between adult males and females, yet there were marked differences between the larval and adult stages. Specific bacterial and fungal taxa are present in the larvae and adults (fed hydrolyzed yeast with sugar) which is likely related to differences in nutritional biology of these life stages. We observed a significant abundance of the Acetobacteraceae at the family level, both in the larval and pupal stages. Conversely, Enterobacteriaceae was highly abundant (> 80%) only in the adults. The majority of fungal taxa present in Qfly were yeasts or yeast- like fungi. In addition to elucidating changes in the microbiome through developmental stages, this study characterizes the Qfly microbiome present at the establishment of laboratory colonies as they enter the domestication process.

Outcome 6: Identify effects of parental microbiome on offspring performance

Nguyen B[†], Than A[†], Dinh H[†], Morimoto J[‡] & Ponton F[‡] (2020) Parental microbiota modulates offspring development, body mass and fecundity in a polyphagous fruit fly. *Microorganisms* 8 (9): 1289

The commensal microbiota is a key modulator of animal fitness, but little is known about the extent to which the parental microbiota influences fitness-related traits of future generations. We addressed this gap by manipulating the parental microbiota of a polyphagous fruit fly (*Bactrocera tryoni*) and measuring offspring developmental traits, body composition, and fecundity. We generated three parental microbiota treatments where parents had a microbiota that was non-manipulated (control), removed (axenic), or removed-and-reintroduced (reinoculation). We found that the percentage of egg hatching, of pupal production, and body weight of larvae and adult females were lower in offspring of axenic parents compared to that of non-axenic parents. The percentage of partially emerged adults was higher, and fecundity of adult females was lower in offspring of axenic parents relative to offspring of control and reinoculated parents. There was no significant effect of parental microbiota manipulation on offspring developmental time or lipid reserve. Our results reveal transgenerational effects of the parental commensal microbiota on different aspects of offspring life-history traits, thereby providing a better understanding of the long-lasting effects of host–microbe interactions.

Outcome 7: Assess stability of sterile Qfly microbiome after release into the field

Horlick J[‡], Morimoto J[‡], Majumder R[†], Biswas JMH[†], Mainali B[‡], Taylor PW, Ponton F[‡], Rempoulakis P[‡] & Chapman T (manuscript) Sterile males released as part of sterile insect technique do not acquire wild microbiome**.**

Microbiome manipulations can improve sexual competitiveness of sterilised males used in the biological control of insect pests. Theory predicts, and recent empirical suggests that microbiome manipulations are unlikely to be long-lasting because released sterile males will interact with and acquire microbiome profile from their environment, thereby eliminating any potential benefits of laboratory manipulations. As a result, released sterile males' microbiome should resemble the microbiome of wild males over time. Here, we used Next-Generation-Sequencing and large open-field trials in a natural population of the fruit fly *Bactrocera tryoni* ('Queensland fruit fly') to address this gap. Our results show that even though microbiome diversity is similar between sterile and wild males, sterile males have distinct microbiome composition relative to wild males which is constant over time. This is the first empirical evidence that released sterile males do not to acquire wild microbiome. Our findings have broad significance to applications of microbiome manipulations to improve sustainable control of biological pests.

Outcome 8: Characterize functional impacts of aggregation for larval nutrition

Morimoto J[‡], Nguyen B[†], Tabrizi ST[‡], Ponton F[‡] & Taylor PW (2018) Social and nutritional factors shape larval aggregation, foraging, and body mass in a polyphagous fly. *Scientific Reports* 8:14750

The majority of insect species have a clearly defined larval stage during development. Larval nutrition is crucial for individuals' growth and development, and larval foraging success often depends on both resource availability and competition for those resources. To date, however, little is known about how these factors interact to shape larval development and behaviour. Here we manipulated the density of larvae of the polyphagous fruit fly pest *Bactrocera tryoni* ('Queensland fruit fly'), and the diet concentration of patches in a foraging arena to address this gap. Using advanced statistical methods of machine learning and linear regression models, we showed that high larval density results in overall high larval aggregation across all diets except in extreme diet dilutions. Larval aggregation was positively associated with larval body mass across all diet concentrations except in extreme diet dilutions where this relationship was reversed. Over time, larvae in low-density arenas also tended to aggregate while those in high-density arenas tended to disperse, an effect that was observed for all diet concentrations. Furthermore, larvae in high-density arenas displayed significant avoidance of low concentration diets – a behaviour that was not observed amongst larvae in low-density arenas. Thus, aggregation can help, rather than hinder, larval growth in high-density environments, and larvae may be better able to explore available nutrition when at high-density than when at low-density.

Outcome 9: Assess ability of larvae to tolerate variable nutritional environments

Morimoto J[‡], Tabrizi ST[‡], Lundbäck I, Mainali B[‡], Taylor PW & Ponton F[‡] (2019) Larval foraging decisions in competitive heterogeneous environments accommodate diets that support egg-to-adult development in a polyphagous fly. *Royal Society Open Source* 6: 190090

In holometabolous insects, larval nutrition is a key factor underpinning development and fitness. Heterogeneity in the nutritional environment and larval competition can force larvae to forage in suboptimal diets, with potential downstream fitness effects. Little is known about how larvae respond to competitive heterogeneous environments, and whether variation in these responses affects current and next generations. Here, we designed nutritionally heterogeneous foraging arenas by modifying nutrient concentration, where groups of the polyphagous fruit fly *Bactrocera tryoni* could forage freely at various levels of larval competition. Larval foraging preferences were highly consistent and independent of larval competition, with greatest foraging propensity for high (100%) followed by intermediate (80% and 60%) nutrient concentration diets, and avoidance of lower concentration diets (less than 60%). We then used these larval preferences (i.e., 100%, 80% and 60% diets) in fitness assays in which larvae competition was maintained constant, and showed that nutrient concentrations selected by the larvae in the foraging trials had no effect on fitness-related traits such as egg hatching and pupation success, adult flight ability, sex ratio, percentage of emergence, nor on adult cold tolerance, fecundity and next-generation pupal weight. These results support the idea that polyphagous species can exploit diverse

hosts and nutritional conditions with minimal fitness costs to thrive in new environments.

Outcome 10: Characterize functional impacts of larval aggregation for adult performance

Morimoto J[‡], Nguyen B[†], Dinh H[†], Than AT[†], Taylor PW & Ponton F[‡] (2019) Crowded developmental environment promotes adult sex-specific nutrient consumption in a polyphagous fly. *Frontiers in Zoology* 16: 4

The fitness of holometabolous insects depends largely on resources acquired at the larval stage. Larval density is an important factor modulating larval resource-acquisition, influencing adult survival, reproduction, and population maintenance. To date, however, our understanding of how larval crowding affects adult physiology and behaviour is limited, and little is known about how larval crowding affects adult non-reproductive ecological traits. Here, larval density in the rearing environment of the polyphagous fruit fly *Bactrocera tryoni* ('Queensland fruit-fly') was manipulated to generate crowded and uncrowded larval treatments. The effects of larval crowding on pupal weight, adult emergence, adult body weight, energetic reserves, fecundity, feeding patterns, flight ability, as well as adult predation risk were investigated. Adults from the crowded larval treatment had lower adult emergence, body weight, energetic reserves, flight ability and fecundity compared to adults from the uncrowded larval treatment. Adults from the crowded larval treatment had greater total food consumption (i.e., consumption of yeast plus sucrose) relative to body weight for both sexes compared to adults from the uncrowded treatment. Furthermore, males from the crowded treatment consumed more yeast relative to their body weight than males from the uncrowded treatment, while females from the crowded treatment consumed more sucrose relative to their body weight than females from the uncrowded treatment. Importantly, an interaction between the relative consumptions of sucrose and yeast and sex revealed that the density of conspecifics in the developmental environment differentially affects feeding of adult males and females. We found no effect of larval treatment on adult predation probability. However, males were significantly more likely to be captured by ants than females. We show that larvae crowding can have important implications to ecological traits in a polyphagous fly, including traits such as adult energetic reserve, flight ability, and adult sex-specific nutrient intake. Our findings contextualise the effects of larval developmental conditions into a broad ecological framework, hence providing a better understanding of their significance to adult behaviour and fitness. Furthermore, the knowledge presented here can help us better understanding downstream density-dependent effects of mass rearing conditions of this species, with potential relevance to Sterile Insect Technique.

Outcome 11: Assess impacts of larval carbohydrates on development and quality

Morimoto J[‡], Nguyen B[†], Lundbäck I, Tabrizi ST[‡], Ponton F[‡] & Taylor PW (2020) Effects of carbohydrate types on larval development and adult traits in a polyphagous fruit fly. *Journal of Insect Physiology* 120: 103969

Nutrition is a major mediator of insect life-history trait expression. While the role of macronutrient (carbohydrate and protein) balance on trait expression has received substantial attention, the implications of different classes of specific macronutrients remains virtually unexplored. Here, we addressed this gap by varying the type of carbohydrate in larval diets of the polyphagous fruit fly *Bactrocera tryoni* (aka 'Queensland fruit fly'). Sourcing insects from a colony maintained using larval diets that contain sucrose, we assessed the effects of sucrose, maltose, and lactose on larval development and adult traits. Replacement of sucrose with lactose resulted in slow larval growth, as well as decreases in pupation, adult emergence and adult body weight for both sexes, although adult lipid reserves were unaffected. Sucrose and maltose were equivalent in terms of larval growth, pupation, adult emergence and adult weight of both sexes. Surprisingly, adults from larvae reared on diets containing maltose had lower lipid reserves than adults from larvae reared on diets containing either lactose or sucrose. The sex ratio of adults at emergence from larvae reared on diets containing lactose and maltose was balanced, but was female-biased in adults from larvae reared on diets containing sucrose. Our results show that carbohydrate sources are not equivalent for development of the Queensland fruit fly, affecting both larval development and adult traits. These findings have implications for understanding the ecology of this highly polyphagous species which infests fruits with highly diverse carbohydrate contents, as well as for the rearing and management of this pest species.

Outcome 12: Establish impacts of larval rearing environment on adult fitness

Nguyen B[†], Ponton F[‡], Than A[†], Taylor PW, Chapman T & Morimoto J[‡] (2019) Interactions between ecological factors in the developmental environment modulate pupal and adult traits in a polyphagous fly. *Ecology & Evolution* 9: 6342-6352

In holometabolous insects, adult fitness depends on the quantity and quality of resource acquired at the larval stage. Diverse ecological factors can influence larval resource acquisition, but little is known about how these factors in the larval environment interact to modulate larval development and adult traits. Here, we addressed this gap by considering how key ecological factors of larval density, diet nutritional composition, and microbial growth interact to modulate pupal and adult traits in a polyphagous tephritid fruit fly, *Bactrocera tryoni* (aka "Queensland fruit fly"). Larvae were allowed to develop at two larval densities (low and high), on diets that were protein-rich, standard, or sugar-rich and prepared with or without preservatives to inhibit or encourage microbial growth, respectively. Percentage of adult emergence and adult sex ratio were not affected by the interaction between diet composition, larval density, and preservative treatments, although low preservative content increased adult emergence in sugar-rich diets but decreased adult emergence in protein-rich and standard diets. Pupal weight, male and female adult dry weight, and female (but not male) body energetic reserves were affected by a strong three-way interaction between diet composition, larval density, and preservative treatment, whereby in general, low preservative content increased pupal weight and female lipid storage in sugar-rich diets particularly at low-larval density and differentially modulated the decrease in adult body weight caused by larval density across diets. Our findings provide insights into the ecological factors modulating larval development of a polyphagous fly species and shed light into the ecological complexity of the larval developmental environment in frugivorous insects.

Outcome 13: Assess the role of microbiome in larval performance

Morimoto J[‡], Nguyen B[†], Tabrizi ST[‡], Lundbäck I, Taylor PW, Ponton F[‡] & Chapman TA (2019) Commensal microbiota modulates larval foraging behaviour, development rate and pupal production in *Bactrocera tryoni*. *BMC Microbiology* 19: 286

Commensal microbes can promote survival and growth of developing insects, and have important fitness implications in adulthood. Insect larvae can acquire commensal microbes through two main routes: by vertical acquisition from maternal deposition of microbes on the eggshells and by horizontal acquisition from the environment where the larvae develop. To date, however, little is known about how microbes acquired through these different routes interact to shape insect development. In the present study, we investigated how vertically and horizontally acquired microbiota influence larval foraging behaviour, development time to pupation and pupal production in the Queensland fruit fly ('Qfly'), *Bactrocera tryoni*. Both vertically and horizontally acquired microbiota were required to maximise pupal production in Qfly. Moreover, larvae exposed to both vertically and horizontally acquired microbiota pupated sooner than those exposed to no microbiota, or only to horizontally acquired microbiota. Larval foraging behaviour was also influenced by both vertically and horizontally acquired microbiota. Larvae from treatments exposed to neither vertically nor horizontally acquired microbiota spent more time overall on foraging patches than did larvae of other treatments, and most notably had greater preference for diets with extreme protein or sugar compositions. The integrity of the microbiota early in life is important for larval foraging behaviour, development time to pupation, and pupal production in Qflies. These findings highlight the complexity of microbial relations in this species, and provide insights to the importance of exposure to microbial communities during laboratory- or mass-rearing of tephritid fruit flies.

Outcome 14: Assess impacts of microbiome on adult nutrition

Nguyen B[†], Dinh H[†], Morimoto J[‡] & Ponton F[‡] (manuscript) Sex-specific effects of the microbiota on adult carbohydrate intake and body composition in a polyphagous fly.

The microbiota influences hosts' health and fitness. The extent to which the microbiota affects host' foraging decisions and life history traits in both sexes similarly remains however to be fully understood. Our study explored the effects of manipulating the microbiota on feeding performance and phenotypic traits at larval and adult stages

of the polyphagous fruit fly *Bactrocera tryoni*. We generated three treatments: control (non-treated microbiota), axenic (removed microbiota), and reinoculated (axenic individuals which had their microbiota re-introduced). We found that axenic larvae and immature (i.e., newly emerged) adults were lighter compared to control and reinoculated individuals. Additionally, we found a sex-specific effect of the microbiota manipulation on carbohydrate intake and body composition of mature adults (10 days old). Axenic males ingested less carbohydrate, had lower body weight and total body fat relative to control and reinoculated ones. Carbohydrate intake was however overall higher in axenic females than in control and reinoculated ones but body weight and lipid reserve were similar across treatments. Axenic females produced fewer eggs than control and reinoculated females. Our findings corroborate the cumulative body of evidence on the far- reaching effects of microbiota in insects and show for the first time a sex-specific effect of microbiota on feeding behaviour in Tephritidae. Our results also underline the dynamic relationship between the microbiota and the host as reinoculating microbes restores some traits that were affected in axenic individuals.

Outcome 16: Assess impacts of nutrition on immune function

Dinh H[†], Lundbäck I, Kumar S[‡], Than AT[†], Morimoto J[‡] & Ponton F[‡] (manuscript) Sex-specific effects of protein-rich diet promotes higher bacterial load and lower survival after infection in a polyphagous fly.

Nutrition is a central factor influencing immunity and resistance to infection, but the extent to which nutrition during development affects adult responses to infections is poorly documented. Our study investigated the effects of larval diet on the survival, pathogen load, and food intake of adult fruit flies after bacterial septic infection. Effects on development and adult physiological traits were also measured. We found a strong sex-specific effect of larval diet on survival post-infection; with a greater survival rate and lower bacterial load for infected females- but not infected males- fed the sugar-biased larval diet compared with females fed protein-biased larval diet. This might be linked to the different macronutrient intakes that were observed between infected males and females when given a choice between diets. While macronutrient intake was comparable between larval diets for infected males, infected females reared on the sugar-biased larval diet ingested less food than those on the protein-biased larval diet. Larval diet also affected developmental time, adult body weight and body reserves. Our results highlight the importance of the interaction between developmental diet and sex on infection outcomes in adulthood.

Outcome 16: Identify links between nutrition and immunity from infection

Dinh H[†], Mendez V[‡], Tabrizi ST[‡] & Ponton F[‡] (2019) Macronutrients and infection in fruit flies. *Insect Biochemistry and Molecular Biology* 110: 98-104

Nutrition and infection are closely linked. While it is now well established that hosts can modulate their nutrition after being infected, the extent to which this change in foraging provides the host with a greater fitness remains to be fully understood. Our study explored the relationships between dietary choice, macronutrients intake [i.e., protein (P) and carbohydrate (C)], infection, survival rate and growth of pathogenic bacterial population in the true fruit fly *Bactrocera tryoni*. Results showed that flies injected with the bacterium *Serratia marcescens* decreased their macronutrient intake and shifted their diet choice to carbohydrate-biased diet compared to naïve individuals. Interestingly, flies injected with either PBS (i.e., sham-infected) or heat-killed bacteria also reduced food intake and modulated diet choice but only for a day after injection. When infected flies were restricted to the diet they selected (i.e., PC 1:8), they survived better the infection than those restricted to a protein-biased diet (i.e., PC 1:5). In addition, we did not observe any growth of pathogen load in infected flies fed PC 1:8 for the first 3 days post-infection. Finally, a decrease in lipid body reserves was found in flies injected with live bacteria and, interestingly, this loss of body lipid also occurred in flies injected with heat-killed bacteria, but in a diet- dependent manner. Our results indicated that *B. tryoni* flies modulated their macronutrient intake and decreased the negative effects of the infection on their survival ("nutritional self-medication") the first days following the infection.

Outcome 17: Assess impacts of parental nutrition on immune function

Dinh H[†], Nguyen B[†], Morimoto J[‡], Lundbäck I, Kumar S[‡] & Ponton F[‡] (manuscript) Transgenerational effects of macronutrient balance on offspring pathogen resistance and life history traits in fruit fly.

Environmental conditions experienced by parents can influence next generations, with parental nutritional conditions playing an important role in shaping offspring phenotypes. However, our understanding of transgenerational effects of parental diet on the pathogen resistance of offspring is poorly documented. In this study, we addressed this knowledge gap by manipulating the diet quality of parents (i.e., mother, father, or both) and measuring transgenerational effects on offspring development and survival after pathogen immune challenge. Our results showed that maternal, but not paternal, diet had a sex-specific effect on offspring resistance, with sons (but not daughters) from mothers fed either carbohydrate- or protein-biased diets being less resistant to infection than sons from mothers fed balanced diets. Interestingly, this effect was reversed when the diet of both parents was manipulated, whereby sons from parents fed either carbohydrate- or protein-biased diets had higher survival upon pathogen infection than sons from parents fed balanced diets. Diet manipulation of mother, father, or both parents had no effect on offspring developmental traits with the exception of egg hatching rate, which decreased when mothers were fed a protein-biased diet. The results emphasize the complexity of the transgenerational effects of parental diet on offspring pathogen resistance and provide insights into the long-lasting implications of parental nutrition to future generations' fitness.

Outcome 18: Assess potential of immune priming to protect released flies

Dinh H[†], Tabrizi ST[‡] & Ponton F[‡] (manuscript) No evidence of oral immune priming in the fruit fly *Bactrocera tryoni*

Immune priming is an enhanced protection of the host upon a secondary exposure to a previously experienced pathogen, and has been observed widely in invertebrates. In the present study, we aimed to investigate oral priming across developmental stages (i.e., ontogenic priming) and in adult stage using the fruit fly *Bactrocera tryoni* and the pathogenic bacterium *Serratia marcescens*. In a first experiment, we measured ontogenic immune priming by feeding larvae either heat-killed or live *S. marcescens* and injecting them with the same pathogen at adult stage. In a second experiment, we measured oral immune priming at adult stage by feeding adult females with either live or heat-killed *S. marcescens* and injecting them with the same bacterium two days following priming. In both experiments, survival rate of challenged flies was used as a proxy of pathogen resistance. We observed no evidence of immune priming, with survival rates are comparable between primed and non-primed flies.

Theme 3: Pre-release treatments and release methods

Project 1: Pre-release treatments

Summary

Tephritid flies commonly have a long post-emergence development period such that when flies are released as immature adults in SIT programs they need to forage and survive in the field for many days before maturing and participating in mating activity. With high field mortality rates, this means that few of the released flies might survive long enough to reach maturity, or may fail to secure the nutrition they need to support reproductive development. As a consequence, very few of the released sterile flies might actually participate in SIT.

Pre-release treatments have been a major focus of SIT research for numerous overseas species, especially medfly and melonfly. In Australia, there has been substantial work conducted on the value of yeast hydrolysate as a prerelease supplement that increases reproductive development and mating success, increases male ability to inseminate females, increases the male ability to prevent females from remating, and enhances longevity. These patterns have been borne out in field releases led by NSW DPI (MT06049). There are many additional pre-release supplements that might be considered to enhance the post-release performance of Qflies and thereby enhance efficacy of SIT. For example, a juvenile hormone analogue, methoprene, has been found to accelerate maturation

and improve mating performance in several fruit fly species, including Qflies (CT05002). The methods used in previous studies are not practical for SIT application, however, owing to the use of toxic and flammable carriers for the hormone. Raspberry ketone has also shown promise as a pre-release treatment (CT05002), but the full potential is yet to be explored. This project investigates in detail the potential application of pre-release supplements to enhance the post-release performance of sterile Q-flies, and develop practical methods that can be adopted in operational programs.

Initial studies were with raspberry ketone, and provide the first findings for any fruit fly species to consider the application of this metabolic enhancer in SIT programs. Oral raspberry ketone supplements are found to accelerate development of reproductive organs (Outcome 1), result in mating and much younger ages (Outcome 2), result in matings that are effective at inducing sexual inhibition in mates (Outcome 3). While much of the effect of raspberry ketone is likely owing to accelerated metabolism, effects may also in part be due to the presence of raspberry ketone in the pheromone blend emitted by males to attract mates (Outcome 4). In addition to positive effects on development rate and sexual performance, oral raspberry ketone supplements are found to reduce responsiveness of male Qflies to cuelure traps (Outcome 5), with this effect apparent mediated by changes in central processing of sensory input rather than changes in receptor sensitivity (Outcome 6). Inhibition of cuelure response in sterile Qflies potentially enables simultaneous application of SIT and MAT, which would vastly improve the efficacy of SIT as wild males are eliminated and continually replaced by sterile males (reducing the number of sterile flies required for a level of efficacy by up to 95%; Barclay et al. 2014 Annals of the Entomological Society of America). Raspberry ketone pre-release supplements can even provide some protection against predators (Outcome 7). Contrasting the positive effects of raspberry ketone supplements on development, cuelure response, and anti-predator defense, there are also some risks involved. In particular, presumably owing to high metabolic rates driving high nutrient demand, Qflies receiving raspberry ketone supplements exhibit elevated vulnerability to starvation (Outcome 8). Operationally, the extent to which this is likely to impact SIT programs will depend on the environment where releases are carried out. In towns or orchards, with ample foliage and irrigation, it is unlikely that conditions would expose the released flies to starvation conditions, but releases in less hospitable environments could.

In a parallel line of inquiry, this project also explored the potential value of methoprene supplements, with many findings in common with studies of raspberry ketone. Methoprene supplements accelerate development (Outcome 9) and increase mating propensity of flies at young ages (Outcome 10). Positive effect of methoprene on development and mating were initially conducted in the laboratory, but field cage studies provided a strong confirmation (Outcome 11). Having found strong effects, this project explored alternative economic sources of methoprene, finding that a low-cost mosquito larvicide was as effective as analytical grade methoprene at a fraction of the cost (Outcome 12). Successful SIT requires that matings not only occur but that they be effective at inducing sexual inhibition in mates in order to protect against subsequent potential matings by fertile wild males. Methoprene-treated makes were found to be as effective as naturally matured males at inducing sexual inhibition in their mates (Outcome 13); that is, increased mating propensity does not come at a cost of diminished efficacy of matings. As for raspberry ketone supplements, there are some risks associated with methoprene pre-release supplements in form of elevated vulnerability to starvation (Outcome 14).

Raspberry ketone is a known metabolic enhancer with very general effects, for example being included as an active ingredient in human weight-loss products, and the effects of methoprene closely mirror those of raspberry ketone in terms of accelerated development and high early mating propensity, and even in similar elevated risks of starvation should the sterile flies be released in particularly inhospitable locations. Drawing on the possibility that the effects on these life history traits are driven by a general elevation of metabolism, the possibility that other stimulants might yield similar effects was considered. Supporting this general idea, studies of caffeine as a pre-release supplement yielded effects on development and mating that closely matched those of raspberry ketone and methoprene (Outcome 15).

With robust findings in hand from laboratory and field cage studies, the next step was to test the effects of raspberry ketone and methoprene supplements in field releases. At the outset of HG14033 the SITplus program had planned to adopt the historical standard of releasing flies two to three days after emerging when at an early stage of sexual development and the investigations of raspberry ketone, methoprene and caffeine pre-release supplements sought to shorten the post-release development period so that as many flies as possible reached maturity after release and participated in SIT. Field releases confirmed high levels of effectiveness of both raspberry ketone and methoprene supplements, with both treatments yielding very substantial increases in the

number of sterile male Qflies surviving and attaining sexual maturity after release (Outcome 16). Subsequently, however, a significant change was made in the post-emergence handling of sterile Qflies. Based on the findings of Theme 6 Project 1, which indicated massive benefits of holding the flies until five days of age before release, trial operational releases in FF17001 adopted release at five days of age. The question then arose of whether raspberry ketone or methoprene pre-release supplements provide benefits above those already achieved by the extended pre-release holding period. In field releases, while very significant benefits of both supplements were found when the flies were released at two days of age (Outcome 16), no benefits were found when the flies were released at five days of age (Outcome 17) owing to the already very high performance of flies released at this age.

In addition to effects of metabolic enhancers, Theme 3 Project 1 considered the possibility that cheap plant-based sources of protein might provide a viable alternative to the comparatively expensive yeast hydrolysate that is used to feed adult flies in mass rearing facilities and before release. While plant-based proteins have proven effective with some *Anastrepha* fruit flies, none of the tested plant-based proteins was adequate to support reproductive development in Qfly and hence are not currently viable as an economical adult diet for pre-release holding or for adult colony maintenance in the factory (Outcome 18).

As a capstone, the learnings of Theme 3 Project 1 are included as core components of a review of pre-release protocols for fruit fly SIT in an International Atomic Energy Agency book (Outcome 19). The research conducted under Theme 3 Project 1 places Qfly SIT at the forefront of efforts to improve post-emergence handling to maximise performance of sterile flies after release.

Conclusions & Recommendations

- Raspberry ketone, methoprene and caffeine are all effective at accelerating development of Q-flies and could be applied in operational SIT programs under the historical conditions of 2 – 3 day pre-release holding period.
- Releasing flies at five days of age (See Theme 3 Project 2) yields such massive improvements in field performance of sterile Qflies that additional supplements provide no incremental benefit to development
- Males treated with raspberry ketone and methoprene are fully capable of inducing sexual inhibition in their mates, even when mating at unusually young ages
- Mosquito larvicides can provide a cheap source of methoprene
- Raspberry ketone is incorporated in pheromone, and increases amount of pheromone produced
- Raspberry ketone feeding reduces cuelure response, potentially enabling simultaneous SIT and MAT that would vastly increase the efficacy of SIT
- Raspberry ketone treatment provides some protection from predators by aversion
- Males treated with raspberry ketone or methoprene have increased vulnerability to nutritional stress and so these treatments should be avoided when releasing flies in dry environments
- Plant-based proteins do not provide an alternative to yeast hydrolysate for pre-release nutrition

Achievements

Outcome 1: Establish potential of Raspberry Ketone for accelerated development

Akter H[†] & Taylor PW (manuscript) Raspberry ketone supplement accelerates reproductive organ development of male Queensland fruit fly, *Bactrocera tryoni*.

Raspberry ketone (RK), 4-(4-hydroxyphenyl)-butan-2-one, is highly attractive to mature males of some fruit flies (Tephritidae), including Queensland fruit fly *Bactrocera tryoni* (Froggatt) ('Q-fly'). Mature male Q-flies that feed on RK analogues gain elevated metabolism and increased sexual performance. Although immature males are not attracted to RK, by mixing RK in food of recently emerged adults a recent study has exploited these metabolic effects of RK to accelerate the emergence of sexual activity in developing male Q-flies. Previous studies only considered effects of RK-supplementation on mating, so the possibility exists that RK only affects behaviour without accelerating development of reproductive organs. The present study assesses the effects of RK supplements on reproductive organ development in male Q-flies. Recently emerged flies were treated for 48 hours with RK (0% control, 1.25%, 5%) mixed in a diet of sugar or sugar+yeast hydrolysate (YH)(3:1), and were provided

only sugar thereafter. For flies fed sugar+YH, RK supplements accelerated the development of testes and ejaculatory apodemes, indicating that the early matings of RK-supplemented male Q-flies are supported by corresponding development of reproductive organs. For flies fed sugar only, however, RK did not affect testes development and suppressed development of ejaculatory apodemes. In the absence of the nutritional support of YH, RK supplements appear to impose metabolic costs that result in suppressed development of some reproductive organs. Pre-release RK supplements show promise for sterile insect technique programs, although should be considered together with nutrition as a combined treatment.

Outcome 2: Assess potential of Raspberry Ketone supplements for enhanced and early mating performance

Akter H[†], Méndez V[‡], Morelli R[‡], Perez J[‡] & Taylor PW (2017) Raspberry ketone supplement promotes early sexual maturation in male Queensland fruit fly, *Bactrocera try*oni (Diptera: Tephritidae). *Pest Management Science* 73: 1764–1770

Raspberry ketone (RK) is highly attractive to sexually mature, but not immature, males of many Bactrocera species, including Queensland fruit fly ('Qfly', *Bactrocera tryoni*), and acts as a metabolic enhancer in a wide diversity of animals. We considered the possibility that, as a metabolic enhancer, RK in adult diet might accelerate sexual maturation of male Qflies. Recently emerged adult Qfly males (0-24 hrs old) were exposed to RK-treated food for 48 hours and were then provided only sugar and water. Four doses of RK (1.25, 2.5, 3.75 and 5 %) along with 0% control were tested with two types of food: sugar alone and sugar mixed with yeast hydrolysate (3:1). For flies tested when 4 - 10 days old, all RK doses increased mating probability of flies fed sugar mixed with yeast hydrolysate but did not show any effect in mating probability of flies fed only sugar. No effects of RK were found for flies tested when 10 - 30 days old for either diet group. There was no evidence that RK affected longevity at any of the doses tested. Feeding of RK together with yeast hydrolysate to immature Qfly increases mating propensity at young ages and accordingly shows significant potential as a pre-release supplement that might increase the proportion of released flies that attain sexual maturation in Sterile Insect Technique programs.

Outcome 3: Assess remating by mates of Raspberry Ketone treated males

Akter H[†] & Taylor PW (2018) Sexual inhibition of female Queensland fruit flies mated by males treated with raspberry ketone supplements as immature adults. *Journal of Applied Entomology* 142: 380-387

Raspberry ketone (RK) dietary supplements accelerate the emergence of sexual behaviour in developing Queensland fruit fly (Q-fly) males, and show promise as a pre-release supplement for use in sterile insect technique (SIT) programs. However, the value of RK supplements in SIT programs would be greatly reduced if RK-treated males are ineffective at inducing sexual inhibition in mated females. To test the effectiveness of matings by RK-treated males, we here investigate the remating propensity of females mated by RK-treated (1.25% or 5% RK in food) and RK untreated (control) males. Tested males received RK supplements mixed in sugar and yeast hydrolysate for two days after emerging and then received only sugar. To test for male age-dependent effects, virgin females were mated to treated and untreated males that were 6, 8, 10, 20 or 30 days old. To test for persistence of sexual inhibition, mated females were tested for re-mating propensity at 1, 7 or 15 days after their first mating. RK treated males did not differ from control males in copula duration and females mated by RK treated males did not differ from those mated by control males in remating propensity, second copula latency or second copula duration. RK-treated Q-fly males not only mate at younger ages but their matings are as effective as those of untreated controls at inducing sexual inhibition in mates.

Outcome 4: Assess effects of Raspberry Ketone on pheromone composition

Akter H[†], Pérez J[‡] & Park SJ[‡] (2021) Raspberry ketone supplements provided to immature male Queensland fruit fly, *Bactrocera tryoni* (Froggatt), increase the amount of volatiles in rectal glands. *Chemoecology* 31: 89-99

Raspberry ketone (RK) supplements provided together with sugar and yeast hydrolysate accelerate sexual maturation and increase mating success of Queensland fruit fly ('Qfly') males. However, the mechanisms underlying this enhanced mating ability are currently unknown. Volatiles are an important element of Qfly sexual

calling and courtship and so changes in volatiles quantity or quality may be involved, and the present study investigated this possibility. Flies were fed a diet of sugar only (S) or yeast hydrolysate mixed with sugar (YH + S) (1:3) that contained 0% RK (control) and 5% RK (treated) for 2 days after emergence. Volatile compounds were extracted from rectal glands when flies were 6, 8, 10, 20, and 30 days old. Males fed on RK exhibited a significant increase in total volatile production in rectal glands compared to RK-unfed males (control). Males fed on RK with YH + S produced significantly higher amounts of volatiles than males fed on RK with sugar only. Males fed on YH + S diet produced more volatiles in the presence of RK compared to males fed on YH + S diet only. Two compounds, N-(3-methylbutyl)acetamide and N-(3-methylbutyl)propanamide were dominant in endogenously produced rectal gland volatiles comprising ca. more than 90% of the total amount in both RK-fed and control males. Considering exogenous and endogenous compounds together, unaltered RK was dominant along with these two endogenous compounds in RK-fed males in rectal gland until 30 days of age in both diet groups.

Outcome 5: Assess effects of Raspberry Ketone on cuelure response

Akter H[†], Adnan S[†], Morelli R[‡], Rempoulakis P[‡] & Taylor PW (2017) Suppression of cuelure attraction in male Queensland fruit fly provided raspberry ketone supplements as immature adults. *PLoS ONE* 12(8) :e0184086

Tephritid fruit flies are amongst the most damaging insect pests of horticulture globally. Some of the key fruit fly species are managed using the sterile insect technique (SIT), whereby millions of sterile males are released to suppress reproduction of pest populations. Male annihilation technique (MAT), whereby sex specific lures are used to attract and kill males, is often used to reduce wild male numbers before SIT programs commence, providing released sterile males an increased numerical advantage. Overall program efficacy might be improved if MAT could be deployed simultaneously with SIT, continuously depleting fertile males from pest populations and replacing them with sterile males. However, such 'male replacement' requires a means of suppressing attraction of released sterile males to lures used in MAT. Previous studies have found that exposure of some fruit flies to lure compounds as mature adults can suppress subsequent response to those lures, raising the possibility of pre-release treatments. However, this approach requires holding flies until after maturation for treatment and then release. The present study takes a novel approach of exposing immature adult male Queensland fruit flies (*Bactrocera tryoni*, or 'Qfly') to raspberry ketone (RK) mixed in food, forcing these flies to ingest RK at ages far younger than they would naturally. After feeding on RK-supplemented food for two days after emergence, male Qflies exhibited a reduction in attraction to cuelure traps that lasted more than 20 days. This approach to RK exposure is compatible with current practises, in which Qflies are released as immature adults, and also yields advantages of accelerated reproductive development and increased mating propensity at young ages.

Outcome 6: Assess mediation of raspberry ketone-induced reduction in cuelure response

Biswas JM[†], Mainali B[‡], Park SJ[‡], Taylor PW & Rempoulakis P[‡] (2020) Electrophysiological responses to cuelure of raspberry ketone-fed Queensland fruit flies. *Journal of Economic Entomology* 113: 2832-2839

The sterile insect technique (SIT) and male annihilation technique (MAT) are important tools for the control of Queensland fruit fly (Q-fly), *Bactrocera tryoni* (Froggatt), a major insect pest of horticultural crops in Australia. In MAT, mature Q-fly males are attracted to a toxic bait or trap using Cuelure, a synthetic analogue of raspberry ketone (RK). Substantial improvements in control could be achieved by simultaneous use of SIT and MAT, but this requires suppression of the Cuelure response in released sterile flies. Recent studies report that pre-release feeding with RK during the first 48 hours after emergence can reduce the response of mature Q-fly males to Cuelure, but the mechanism underpinning this is unknown. Here, to test whether reduced sensory sensitivity to Cuelure is involved, we evaluated the effects of RK supplements, adult diet (yeast-supplemented diet throughout adult stage vs. yeast-supplemented diet only for 48 hours), and age on electroantennogram (EAG) and electropalpogram (EPG) responses of Q-flies to Cuelure stimuli. EAG responses did not vary with RK supplements, sex, or age of Q-flies that were fed a yeast-supplemented diet throughout the adult stage, but the responses of Q-flies fed yeast-supplemented diet only for 48 hours decreased with age. EPG responses of both sexes of Q-flies were affected by RK supplements, age, and their interaction, but without patterns that might indicate reduced maxillary palp response of RK supplemented flies to Cuelure. Our findings do not support the hypothesis that reduced Cuelure response of male Q-flies fed RK supplements is explained by reduced electrophysiological response in antennae or maxillary palps.

Outcome 7: Assess potential anti-predator functions of raspberry ketone feeding

Kempraj V[†], Park SJ[‡], Mendez V[‡] & Taylor PW (manuscript) Predator aversion as a benefit of raspberry ketone consumption in the Queensland fruit fly, *Bactrocera tryoni*, and predator learning as an effective countermeasure.

Males of many dacine fruit flies feed on biologically active phytochemicals, including raspberry ketone, methyl eugenol, and zingerone, and gain substantial benefits in mating success. Phytochemical feeding may also affect fruit fly interactions with predators. We here find that male *Bactrocera tryoni* (Queensland fruit fly) may gain protection from a jumping spider predator, *Opisthoncus quadratarius*, after feeding on raspberry ketone but also that after exposure to raspberry ketone-fed (RK+) B. tryoni these predators learn to handle *B. tryoni* effectively as a noxious prey. When naïve spiders were repeatedly provided RK+ flies as prey, they greatly increased their hunting success with RK+ flies. Observations of hunting behaviour suggest that the spiders learned to deploy attack from in front of the fly, an approach that is commonly used by jumping spiders to avoid noxious defenses of chemically defended prey such as ants. This change in predatory behaviour was not permanent, as after subsequently being repeatedly provided flies that had not been fed raspberry ketone, capture success returned to levels similar to those of naïve spiders. Our findings provide a new dimension to understanding the biology of raspberry ketone feeding by male *B. tryoni* and also highlight a role for learning in the response of a generalist jumping spider predator.

Outcome 8: Assess impacts of Raspberry ketone on longevity and vulnerability to starvation

Akter H[†], Rempoulakis P[‡], Inskeep J[†] & Taylor PW (manuscript) Raspberry ketone feeding affects the tolerance of Queensland fruit fly *Bactrocera tryoni* (Froggatt) to nutritional and desiccation stress.

Queensland fruit fly (Q-fly) males exhibit accelerated sexual maturation when their full diet is supplemented with raspberry ketone (RK) for two days following emergence. However, there may be risks associated with such accelerated development, such as increased vulnerability to starvation or desiccation. The present study tests these possibilities. Flies were fed for 48 hours with a diet of sugar mixed with yeast hydrolysate (3:1) that contained 0% RK (control), 1.25% RK (low dose) or 5% RK (high dose). To test vulnerability to starvation, flies were set up in group cages under three conditions - yeast hydrolysate+sugar+water (full diet); with 'water only', and with 'no food or water' - for both control and RK-treated flies. To test vulnerability to desiccation, flies were individually housed in glass vials containing 4-5 grains of silica gel. To analyse the water and lipid storage in Q-fly under different nutritional stresses another group identical to the survival in starvation study was set up. Desiccated flies were also subjected to analysis of water and lipid storage in relation to RK dose, sex and body size. To assess the RK effect on initial lipid and water level in Qfly one group of flies were separated and analysed immediately after RK treatment without exposure to any stress. Overall females were more resistant to starvation and desiccation compared to males. Raspberry ketone-fed flies were more susceptible than control flies to desiccation, however, RK-fed flies survived longer compared to control flies in starvation ('no food or water' and 'water only'). Lipid level decreased significantly in starved flies ('no food or water' and 'water only') while RK-fed flies lost significantly more lipid compared to control. However, in desiccated flies, lipid did not change significantly. On the other hand, water level decreased significantly in both desiccated and starved flies. Body size had effect on initial water content but no effect on initial lipid reserve. Body size did not show any significant effect on the survival, water content, water changes and lipid changes in desiccated flies but lipid reserve was affected by body size in this group of flies. In starved flies also body size affected water content ('no food or water' and 'water only'), lipid reserve ('no food or water') and water changes ('water only') but did not affect lipid changes. Results are discussed in the context of the possible use of RK as pre-release supplementation in SIT application.

Outcome 9: Assess potential of oral methoprene treatment for accelerated development

Adnan SM[†], Perez-Staples D & Taylor PW (2020) Dietary methoprene treatment promotes rapid development of reproductive organs in male Queensland fruit fly. *Journal of Insect Physiology* 126: 1040904

Methoprene supplements added to diets of yeast hydrolysate and sugar promote early expression of sexual behaviour and mating in male Queensland fruit fly (*Bactrocera tryoni*; 'Q-fly') and show promise as a pre-release treatment for sterile insect technique programs. Currently it is not known whether the early mating behaviour of

methoprene-treated male Q-flies is only behavioural or is coupled with accelerated development of reproductive organs. Accordingly, the present study investigates whether incorporation of methoprene into diets of yeast hydrolysate and sugar (1:3) or sugar alone, accelerate development of testes, ejaculatory apodeme, and accessory glands in male Q-flies and ovaries in females. All organs increased in size as the flies aged and matured, and development rate of all organs was far greater when the flies were provided yeast hydrolysate in addition to sugar. Incorporation of methoprene into diets containing yeast hydrolysate was found to strongly accelerate development of testes and ejaculatory apodeme, but not accessory glands, in males. In the absence of yeast hydrolysate, methoprene treatment had only a modest effect on male organ development. In contrast to males, development of ovaries in female Q-flies did not respond to dietary methoprene supplements, regardless of whether they were fed yeast hydrolysate and sugar or sugar alone. These findings of diet-dependent effects of methoprene supplements on reproductive organs are a close match to previous studies investigating effects of methoprene supplements on mating behaviour. Overall, methoprene supplements substantially enhance the positive effects of protein rich adult diet on the early expression of sexual behaviour and accelerate development of reproductive organs in male, but not female, Q-flies. Methoprene supplements added to pre-release diets of yeast hydrolysate and sugar show promise as a means of accelerating reproductive development of Q-flies released in sterile insect technique programs, and may also bias operational sex ratio in favour of males.

Outcome 10: Establish potential of methoprene treatment as a means of increasing mating performance

Adnan SM[†], M**é**ndez V[‡], Morelli R[‡], Akter H[†], Farhana I[†] & Taylor PW (2018) Dietary methoprene supplement promotes early sexual maturation of male Queensland fruit fly *Bactrocera tryoni*. *Journal of Pest Science* 91: 1441-1454

Sterile insect technique (SIT) is an environmentally benign pest management technique that relies on released sterile male insects mating with, and curtailing reproduction of, wild females. However, for species with high mortality rates and long adult maturation phases, a large proportion of the released insects can die before maturing and so fail to contribute to SIT. To counter this problem, inclusion of yeast hydrolysate in pre-release diets and treatment of pupae or adults with methoprene, a juvenile hormone analogue, have been investigated as means of accelerating development of some fruit flies, including Queensland fruit fly, *Bactrocera tryoni* (Froggatt) ('Q-fly'). Methoprene has most often been administered topically in acetone solution, which is toxic, flammable, and impractical for operational settings. As a practical alternative, we incorporated methoprene (0, 0.05, 0.1, and 0.5%) into Q-fly adult diet of sugar only or sugar mixed with yeast hydrolysate for two days, and then provided sugar only for the rest of the trial period. Mating performance of males and females was tested from 4 to 30 days of age. Flies provided sugar mixed with yeast hydrolysate had increased mating propensity in comparison to flies that were provided sugar only. At all ages and for both diets, all methoprene doses increased male mating probability. Methoprene treatment did not affect copula latency of males that received yeast hydrolysate, but males that received only sugar mated earlier if they had received 0.05% methoprene. Methoprene treatment of males was also associated with longer copulations, which may affect fertility of females that later remate. Females differed from males in that methoprene treatment did not significantly affect mating probability or latency, but resembled males in that methoprene treatment resulted in longer copulations. Sex differences in response to methoprene may lead to male-biased operational sex ratio when bisex Q-fly strains are used in SIT. Yeast hydrolysate increased longevity of both males and females, but methoprene treatment did not affect longevity. Overall, findings of the present study indicate that Q-fly sexual maturation can be accelerated, and SIT might hence be enhanced, by incorporation of methoprene and yeast hydrolysate in pre-release diet.

Outcome 11: Field cage confirmation of methoprene as a means of increasing mating performance

Adnan SM[†], Inskeep J[†], Farhana I[†], Rempoulakis P[‡] & Taylor PW (2020) Dietary methoprene enhances sexual competitiveness of sterile male Queensland fruit flies in field cages. *Journal of Pest Science* 93: 477–489

Queensland fruit flies *Bactrocera tryoni* (Froggatt) have a long adult maturation phase which, together with high mortality rates, can substantially reduce number of released flies that survive to mature and contribute to sterile insect technique (SIT) programs. This constraint on SIT can potentially be addressed by incorporating methoprene, a juvenile hormone analogue, into an adult diet of sugar and yeast hydrolysate for two days after emergence. Methoprene treatments have been found to accelerate sexual development of male Queensland fruit fly, resulting

in increased mating propensity of 5-7-day old males in no-choice laboratory trials. Before considering deployment of methoprene as a pre-release treatment in SIT it is necessary to demonstrate mating competitiveness and compatibility of methoprene-treated flies under field-like conditions. In the present study, we assessed whether methoprene treatment increases ability of sterile males (5 and 7 days old) to compete with mature (wild or laboratory) males for matings with mature (wild or laboratory) females in field cages. We also investigated mating compatibility to test for sexual isolation between sterile flies and mature (wild or laboratory) fertile flies. In mating competitiveness tests, methoprene-treated males of either age outperformed mature wild or laboratory males for matings with mature wild or laboratory females, respectively. Untreated 5 and 7 day old males were less competitive than mature wild or laboratory mature males, and hence had lower relative sterility indexes. Methoprene-treated males mated earlier in the evening and continued mating for longer than untreated sterile males and mature wild or laboratory males. In mating compatibility trials, methoprene-treated males mated equally with methoprene-treated females and mature females whereas methoprene-treated females tended to mate more often with mature males than with methoprene-treated males. However, untreated flies of both sexes exhibited substantial sexual isolation. Pairings that comprised methoprene-treated males and mature females had shorter mating latency and longer copulations than other pairings. Unlike males, methoprene-treated females did not exhibit changes in mating latency or duration. Overall, the present study supports the use of pre-release dietary methoprene treatment in Queensland fruit fly SIT.

Outcome 12: Identify an economical source of methoprene

Adnan SM[†], Farhana I[†], Inskeep J[†], Rempoulakis P[‡] & Taylor PW (2019) Accelerated sexual maturation in methoprene-treated sterile and fertile male Queensland fruit flies, and mosquito larvicide as an economical and effective source of methoprene. *Journal of Economic Entomology* 112: 2842-2849

Queensland fruit flies *Bactrocera tryoni* ('Q-fly') have long adult pre-reproductive development periods, which can present challenges for sterile insect technique (SIT) programs. Holding the sterile flies in release facilities is expensive for control programmes. Alternatively, releases of sexually immature males can lead to substantial mortality of sterile males before they mature. Recent studies have reported effectiveness of dietary supplementation with a mosquito larvicide (NOMOZ®) that contains S-methoprene, a juvenile hormone analogue, for accelerating sexual development of fertile Q-fly males. However, it is not known whether effects on sterile flies are comparable to effects on fertile flies, or whether effects of methoprene-containing larvicide are comparable to effects of analytical standard methoprene such has been used in most studies. Here we address both knowledge gaps, investigating the effects of analytical standard methoprene and NOMOZ® mixed with food and provided for 48 hours following emergence on sexual development and longevity of fertile and sterile Q-flies. Compared with controls, fertile and sterile male Q-flies that were provided diets supplemented with methoprene from either source exhibited substantially accelerated sexual development by 2 - 3 days and longer mating duration. Unlike males, females did not respond to methoprene treatment. While fertile and sterile flies were generally similar in sexual development and response to methoprene treatment, sterile flies of both sexes tended to have shorter copula duration than fertile flies. Neither methoprene supplements nor sterilisation affected longevity of flies. The present study confirms effectiveness of dietary methoprene supplements in accelerating sexual development of both fertile and sterile male (but not female) Q-flies, and also confirms that low-cost mosquito larvicides that contain methoprene can achieve effects similar to those for high-cost analytical grade methoprene as pre-release supplements for Q-fly SIT.

Outcome 13: Assess remating by mates of methoprene-treated males

Adnan SM[†], Farhana I[†], Rempoulakis P[‡] & Taylor PW (2020) Methoprene-induced matings of young Queensland fruit fly males are effective at inducing sexual inhibition in females. *Journal of Applied Entomology* 144: 500-508

Pre-release dietary treatment with methoprene, a juvenile hormone analogue, decreases the age at which male Queensland fruit flies mature and hence may decrease the post-release delay until released sterile flies participate in sterile insect technique (SIT) programs. However, if matings of young methoprene-treated males are not effective at inducing sexual inhibition in their mates, then this treatment may not enhance SIT. The present study investigates efficacy of matings of methoprene-treated males at inducing sexual inhibition in their mates. Methoprene incorporated into a diet of sugar and yeast hydrolysate (w/w 3:1) for 48 hrs after emergence resulted

in significantly increased male mating propensity when flies were less then 10 days of age, but not when older, and longer copulations. Copula latency did not vary with methoprene treatment but did decrease with age. The matings of young methoprene-treated males were effective at inducing sexual inhibition in their mates, matching the efficacy of untreated mature males. Regardless of treatment, females had reduced tendency to remate if their first mate was 15 days of age than if their first mate was younger (6, 8 days) or older (20, 25, 30 days). Females mated by methoprene-treated males that did remate tended to remate later in the day than females mated by untreated males. Also, second copula durations of females first mated by a 6 - 10 day old male were shorter if the male was methoprene treated. These patterns in remating females may indicate greater efficacy of the initial mating of methoprene-treated males. Overall, we find that the additional matings of young methoprene-treated male Queensland fruit flies are effective at inducing sexual inhibition in their mates. This finding supports the incorporation of methoprene into pre-release diet for SIT.

Outcome 14: Assess risks of methoprene treatment for ecological competence

Adnan SM[†], Farhana I[†], Rempoulakis P[‡] & Taylor PW (manuscript) Methoprene treatment increases activity, starvation and desiccation risk of Queensland fruit fly.

Juvenile hormone is an important regulator of sexual development in insects, and application of methoprene, a juvenile hormone analogue, together with access to a protein-rich diet, has been found to accelerate sexual maturation of several tephritid fruit fly species including Queensland fruit fly *Bactrocera tryoni* ('Q-fly'). Such accelerated development is a potentially valuable means to increase participation of released males in sterile insect technique programs. However, there is a risk that benefits of accelerated maturation might be countered by increased vulnerability to starvation and desiccation. The present study investigates this possibility. After emergence, flies were treated with three levels of methoprene (0, 0.05%, and 0.5%) incorporated into a diet of sugar and yeast hydrolysate for two days after emergence. Survival of groups and individual flies was assessed under conditions of food stress, food and water stress, and *ad libitum* access to diet, and survival of individual flies was also assessed under desiccation stress. Most flies provided *ad libitum* access to diet were still alive at 7 days, whereas all stressed flies died within 4 days. Desiccation stressed flies had the shortest survival followed by food and water stress, and then food stress. Methoprene supplements increased susceptibility of flies to each stress. Flies subjected to food and water stress had the least lipid reserves at death, whereas flies subjected to desiccation stress retained the least water reserves. To investigate mechanisms that might underlie reduced survival under stress, we also quantified activity level of flies that were subjected to food and water stress and desiccation stress. Activity level was greater for flies provided methoprene, but did not vary with stress type or sex, suggesting that increased vulnerability of flies to stress is related to elevated metabolism associated with elevated activity. Deleterious effects of methoprene supplements on stress tolerance indicate a need for careful consideration of the conditions that will be encountered by flies in the field before deploying methoprene as a pre-release treatment in Q-fly sterile insect technique programs.

Outcome 15: Assess potential use of caffeine supplements to accelerate development

Adnan SM[†], Farhana I[†], Park SJ, Rempoulakis P[‡] & Taylor PW (2020) Caffeine as a promotor of sexual development in sterile Queensland fruit fly males. *Scientific Reports* 10: 14743

Sterile insect technique (SIT) is an environmentally benign pest management technique that involves releasing millions of sterile insects to suppress reproduction of pest populations. Many fruit flies, including Queensland fruit fly (*Bactrocera tryoni* Froggatt, 'Q-fly'), have long adult maturation periods such that pre-maturation mortality can greatly reduce abundance of sexually active sterile males and impede SIT efficacy. Q-fly is the most difficult and costly challenge to market access for Australia's horticulture industries, and has been targeted for intensive use of SIT program. We here demonstrate potential of pre-release caffeine supplements as a novel means to accelerate sexual maturation in male Q-fly. In mating trials, analytical caffeine was very effective at accelerating sexual maturation, while no positive effects of caffeine-containing instant coffee or guarana supplements were detected. In parallel, development of testes and ejaculatory apodemes was accelerated in males provided analytical caffeine but not instant coffee or guarana. High doses of guarana and instant coffee reduced longevity while even the highest doses of analytical caffeine did not affect longevity. Pre-release caffeine supplements promote sexual maturation in Q-flies, and similar benefits are expected in other fruit flies having long adult maturation periods.

Outcome 16: Assess field performance of raspberry ketone- and methoprene- supplemented flies

Biswas JH[†], Mainali B[‡], Inskeep JR[†], Cross D, Benelli M[†], Allen AP, Taylor PW & Rempoulakis P[‡] (in press) Pre-release dietary supplements of methoprene and raspberry ketone increase field abundance of sterile Queensland fruit flies. *Journal of Economic Entomology*

The sterile insect technique (SIT) is a sustainable pest management tool based on the release of millions of sterile insects that suppress reproduction in targeted populations. Success of SIT depends on survival, maturation, dispersal, and mating of released sterile insects. Laboratory and field cage studies have demonstrated that dietary supplements of methoprene and raspberry ketone (RK) promote sexual maturation of adult Queensland fruit fly, *Bactrocera tryoni* (Froggatt), and may hence shorten the delay between release and maturity in the field. We investigated the effects of methoprene and RK dietary supplements on field abundance of sexually mature sterile Q-flies relative to untreated flies fed only sugar and yeast hydrolysate before release at two days of age. Compared with untreated flies, more methoprene- and RK-treated flies were recaptured in cuelure traps to which only sexually mature males are attracted. At distances of 100 and 200 m from the release point recapture rates were higher for methoprene- and RK-treated flies than for untreated flies, but at 300 m recapture rates were low and were similar for treated and untreated flies. Rainfall, relative humidity, wind speed, and wind direction did not affect recapture rates, but temperature was positively correlated with recapture rates for all treatments. There was a strong correlation between the number of sterile and wild flies caught in traps, indicating co-location in the field. Dietary supplements of methoprene and RK can substantially increase abundance of sexually mature sterile male Q-flies in the field following release as two-day-old immature adults.

Outcome 17: Assess effects raspberry ketone and methoprene supplements with extended pre-release holding

Biswas JH[†], Rempoulakis P[‡], Benelli M[†], Adnan SM[†], Allen AP, Taylor PW & Mainali B[‡] (manuscript) Raspberry ketone and methoprene as pre-release dietary supplements for Queensland fruit fly sterile insect technique with extended pre-release holding: effects on field abundance and dispersal of mature sterile males.

In Sterile Insect Technique (SIT) programs, the released sterile insects must attain sexual maturity at an early adult age so that a large proportion survive to mature and contribute to reducing reproduction of pest populations. Previous field studies based on release of adult Queensland fruit fly, *Bactrocera tryoni* (Froggatt) ('Q-fly'), at 2 days of age found a significant increase in the recapture of mature male Q-flies when they were provided methoprene or raspberry ketone (RK) before release. SIT for Q-flies has subsequently adopted release at 5 days of age; there is now a need to re-assess the merit of methoprene and RK supplements in this context. We assessed field abundance and dispersal of mature sterile male Q-flies that had been held for 5 days on pre-release standard diets of sugar and yeast hydrolysate (3:1) (control) with and without methoprene and RK supplements. Overall, the proportion of control and RK treated flies recaptured was higher than the proportion of methoprene-treated flies recaptured. We found no evidence that either methoprene or RK supplements yielded improvements over the control diet alone in terms of abundance and dispersal of mature sterile male Q-flies. Laboratory studies indicate that while the doses of methoprene and RK used in field releases at 2 days of age did not affect male longevity when sugar and water were provided, these doses resulted in reduced longevity when no sustenance was provided. Further, all doses of methoprene and RK used in field releases at 5 days of age reduced male longevity regardless of sustenance. At 5 days of age the flies are already in an advanced state of maturity when released and in this context, it appears that additional pre-release treatments of methoprene and RK provide no further improvement and may even be detrimental when nutrition is scarce.

Outcome 18: Conduct quality control assessment of flies maintained using plant-based proteins

Gaire S[†], Biswas MJH[†], Rempoulakis P[‡], Crisp P, Taylor PW & Mainali BP[‡] (manuscript) Evaluation of plant-based proteins on the performance of adult Queensland fruit flies.

Efficient mass production of insects is central to success of SIT programs. While developing mass rearing strategies, development of cost-effective diet that help reduce the production cost without compromising insect quality is imperative as artificial diets represent one of the main costs of insect mass production. Adults of the tephritid fruit fly are reared on yeast hydrolysate (YH) and sugar in the fruit fly factories across the globe. The adult diets provide

source of protein and carbohydrate for growth, development, survival and reproduction. The source of protein, yeast hydrolysate, is not only expensive but consistency in terms of quality and supply is another major issue. Since Qfly is anautogenous, the females require protein for egg maturation and the males require protein for reproductive development as well as production of pheromone. Owing to inconsistency in the supply and quality of YH, and its expensiveness, a search of an alternative protein source to yeast hydrolysate is imperative. A study was conducted to assess potential of plant-based proteins as an economical alternative to yeast in rearing and rear-out of Qfly (such materials are effective in some other fruit flies). Plant-based proteins – chickpea, hemp and soybean powder were provided alongside sugar to the newly emerged Qflies and their effect on development and reproductive performance of the flies in comparison to YH was investigated. The analyses revealed that no plant-based protein was comparable to YH as a protein source to adult flies in terms of mating and female fecundity. The flies that were fed on plant-based proteins such as chickpea, hemp, and soybean powder failed to mate, with some exceptions of those fed chickpea, even after 20 days of rearing. The YH fed adults started mating day 6 onwards, with mating percentage of 90% of the tested population on day 10 while the chickpea fed flies achieved maximum mating percentage of 40% when the flies were 20 d old. This means the sole use of plant-based protein plus sugar as an adult diet for mass production is unfeasible. Nonetheless, mixing of the plant-based proteins with YH may be palatable to the adult Qfly and will be as good as sole YH in terms of reproductive performance. Reduced proportion of YH in the adult diet would help reduce significant cost involved in the Qfly production system.

Outcome 19: Review of pre-release treatments for SIT

Pereira R, Yuval B, Liedo P, Teal PEA, Shelly TE, McInnis DO, Haq I, Taylor PW & Hendrichs J (2021) Improving post-factory performance of sterile male fruit flies in support of the sterile insect technique. pp. 101–124 in: Dyck VA, Hendrichs J & Robinson AS (eds.), Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Second Edition, CRC Press, Boca Raton, Florida, USA.

The sterile insect technique (SIT) is being applied against tephritid fruit fly pests in many areas of the world. Currently fruit fly factories have a capacity to produce and sterilize several billion sterile insects per week and to make them available for shipment to their final destinations. There, at sterile fly emergence and release facilities, the emerging flies are fed and held close to maturity, and then collected for area-wide release. While much research effort has been invested in improving mass-rearing and quality control procedures at the fly factory level, the post-factory handling of sterile flies has received much less attention. However, research conducted mainly from 2000 onwards, has focused on developing and validating ways to improve sterile male performance through better management during the critical period that starts with the arrival of pupae at the fly emergence and release facility and ends with the release of the sterile flies in the field. This chapter summarizes the progress made in this area for fruit fly species from the genera *Anastrepha*, *Bactrocera*, *Ceratitis* and *Zeugodacus* against which the SIT is being applied. To increase the effectiveness of fruit fly SIT programmes, exposure of sterile males to nutritional, hormonal and semiochemical supplements has been assessed to improve sterile male performance and to enhance post-factory handling and release methods. Incorporation of protein and juvenile hormone into pre-release *Anastrepha* spp. diets significantly accelerates sterile male maturation and improves sexual performance among several species, while improved and probiotic adult diets and semiochemical treatments using ginger root oil or citrus oils in *Ceratitis capitata*, and methyl eugenol and raspberry ketone in *Bactrocera* and *Zeugodacus* species, significantly increases sterile male mating competitiveness. Some of these treatments and improvements have been transferred to and are being applied routinely in operational programmes. However, these efforts need to be further strengthened to assess the interaction of different environmental and holding conditions, supplements and release systems, to be able to further improve the performance of mass-produced sterile males, a critical component for increasing the effectiveness of operational programmes.

Project 2: Release methods

Summary

Release methods are of massive importance to the efficacy of an SIT program. Historical practices for Q-fly have mostly released adult flies from bins. In such 'ground release' methods, irradiated pupae are placed in plastic bins for emergence. After emerging, the flies are supplied water and sugar and in some programs have also been provided yeast hydrolysate. Once at release age, the bins are transported to the field and opened. This method is

effective, but it is somewhat cumbersome, costly and labour intensive, requiring rear out facilities close to release sites to minimise road transportation of emerged adults. In some SIT programs, pupal releases have been considered as an alternative that eliminates the need for rearing out facilities. In pupal release methods, bins or other containers of pupae are placed in the field. The flies emerge from their pupal cases and, once their cuticle hardens, exit the container to disperse in the field. Some overseas SIT programs rely on aerial releases, usually from fixed wing aircraft, although sometimes from helicopter. In aerial release programs, pupae are usually held in specialised racked cages, where the adults emerge and are provisioned with water and sugar, and sometimes also yeast hydrolysate. Once at release age, the cages of flies are transferred to a cool room in which the flies are immobilised. Once immobile, the flies are removed from the rearing out racks and transferred to a refrigerated box. The box of chilled adults is then attached to a motorised release machine in an aircraft. As the aircraft flies, the sterile insects are slowly fed through a shoot and discharged. As the flies descend they are dispersed, and then disperse further once at ground level. Having considered the available options, the SITplus program technical advisory committee decided on aerial release as the sole method for investigation and Theme 3 Project 2 hence focused on trialing and refining the required equipment.

Conclusions & Recommendations

- Aerial release systems developed for the SITplus program are effective
- Releases should be carried out as soon as practicable after chilling and packing of adult flies to minimise deleterious effects
- To support alternative small-scale and local implementations of Qfly SIT, consideration should be given to the development and testing of pupal release methods

Achievements

Outcome 1: Trial aerial release protocols

An aerial release unit was designed and constructed in conjunction with WrightsAir (www.wrightsair.com.au/) and fitted to a Cessna 207 fixed wing aircraft. The unit is of a design that can be readily transferred between similar aircraft, thereby ensuring that aerial release programs will not be constrained by the availability of a specific aircraft. Being able to install and remove the aerial release unit also means that the aircraft does not need to be solely dedicated to the release program, and can the repurposed for other activities when not releasing sterile insects. A software program was developed to operate the aerial release unit once airborne, to enable starting and stopping of release as the aircraft enters and exits the release area and to adjust release rates to accommodate changes in aircraft speed without operator input. Of importance for cost considerations, this design enables one person operation. The aerial release unit was assessed in a series of trial flights over Goolwa and Adelaide to test and adjust equipment and to set the release auger speeds. The aerial release unit has been used in the releases of FF17001 to great effect, and without failure, through two full seasons of releases in Cobram (VIC) and Hillston (NSW).

Outcome 2: Establishing effect of pre-release chilling on Qfly quality

Gaire S[†], Biswas MJH[†], Benelli M[†], Rempoulakis P[‡], Taylor PW & Mainali BP[‡] (in press) Effect of chilling on quality control parameters of sterile Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Journal of Economic Entomology*

To reduce fly movement and physical damage, in most fruit fly SIT programs sterile flies are chilled to facilitate collection, packing, and transfer to release machines or containers, and during transport to release sites. Chilled release is common for aerial and roving releases of adult flies. In Australia, release sites are often quite distant from rear-out facilities, such that longer transportation times are required than is the case for many SIT programs for other fruit fly species in other countries. In current SIT program flies are held for 5 d rather than 2–3 d before release, and are chilled for up to 5 h at 3–5°C, and even longer periods may be required as SIT operations expand to more remote release sites. Accordingly, there is a need for detailed understanding of the effects of chilling in

this new operational context. To guide SIT procedures, it is important to understand the impact of such practises on performance of sterile Qfly. The present study assesses the effect of chilling temperature and exposure period on quality parameters of sterile Qfly. The effects of two temperature regimes (4°C and 6°C) and six exposure periods (0, 1, 2, 4, 6, and 12h) on chill coma recovery time (CCRT), flight ability, survival under nutritional stress and longevity of both males and females were investigated. Flies chilled at 4°C took longer to recover than that those chilled at 6°C. Flight ability, survival under nutritional stress, and longevity all decreased as chilling period increased but did not differ between the two tested temperatures. At both temperatures and all chilling periods, the flies lived on average more than 28 d. If lifespan of flies in the field is shorter than this, then the reduction in longevity may not have much impact on operational SIT. The trade-offs with fly quality need to be considered when chilling adult Qflies for SIT programs. For most metrics there were no differences between chilling at 4°C and 6°C, but shorter CCRT was found at 6°C. Hence, while both temperatures could be used, where feasible it is suggested that 6°C be adopted. Chilling period had significant negative effects on flight ability, longevity and survival under nutritional stress. When longer chilling periods are required to reach remote release zones, there may be value in increasing the number of flies released to compensate for reductions in quality. Overall, chilling periods should be minimised to preserve quality of Qflies released in SIT programs.

Theme 4: Post-release identification

Once flies are released it is important that the sterile flies be easily and cheaply discriminated from the wild population. Sterile flies misidentified as wild can lead to erroneous outbreak alerts and wild flies misidentified as sterile can lead to delays in response to actual outbreaks. Marking is also important so that changes in pest populations can continue to be monitored during SIT releases, enabling adaptive management decisions about which control practises to implement and where.

Traditionally, identification has been based on the 'self-marking technique', whereby pupae are coated with fluorescent dye powder that is transferred externally to the emerging adults. Unfortunately, these external dyes have been found to significantly reduce survival and quality of sterile Qflies in some studies. In addition to reducing the quality of sterile flies, fluorescent powder dyes may also be imperfect markers, or may be perceived as such by trading partners. Recognizing the real or perceived potential for inadequate marking, loss of marks or even transfer of external dye to wild flies in monitoring traps, Theme 4 assesses the efficacy and tradeoffs of visible dye marking (Project 1), and develops 'back up' methods (Projects 2 – 4).

Project 1: Visible Markers

Summary

Fluorescent dye marking has been an integral part of SIT for fruit flies in general and for Qfly specifically. It helps to estimate population size, survival and dispersal of the released flies by assessing the relationship between marked, released, and recaptured individuals. In addition, the mark, release, and recapture technique has become an important tool for ecological research. Of greatest importance, dyes enable identification of sterile flies so that they are not mis-interpreted as wild flies that might contribute to an outbreak declaration. Fluorescent dyes that have been used to mark sterile Qfly in Australia include Arc Chrome, Fire Orange, Orange, Red, Blaze Orange, Laser Red, Nova Red, and Comet Blue. Some dyes have been reported to be more difficult to identify than others. Marking with dye has been reported to sometimes diminish emergence and survival of Qfly. Although pink and orange dyes are most commonly used in Qfly trials and suppression programs, multiple discriminable dyes are sometimes needed to differentiate flies used in overlapping releases at the same location. While there have been studies investigating effects of different dyes on Qfly at a particular dose, and effects of dose for individual dyes, to more fully evaluate the suitability and use of different dyes, there was a need for studies that compare effects of dose across multiple dyes.

Theme 4 Project 1 assessed the effectiveness of a catalog of dyes as markers, and also assessed impacts of dyes on Qfly performance (Outcome 1). A review was also carried out to assess potential alternative marking methods that could be used with Qfly both routinely and as 'safety nets' in circumstances where dye marking fails to distinguish adequately between wild and sterile flies (Outcome 2).

Conclusions & Recommendations

- All of the tested dyes except for Stella green were similar in all assessed metrics of fly performance and are recommended for use in SIT programs.
- 2g dye/l of pupae is optimal in terms of visibility and fly performance

Achievements

Outcome 1: Establish persistence and visibility of fluorescent dyes

Akter H, Taylor PW & Crisp P (2020) Visibility and persistence of fluorescent dyes, and impacts on emergence, quality, and survival of sterile Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae). *Journal of Economic Entomology* 113: 2800-2807.

Tephritid flies released in sterile insect technique pest management programs are usually marked with fluorescent dyes so that they can be distinguished from wild flies in monitoring traps. Sterile flies are marked with fluorescent dye at the pupal stage, usually 24–48 h before emergence. Dyed flies accumulate the fluorescent dye in their ptilinum during eclosion which can be seen with microscopic examination under UV light. Dyes can have adverse effects on emergence, quality, and survival, which can impact SIT success, and so it is important to identify dyes and doses that maximize marking efficacy while minimizing deleterious effects on fly quality. This study examines the effects of five fluorescent dye products, Fluoro Pink, Fluoro Orange, Stella Green, Arc Chrome, and Astral Pink applied at four dose levels (1, 2, 3, and 4 g/liter) on Queensland fruit fly. All dye products caused a similar dose-dependent reduction in percentage of adult emergence. Incidence of morphological deformity of emerged adults increased with dose, and this trend was similar for all dye products. No effects of dye product or dose were found on survival rates over the first 35 d of adulthood, although females tended to have higher survival than males. Visibility varied with dose and dye product; 1 g/liter dye was less visible than 2, 3, or 4 g/liter, and Stella green had lower visibility than other dyes. All of the tested dyes except for Stella green were similar in all assessed metrics of fly performance and are recommended for use in SIT programs.

Outcome 2: Review available marking methods

Dominiak B, Taylor PW & Rempoulakis P‡ (manuscript) Marking and identification methodologies for mass releases of sterile Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae). An overview.

The Sterile Insect Technique (SIT) is a most promising methodology for effective control of the Queensland fruit fly (Qfly), *Bactrocera tryoni*, a major horticultural pest of Australia. The Qfly SIT has a long history, with a significant amount of research invested, and numerous releases, mainly in NSW and Victoria. In SIT operations worldwide, a reliable insect marking technique is a prerequisite for the successful discrimination among wild and laboratory reared flies in the field. For quarantine purposes, often same requirements apply for declared pest free areas following successful SIT releases programs. A great amount of variation in dye performance and visibility has been recorded from several Qfly SIT release projects in the past. Here we review the past experience from those operations, and the current and developing methods for the marking and identification of Queensland fruit flies that have been mass reared and irradiated for sterile releases. Problems arising from less successful use of dyes are highlighted, and novel methodologies that have the potential to substitute the dye in future operations are presented and discussed.

Project 2: Identification through biomarkers

Summary

Biomarkers of irradiation exposure were investigated for their application in the post-release identification of irradiated fruit flies. This project follows up from a previous project (VG09160) that identified a persistent biomarker of irradiation-damage in Qfly, γH2Av (Siddiqui et al. 2013 Mutagenesis). This protein biomarker was

used to develop Western Blot and ELISA methods that showed significant promise as a means of identifying irradiated flies. Building on the success of this previous project, this project confirms this protein marker can be used to confirm whether unmarked flies caught in monitoring traps are released sterile flies or are instead from the wild population. This work commenced with the basic development of γH2AX as a marker of prior IR exposure in Qfly, with potential applications both in the detection of irradiation damage in insects found in shipments of purportedly irradiated produce, and then demonstrated a dose response that would enable estimation of dose received from the γH2AX signal. This dose response was developed as a tool for dosimetry that was used to refine X-irradiation procedures for sterilisation of Qfly at the pupal stage (Theme 2 Project 2). Further, the assay developed for dosimetry identified a strong and persistent γH2AX signal that was almost undiminished 30 days after irradiation (for details, see Theme 2 Project 2).

Conclusions & Recommendations

- γH2AX provides a reliable marker of irradiation that can be used to distinguish sterile and wild flies if visible markings prove ambiguous
- A tool could be developed based on γH2AX for routine use in SIT programs for Qfly, and potentially other insects

Achievements

Outcome 1: Develop a general marker of irradiation in insect tissues

Siddiqui MS[‡], Taylor PW & Crisp P (2020) Gamma-H2AX: a promising biomarker for fruit fly phytosanitary irradiation exposure. Chapter 11 in Perez-Staples D, Diaz-Fleischer F, Montoya P, Vera MT, Area-Wide Management of Fruit Fly Pests, CRC Press, London, UK.

DNA double-strand breaks (DSBs) are one of the most biologically significant DNA damage lesions. Exposure to ionising radiation (IR) causes DSBs in living organisms, which trigger intrinsic DNA repair mechanisms. Phosphorylation of the C-terminal of the core histone protein H2AX (termed γH2AX when phosphorylated) is an early known response to DNA DSBs. Quantification of the γH2AX response offers a highly sensitive and specific assay for detecting DSB formation and repair. Post-harvest exposure to IR of 150–400 Gy is an increasingly prominent phytosanitary measure in a variety of Australian (and imported) fruit. The radiation-induced γH2AX response has been shown to be highly persistent in the Queensland fruit fly ('Q-fly'; *Bactrocera tryoni*), Australia's most economically damaging insect pest of horticultural crops, lasting at least 17 days after exposure to IR. The presence of persistent γH2AX, indicating ongoing repair of impaired DNA, can be used to assess irradiation exposure in fruit flies. A direct and reliable assay using γH2AX as a marker of prior IR exposure in fruit flies has the potential to facilitate domestic and international trade in commodities that have been irradiated for disinfestation.

Project 3: Isotope ratio analysis

Summary

Methods based on Isotope Ratio Analysis (IRA) provide a timely opportunity to substantially improve the cost effectiveness, speed and reliability of identification processes that underpin Qfly outbreak declarations and pest management responses. In this project we develop cheap, fast and reliable IRA protocols for identifying sterile flies as an alternative to the comparatively expensive, slow and complicated microsatellite DNA methods (which will likely become unreliable if we are successful in ameliorating loss of wild type genetic material in domesticated Qflies). In brief, IRA procedures work because flies truly are what they eat; IRA procedures can identify whether a fly developed in fruit (wild) or in artificial media (sterile) through differences in the types of carbon that their bodies are composed of. This is because, due to differences in photosynthetic pathways, the carbon isotope signature from cane sugar used in the mass-rearing diet of sterile flies is distinctly different from the carbon isotope signature found in fruit.

Based on these differences in carbon isotope signatures, IRA procedures have been used to identify the addition of cane sugar into Australian fruit juices and can similarly be used to identify the presence of carbon from cane sugar in the bodies of mass-reared flies. By simply sampling a small part of the fly's body in an Isotope Ratio Mass Spectrometer (IRMS), IRA procedures reveal the body's carbon composition and yield clear identification. We assess IRA as a method for discerning sterile and wild Q-flies such that it could be implemented as a safeguard method, to complement dyes. Of note, the cost of the analysis is very competitive for the degree of protection that would be achieved against misidentifications.

Conclusions & Recommendations

- Intrinsic $\delta^{13}C$ analysis offers a precise tool to discriminate between sterile and wild Q flies in SIT programs
- $\delta^{15}N$ values, which can be analysed together with $\delta^{13}C$, do not provide a stand-alone method of discriminating between sterile and wild flies but give other insights into diet
- ^{13}C isotope enrichment of diets can be used to increase signal strength, although it is an expensive approach

Achievements

Outcome 1: Assess stable isotopes for reliable discrimination of sterile flies from the wild

Mainali BP‡, Allen A, Taylor PW & Rempoulakis P‡ (in press) Stable isotopes for reliable identification of wild and mass-reared Queensland fruit flies in sterile insect technique programs. *Journal of Pest Science*

In SIT, it is imperative to reliably discriminate released sterile Qflies from wild flies in monitoring traps for effective operations. Stable isotopes provide a permanent chemical marker to discriminate sterile and wild flies when dye marking is unclear. In this study, we compared the isotopic ratios of carbon and nitrogen between Qflies reared on different larval diets and wild flies collected from diverse locations in Australia and New Caledonia. Finally, we conducted a release–recapture study to corroborate differences in stable isotope C and N ratios in laboratory-reared and wild Q-flies. The $\delta^{15}N$ values obtained from wild and laboratory Qflies showed high variability that is likely related to the food source of the larval and/or adult stage and do not offer an effective means to discriminate between sterile and wild Q-flies. The $\delta^{13}C$ values of examined wild Q-flies ranged from − 27.46 to − 24.37‰ VPDB, whereas those from laboratory-reared, released and recaptured Q-flies ranged from − 25.73 to − 19.26‰ VPDB. Differences in $\delta^{13}C$ values resulted in 100% correct classification of wild flies and 96.88% correct classification of released flies. Intrinsic $\delta^{13}C$ values offer a precise tool to discriminate between released sterile flies and wild flies in SIT programs, regardless of the composition of the larval diet or adult pre-release diet. We suggest isotope ratio tests as a safeguard when fluorescent dyes fail to reliably classify captured Qflies as wild or sterile. The use of $\delta^{15}N$ values does not provide a stand-alone method of discriminating between laboratory and wild Q-flies.

Outcome 2: Assess isotopic enrichment for enhanced marking

Mainali BP‡, Allen A, Taylor PW & Rempoulakis P‡ (manuscript) Assessment of isotopic enrichment method for persistent marking of Queensland fruit fly for use in sterile insect technique.

Discrimination between wild and sterile flies is pivotal to success of sterile insect technique. Stable carbon isotope ratios are proposed as a precise tool to discriminate between wild and sterile Queensland fruit fly (Qfly) adults. A previous study conducted on Qfly demonstrated successful use of $\delta^{13}C$ value in discriminating wild from the sterile but with limited knowledge on persistency of the $\delta^{13}C$ value once the released sterile adults start foraging on C3 plants. We conducted an enrichment study by incorporating commercially available ^{13}C glucose in gel larval diet to understand changes in isotopic incorporation into body tissues following metamorphosis in a holometabolous Qfly with a major objective to isotope labelling of the Qfly to be more meaningful in the sterile insect technique (SIT) in terms of precision and persistence. We compared the changes in isotopic values of Q-fly produced from enriched and control diets at pre-imaginal stages and at different adult ages (0, 5, 10, 30 and 60 d). Our results suggest that the ^{13}C isotope enrichment through larval diet is sufficiently incorporated and fixed into body tissues and retention

of the isotopes were at a level significantly higher than the control. We also found age dependent depletion and shift of ^{13}C isotope towards the natural abundance level but since the $\delta^{13}C$ values of adult Qfly from enriched diet were significantly above the control Qfly and that of the wild flies reported in a previous study, we recommend use of enrichment as a precise and persistent tool when the releases are done to create barrier zones in Qfly eradicated zone.

Project 4: Genetic biomarkers

Summary

It is a challenge to develop a genetic biomarker technology that is shared by all released sterile flies, while at the same time seeking to retain genetic diversity during mass rearing. As part of the geographical variation analysis, our first approach was to use a genome-wide marker system (DArTseq technology) to identify population-specific genetic markers. The DArTseq analysis indeed revealed significant population differentiation, but none of the markers were diagnostic for any Qfly population. Instead, the observed genetic differentiation was due to differences in the relative proportion of allelic variants between populations (see Theme 5, Project 2). This was also true when we compared the SITplus factory flies with other Qfly populations, where the highly domesticated factory samples grouped amongst themselves and were differentiated from other populations, but the level of separation was within the margins of other interspecific comparisons. The results suggested that DArTseq (or similar marker systems) alone was insufficient to produce population-specific markers that could be used in identification of mass-reared (sterile) flies, and that population whole-genome re-sequencing might be needed to achieve the required resolution.

Although it should be easier to identify genetic markers that differentiate between species than populations within a species, diagnostic markers for separating members of the tryoni complex (*B. tryoni, B. aquilonis and B. neohumeralis*) were unavailable despite decades of efforts examining mitochondrial and nuclear sequences. We therefore undertook a whole-genome approach to tackle this challenge. This involved construction of high-quality chromosome-level genome assemblies for *B. tryoni* (GenBank GCA_016617805.2), *B. neohumeralis* and *B. jarvisi,* as well as a draft assembly for *B. aquilonis*. With these genomic infrastructures, we are now able to integrate population whole-genome resequencing or DArTseq data and organise these natural polymorphisms in a chromosomal context. Such information can be used in breeding readily identifiable Qfly compatible hybrids for SIT.

While the recent genome resource upgrades provided the critical tools, finding species-specific genetic markers can still be complicated by natural hybridisation / introgression between closely related species in sympatry. This is particularly problematic for the tryoni complex species because sexually compatible taxa co-exist in Queensland and the Northern Territory. For example, signals of bi-directional introgression have been reported between Qfly and NTfly in northern Australia (Theme 5, Project 2). In the case of finding diagnostic markers between Qfly and *B. neohumeralis*, the issue of hybridisation can be circumvented by using *B. neohumeralis* from the Torres Strait Islands and Qflies from the species' southern-most locations in NSW and VIC to define the reference genotype for each species. On the other hand, *B. jarvisi* is taxonomically outside the tryoni complex, one could expect numerous interspecific genetic differences (single nucleotide polymorphisms, small insertion/deletions, large-scale chromosomal rearrangements) between *B. jarvisi* and species within the tryoni complex (e.g., Qfly). These interspecific variations would provide the necessary ingredients for the development of robust diagnostic assays for these economically important group of species.

Conclusions & Recommendations

- *B. tryoni* vs *B. neohumeralis* diagnostic markers can be developed using informative X-linked polymorphisms
- *B. tryoni* vs *B. jarvisi* diagnostic markers can be developed chromosomal inversion polymorphisms
- Hybridisation can yield genetically distinct and readily identifiable types, and if sexually compatible with Qfly could be used in SIT releases

Achievements

Outcome 1: Assess hybridisation potential of Qfly relatives

Popa-Baez AD[†], Lee SF[‡], Rane RV Yeap HL[‡], Taylor PW & Oakeshott JG (manuscript) Genome-wide analysis of sympatric populations of two hybridisable bactroceran species identifies diagnostic genetic markers on putative X-linked genomic scaffolds.

Despite the lack ecological, pheromonal differences and post-zygotic reproductive barrier, *Bactrocera tryoni* and *B. neohumeralis* remain separate species that share an extensive sympatric range from northern Queensland to northern New South Wales. The quest for natural hybrids initially focused on the recovery of phenotypic (callus colour) intermediates, followed by microsatellite and nuclear gene markers, but have so far produced inconclusive results. Furthermore, diagnostic markers differentiating the three members of the tryoni species complex (*B. tryoni*, *B. aquilonis* and *B. neohumeralis*) have not been found despite intensive research efforts. Here we present a genome-wide analysis (DArTseq) on 604 flies (359 *B. tryoni* and 245 *B. neohumeralis*) from 52 populations collected across Australia and Torres Strait Islands, including 10 sympatric populations (Brisbane, Cairns, Cape Tribulation, Cape York, Coen, Cooktown, Mapoon, Mareeba, Rockhampton and Weipa). Clustering analyses of ~25,000 markers showed that across the 10 sympatric populations, two distinct clusters were formed representing members of each species, although one individual from Cape York appeared to have be an intermediate between these two main clusters. Detailed interrogation is underway to assess the likelihood of it being a natural hybrid. Furthermore, considering the *B. neohumeralis* individuals from 12 Torres Strait Island populations and *B. tryoni* individuals from 5 southern populations (VIC and NSW) as the two parental genotypes, we isolated 35 markers that show the highest differentiation between the two species. Based on the new reference *B. tryoni* reference genome, 10 of these 35 markers appeared to be single copy loci, scattered across multiple genomic scaffolds that were not assigned to any autosomes. Most of these putative diagnostic markers are found in a 23 Mb scaffold, which is believed to be a segment of the X-chromosome in *B. tryoni* based on strong *Bactrocera-Drosophila* synteny evidence. Our genome-wide survey of *B. tryoni* and *B. neohumeralis* populations confirmed the weak gene flow between the two taxa despite sympatry and uncovered putative X-linked markers that could be developed into routine species diagnostic assays.

Outcome 2: Jarvis' fruit fly as a potential Qfly hybrid

Southwood D[†], Rane RV, Lee SF[‡], Taylor PW, Ranganathan S & Oakeshott JG (manuscript) The genome of Jarvis' fruit fly (*Bactrocera jarvisi*) provides insight into the evolution of *Bactrocera* species.

Jarvis' fruit fly, *Bactrocera jarvisi*, is a significant pest in the Australian horticultural industry due to its destructive effect on mango crops. A lack of genomic resources has hindered efforts to understand its biology and evolution, including its phylogenetic relationship with other *Bactrocera* species and high heat tolerance. This study presents the first chromosome-level assembly of the *Bactrocera jarvisi* genome, assembled using a hybrid approach of short and long reads, with Hi-C sequencing for scaffolding. The final assembly is a 545.6 Mb genome with a scaffold N50 of 79.1 Mb, with 76.0% of bases anchored onto 5 pseudomolecules. Based on the analysis of universal single-copy orthologues, the genome is 98.1% complete, and has been annotated for 13,505 protein-coding genes. Comparative phylogenomic analysis with seven other tephritid species confirms the phylogeny of *Bactrocera jarvisi* among bactrocerans and provides further indications of branch timing estimates within its lineage. Evidence is presented of significant evolutionary pressures on the Australian bactrocerans, with genes related to heat tolerance and starvation adaptation noted as being positively selected. We also confirm the high degree of chromosomal conservation within higher dipteran species, with 91.3% of *Drosophila melanogaster* orthologues co-occurring in the corresponding Muller element in *Bactrocera jarvisi*. This high-quality *Bactrocera jarvisi* assembly provides a comprehensive resource for this horticultural pest, and the strong *Bactrocera-Drosophila* synteny presented here lays the genomic foundation for extrapolation of functional information from the model insect *D. melanogaster*. We confirm the phylogenetic positioning of *Bactrocera jarvisi* as being more closely related to the tryoni than the dorsalis complex of species, find evidence of a close relationship between *Bactrocera dorsalis* and *Bactrocera latifrons*, and note a rapid diversification of lure response phenotypes among bactrocerans.

Theme 5: Ecological competence

Ability to tolerate adverse environmental conditions in the period following release is critical for success of SIT. The mass-rearing environment is very different from the conditions that flies encounter following release and it is important to understand how flies cope with especially adverse conditions following release into conditions for which they may not be well suited. In this project, we investigated the traits that confer ecological performance following release (e.g., survival, dispersal).

Project 1: Genetics/Genomics of Qfly ecological fitness

Summary

This project in part complements Theme 1 Project 1 and Theme 5 Project 2 investigating differences due to domestication and source population in traits important to the ecological competence of mass-released sterile males. This project investigates the genetic basis of the differences. It focuses on desiccation resistance because the other projects found large variation in this critical trait due to domestication and between source populations.

This project first conducted two genome-wide association studies (GWAS) to identify the regions of the genome encoding the differences in desiccation resistance detected in Project 1. One GWAS involved making a set of isofemale lines from selected field populations, characterising their various levels of desiccation resistance and then conducted crosses between lines at opposite extremes of the resistance spectrum. Progeny from three generations (F4, F5, F6) were then bioassayed for resistance and their genomes sequenced. The second GWAS simply bioassayed and sequenced individuals from a strain recently collected from the field (Sydney). These studies required extensive pre-work to develop isofemale technology (a key genetical tool not previously available for any bactroceran) and a high-quality reference genome (now better than those for either Oriental or Mediterranean fruit fly).

This project then carried out metabolomic analysis of strains of different source population and domestication histories and levels of desiccation resistance. The goal was to identify the specific biochemical processes responsible for the differences in desiccation resistance. This in turn would identify specific causally involved genes within the genomic regions associated with resistance in the GWAS of Outcome 1. Desiccation resistance in other insects is linked to lipid biochemistry, so we specifically targeted the strains' lipidomes. Given that many lipids also used for energy storage, and domestication is known also to affect overall activity levels, we also focused on central metabolism. The lipidomic analysis identified several lipid types and processes which can be used to identify the candidate resistance genes. The genetic/genomic resources developed in this project have also been used in other areas of the program and the metabolic and lipidomic data also represent a versatile resource for future mechanistic work on other SIT-relevant traits that differ between populations or due to domestication.

Conclusions & Recommendations

- Desiccation stress tolerance is variable across individuals and is heritable
- Genes conferring desiccation resistance have been mapped, and can be used as markers in breeding programs
- Variation in desiccation resistance is linked to lipids involved in membrane structure

Achievements

Outcome 1: Establish phenotypic and genetic variance and heritability for stress tolerance

Prasad S[†], Popa-Baez AD[†], Yeap HL[‡], Rane RV, Pandey G, Colombo V[‡], Lee SF[‡], Taylor PW & Oakeshott JG (manuscript) Genetic basis for desiccation resistance in the Queensland fruit fly.

Queensland fruit flies (*Bactrocera tryoni*) are prone to desiccation in field environments and their spatial and temporal abundances are heavily influenced by their resistance to this stress. Substantial variation for desiccation resistance in natural Qfly populations has been reported and that the resistance of most strains declines significantly within a few generations in the laboratory (Theme 1 Project 1). The goal of this project was to elucidate the genetic basis of variation in desiccation resistance.

The first resource developed for this work was a set of isofemale lines from different locations. Isofemale lines are widely used for insect genetic analyses because each such line is established from a single inseminated female so there is relatively little genetic variation within but large differences between them. Previous attempts to make such lines of Qflies have failed but we were able to maintain and screen 12 isofemale lines from four locations (Alice Springs, Mareeba, Narrabri and Sydney) by modifications to culture conditions which substantially improved the productivity of individually housed females. Bioassays of these lines revealed significant differences in desiccation resistance that were stable over generations, indicating a genetic (or epigenetic) basis for the differences. Theme 7 Project 2 has also made use of this resource.

The second resource needed, both for this work and for other projects in the overall program, was a high-quality chromosome-level genome sequence assembly for Qfly. Earlier genomic work by Stuart Gilchrist, John Sved, Marianne Frommer and others had produced a draft assembly (GenBank GCA_000695345.1) but its coverage was incomplete and insufficient for either formal gene annotation or chromosome-level assembly of component fragments. Using a combination of short- and long-read sequencing technologies and genome assembly platforms, we have now achieved a chromosome-level assembly (GenBank GCA_016617805.2) and gene annotation and have made these resources publicly available at the National Center for Biotechnology Information database.

Two genome-wide association studies (GWAS) were carried out to map the genes underlying genetic variation for desiccation resistance in Qfly. In the first experiment we set up crosses between two isofemale lines from opposite extremes of the phenotypic (desiccation resistance) distribution of resistances among all the isofemale lines and then bioassayed >500 progeny individually in F4, F5 and F6 generations for resistance. Groups of these individuals showing different levels of resistance were then subject to whole-genome sequencing. The second experiment took a new wild-caught strain from Sydney which our earlier bioassays had suggested showed relatively high desiccation resistance, and again bioassayed three replicates of 500 flies individually for resistance and sequenced the top and bottom 5% of the phenotypic distribution. Precise genetic markers for desiccation resistance that can be used in breeding programs for SIT strains that preserve their resistance and hence ecological performance when released into the field.

Outcome 2: Define the matrix of correlations among ecological performance traits

Prasad S[†], Yeap HL[‡], Beale D, Pandey G, Colombo V[‡], Taylor M Lee SF[‡], Taylor PW & Oakeshott JG (manuscript) Biochemical basis for desiccation resistance in the Queensland fruit fly.

The biochemical mechanisms associated with desiccation resistance, and the variation in that resistance among populations and during domestication, is investigated. Seven strains were used for this experiment, collected from three different source populations: Sydney, Canberra and Cape Tribulation. These three populations varied significantly in desiccation resistance. From each of these three populations we analysed one recently collected strain (Generations G1-G2 in the laboratory) and one semi-domesticated strain (G12-G28) and for Sydney we used an additional, fully domesticated strain (G100+).

Comprehensive metabolomic profiles were collected from several replicate samples of males from each strain. Males were used because their resistance and metabolomic profiles are less influenced by variation in their reproductive biology than those of females. Two ages were assayed, one- and 19-days post-emergence. One

element of the metabolomics focused on the lipidomes, including free fatty acids, and the other element focused on central metabolism, including key sugars. The lipidomes were prioritised for analysis because lipids have been implicated in desiccation resistance in several other insects. Central metabolism was investigated because of the intimate link between the energy stored in several classes of lipids and its utilisation in central metabolism. Any differences in lipid profiles between strains could therefore have significant flow-on effects on central metabolism. Several hundred individual lipids (15 subclasses) and about three hundred central metabolites were assayed.

Differences have been found between strains from different source locations, but large numbers were found between the newly collected and semi- and fully domesticated strains. These included differences in the total amounts (summed peak area), carbon chain lengths and/or occasionally degrees of unsaturation of several subclasses of polar lipids (mainly phosphatidyl-serines, -ethanolamines and -glycerols), plus some differences in di- and triglyceride contents. Most of the differences were seen in one day old flies, suggesting differences in adult maturation processes. Many of the lipids implicated are involved in membrane structure, which has been shown to impact desiccation resistance in other insects.

Project 2: Regional variation in Qfly fitness genetics/genomics

Summary

Ability to tolerate adverse environmental conditions in the period following release is critical for success of SIT. As such, there is great value in understanding how fitness-linked traits, particularly resistance to abiotic stresses, vary naturally among Qfly populations. This will not only provide knowledge on the level of genetic variation that occurs naturally in these traits (and hence their capacity to respond to selection during rearing) but will also identify the potential importance of matching the characteristics of released flies to the environment in which they will be released. Genetic variation found in nature can be harnessed to produce flies of prescribed attributes. Further, such studies will also allow for some exploration of the degree of 'invasive potential' of flies from different populations, based on their ecological tolerance. The approaches taken here will also allow us to understand changes in fly characteristics that have taken place over the past 100 years during their southward range expansion, which has ancillary value in understanding likely effects of future climate change or likely ability of exotic tropical species to adapt should they establish in Australia.

Understanding of abiotic stress resistance in natural Qfly populations has been fragmentary and earlier findings of geographical differences might have been confounded by taxonomic uncertainties. Furthermore, the most recent genetic (microsatellite markers) survey of Qfly and NTfly population structure was published in 2010 and sampling was limited to mainland Australia. Updated knowledge of regional variation in abiotic stress resistance and population structure was necessary to reveal the current status of existing Qfly populations. These baseline data for Qfly also serve as the operational "wild type" reference for domestication analysis (Theme 1 Project 1) and also as a reference database for diagnosis of incursion origins.

This project investigates the traits that confer ecological performance following release (e.g., survival, dispersal) in Qfly and to produce a comprehensive population structure analysis based on recent and historical samples in Australia and the Pacific region. Populations collected from diverse climatic zones were also used by other SITPlus projects and the reference population clusters generated in this project aided the source-tracing of several Qfly incursions in Australia and New Zealand.

Conclusions & Recommendations

- Qfly populations vary significantly in desiccation stress resistance, although there is no north-south cline
- Population variation in desiccation resistance is stable across years
- Select source populations for SIT strains which have relatively high climatic stress resistance.
- Use of the reference genetic database of Qfly populations generated in this project for source detection of future incursions or outbreaks, to inform risk management with interstate trading of horticultural commodities.

Achievements

Outcome 1: Assess fitness traits that are likely to vary geographically

Popa-Baez A[†], Lee SF[‡], Yeap HL[‡], Prasad SS[†], Schiffer M[‡], Mourant RG, Castro-Vargas C[†], Edwards OR, Taylor PW & Oakeshott JG (2020) Climate stress resistance in male Queensland fruit fly varies among populations of diverse geographic origins and changes during domestication. *BMC Genetics* 21: 135

The highly polyphagous Queensland fruit fly (*Bactrocera tryoni* Froggatt) expanded its range substantially during the twentieth century and is now the most economically important insect pest of Australian horticulture, prompting intensive efforts to develop a Sterile Insect Technique (SIT) control program. Using a "common garden" approach, we have screened for natural genetic variation in key environmental fitness traits among populations from across the geographic range of this species and monitored changes in those traits induced during domestication. Significant variation was detected between the populations for heat, desiccation and starvation resistance and wing length (as a measure of body size). Desiccation resistance was correlated with both starvation resistance and wing length. Bioassay data for three resampled populations indicate that much of the variation in desiccation resistance reflects persistent, inherited differences among the populations. No latitudinal cline was detected for any of the traits and only weak correlations were found with climatic variables for heat resistance and wing length. All three stress resistance phenotypes and wing length changed significantly in certain populations with ongoing domestication but there was also a strong population by domestication interaction effect for each trait. Ecotypic variation in heat, starvation and desiccation resistance was detected in Australian Qfly populations, and these stress resistances diminished rapidly during domestication. Our results indicate a need to select source populations for SIT strains which have relatively high climatic stress resistance and to minimise loss of that resistance during domestication.

Outcome 2: Assess regional variation in fitness traits

Popa-Baez A[†], Catullo R, Lee SF[‡], Yeap HL[‡], Frommer M, Sved JA, Cameron EC, Edwards OR, Taylor PW & Oakeshott JG (2020) Genome-wide patterns of differentiation over space and time in the Queensland fruit fly. *Scientific Reports* 10: 10788.

The Queensland fruit fly, *Bactrocera tryoni*, is a major pest of Australian horticulture which has expanded its range in association with the spread of horticulture over the last ~ 150 years. Its distribution in northern Australia overlaps that of another fruit fly pest to which some authors accord full species status, *Bactrocera aquilonis*. We have used reduced representation genome-wide sequencing to genotype 359 individuals taken from 35 populations from across the current range of the two taxa, plus a further 73 individuals from six of those populations collected 15–22 years earlier. We find significant population differentiation along an east–west transect across northern Australia which likely reflects limited but bidirectional gene flow between the two taxa. The southward expansion of B. tryoni has led to relatively little genetic differentiation, and most of it is associated with a move into previously marginal inland habitats. Two disjunct populations elsewhere in Australia and three on Melanesian islands are each clearly differentiated from all others, with data strongly supporting establishment from relatively few founders and significant isolation subsequently. Resequencing of historical samples from one of the disjunct Australian populations shows that its genetic profile has changed little over a 15-year period, while the Melanesian data suggest a succession of '*island hopping'* events with progressive reductions in genetic diversity. We discuss our results in relation to the control of *B. tryoni* and as a model for understanding the genetics of invasion and hybridisation processes.

Outcome 3: Demonstrate utility of regional genetic variation for incursion tracing

Popa-Baez A[†], Lee SF[‡], Yeap HL[‡], Westmore G, Crisp P, Li D, Catullo R, Frommer M, Sved JA, Cameron EC, Edwards OR, Taylor PW & Oakeshott JG (2021) Tracing the origins of multiple Queensland fruit fly incursions into South Australia, Tasmania and New Zealand. *Biological Invasions*

Incursions of the Queensland fruit fly *Bactrocera tryoni* (Qfly) into areas without permanent Qfly populations present serious threats to the Australian and New Zealand horticultural industries. Identifying the origins of recent incursions will help reduce future threats by enabling the targeting of problematic incursion routes for more stringent quarantine protocols. Here we present an analytical framework based on supervised and unsupervised

machine learning to identify the origins and recent population history of incursion individuals. Our framework is based on a recently developed reference dataset of genome-wide markers for 35 Qfly populations from across the ranges of Qfly and the related taxon *Bactrocera aquilonis* (NTfly). We apply our framework to recent incursions into New Zealand, Tasmania and South Australia. Two distinct Qfly sources were identified for incursions into New Zealand (total 18 individuals), one from the east coast of Australia and one from New Caledonia. All eight recent incursion collections analysed (total 85 individuals) from South Australia and Tasmania most likely originated from just one of six clusters of populations in our reference database, Qfly from the east coast of Australia. None were found to originate from clusters containing NTfly or Qfly/NTfly hybrids in the Northern Territory or north-Western Australia. Several, but not all, of the collections showed signals of small founding population size and two Tasmanian collections each included individuals apparently derived from three different sources within the east coast of Australia. In total, several more incursion events were detected than previously known, although some were founded by relatively few individuals.

Theme 6: Applied landscape ecology

Project 1: Dispersal, maturation and survival

Summary

In SIT operations, Qfly have historically been held for two to three days following emergence at the rear-out centre and then released in the field before while still sexually immature. This holding period provides an opportunity to implement pre-release treatments that can accelerate male development and improve field performance. Sterile flies should be released when they are close to reaching sexual maturity to be immediately ready to mate. Adding supplements such as yeast hydrolysate, raspberry ketone and methoprene can accelerate sexual maturity.

The purpose of this project is to better understand the ecology and behaviour of sterile flies after release as part of an SIT program. Without this information, it is difficult to optimize release parameters such as where, when, and how many to release to maximize the effectiveness of SIT. As new lines of flies are established for the new SIT program, such as by RNAi or gene editing, there will be a need to assess the performance of these lines in the field through simulated releases and then tracking of released flies. Including wild type flies this project provides a valuable benchmark for comparison with mass-reared sterile flies thereby allowing assessment of the effects of domestication and sterilization in an ecological setting. This information will also be of enormous value in understanding how new outbreaks of flies spread in the environment.

Effects of extended holding and yeast hydrolysate supplementation on field performance of Qfly were assessed. While it is a simple intervention, and low-tech, his provided to be one of the most important studies of HG14033. Holding the flies for five days before release, rather than the previous standard of two days, resulted in a 6 – 8-fold increase in survival and maturation (as indicated by recapture rates) (Outcome 1). We also assessed orientation of Qfly when they are statically released from multiple points.

Conclusions & Recommendations

- Owing to 6 – 8-fold increase in survival and maturation, a five-day pre-release holding period is recommended for operational Q-fly SIT programs over the historical two - three day standard
- Pre-release provisioning with yeast hydrolysate is important, greatly increasing survival and maturation after release in the field
- Released sterile flies co-locate with wild flies at landscape scale, demonstrating broadly similar habitat preferences despite some differences in environmental tolerance

Achievements

Outcome 1: Assess effects of extended holding period and pre-release diet on survival and maturation

Biswas MJH[†], Mainali B[‡], Inskeep JR[†], Gaire S[†], Cross D, Stringer LD, Taylor PW & Rempoulakis P[‡] (in press) Extended holding period and yeast hydrolysate in pre-release diet increase abundance of mature sterile Queensland fruit fly males in the field. *Journal of Pest Science*.

For SIT to succeed, it is imperative that the released sterile males survive, disperse, attain sexual maturity, and are sexually competitive against their wild rivals. Q-fly SIT programs have conventionally held adult flies for two to three days and have sometimes fed them only sugar before release, providing little time or nutrition for development prior to release. We investigated whether a 5 d pre-release holding period and provision of yeast hydrolysate (YH) together with sugar in the pre-release diet increases abundance of mature male Q-fly in the field. Indicating increased survivorship and/or maturation, the combination of YH feeding and 5 d pre-release holding period resulted in 6-8 times more recaptures of mature male flies in cuelure traps than was the case for flies released at 2 d with or without YH and for flies released at 5 d without YH. Flies held for 5 d and fed YH were relatively more abundant than flies from other treatments in traps close to the release point and were as abundant as other treatments in traps at the greatest assessed distances. These findings strongly support a recommendation that sterile Q-flies be provided a pre-release diet of YH and sugar and be held for 5 d post eclosion before release. The increased abundance of cuelure-responsive male Q-flies indicates increases in the number that survive until sexual maturity, and hence increases in the numbers available in the field to compete with wild males for mating with wild females. While high rates of maturation are important, the sexually mature males still need to be competitive with wild males for mating with wild females. Further studies are now needed to investigate the effect of an extended pre-release holding period combined with dietary YH on Q-fly mating compatibility with wild females, and competitiveness with wild males.

Outcome 2: Assess co-location of sterile flies with wild flies at landscape scale

Biswas MJH[†], Mainali B[‡], Benelli M[†] & Rempoulakis P[‡] (manuscript) Effect of multiple release points and natural land habitat on the distribution of sterile Queensland fruit fly.

SIT relies on released sterile flies collocating and interacting with wild populations, and so it is important to understand factors that affect their distribution. In particular, distribution may be affected by availability of suitable habitat and abiotic conditions such as wind and temperature. In static ground release SIT programs, number of release points may also affect distribution. A release-recapture study was conducted by statically releasing sterile Q-flies in an open field. The higher recapture rates of sterile and wild Q-fly were achieved in most host plant availability than less host plant or grassland release sites, indicating the field abundance and distribution of sterile and wild Q-fly is affected by natural habitat. The field abundance and distribution of the flies was affected by host plant availability. Spatial distribution of the flies released from four points were relatively homogenous compared flies released from single central point, indicating more even distribution as a result of finer scale release grid. The sterile Q-flies co-localized with wild Q-flies. Wind and temperature had only minor effects on distribution of sterile Qflies.

Project 2: Temperature tolerance and cool storage

Summary

Efficient mass rearing methods are essential for the success of SIT. Mass rearing facilities need not only to reliably produce an adequate number of flies, but also to maintain high fly quality and practical delivery schedule, while at the same time minimising costs. While fly production is usually continuous, demand fluctuates. For example, more frequent releases and larger numbers are required during outbreaks. Cool storage provides a potential means to modulate the supply of insects, and could provide a useful tool for Q-fly SIT programs.

The aim of the project was to evaluate cold storage as a tool for prolonging the developmental time of Qflies at eggs and larval stages. This information is also of critical importance for the development of transportation methods to be used in FF17001. Building on studies of how domestication affects thermal tolerance (Theme 1 Project 1) and studies of regional variation in thermal tolerance (Theme 5 Project 2), this project evaluates cold storage as a tool for prolonging the developmental time while maintaining quality.

Responses of insects to temperatures that are below their optimal level for development have been investigated to increase our understanding of physiology and behaviour, to predict bioclimatic potential and responses to climate change, to develop strategies for disinfesting fruit, and even to establish cryopreservation protocols to store genetic resources. This project investigates the potential use of low temperatures as cool storage to prolong development and thereby modulate Q-fly production schedules. Such cool storage procedures can be useful to modulate production as a routine practice but can also be useful when needed to overcome perturbations in the production chain such as from mechanical failures or disease. In addition, cool storage may prove useful for developing egg shipment protocols for insect rearing programs. To prolong development, insects are usually stored at temperatures below their optimal development temperature, usually above 0 °C and most commonly in the range of 4-15 °C. Countering the benefits of cool storage, a reduction in the survivorship and quality of stored insects is expected, and to be greater for lower temperatures and for longer periods of storage. Accordingly, the benefits of storage duration must be balanced against any reductions in the quality of produced insects, and this needs to be considered in the development of protocols. Studies on Q-fly cold tolerance have focused mainly on survival, distribution and overwintering, pre-release thermal conditioning, and fruit disinfestation. Cool storage protocols have been adopted to synchronise pupal development during mass rearing in Q-fly SIT programs, so that pupae of different chronological age can be irradiated together when at a common physiological age. Although small scale rearing may also benefit from the ability to maintain a reserve of Q-flies, cool storage may provide convenience and economic benefits for the large-scale rearing required by SIT programs, improving the synchronisation of production and demand for field releases. The present study assesses the viability of cool storage of eggs and pupae to prolong developmental time.

Conclusions & Recommendations

- Eggs can be stored at 16 °C to prolong development by up to 6.5 days without reducing egg hatching or development.
- Pupae can be stored at 23 °C to prolong development by 2 days with minimal impact on quality

Achievements

Outcome 1: Assess potential for cool storage of eggs
Benelli M[†], Ponton F[‡] & Taylor PW (2019) Cool storage of Queensland fruit fly eggs for increased flexibility in rearing programs. *Pest Management Science* 75: 1056-1064

The Queensland fruit fly (Q-fly) is Australia's most economically damaging insect pest of fruit crops. The Sterile Insect Technique (SIT) used to suppress outbreaks relies on supply of high-quality flies and this can be assisted by the ability to manipulate production schedules. Cool storage at temperatures that are sufficient to slow development without causing significant somatic damage can provide a valuable means of manipulating production schedules. In this study, we investigate the effect of four storage temperatures (10, 13, 16 and 19 °C) and three exposure times (3, 6 or 9 days) on Q-fly eggs. Egg storage proved effective in prolonging the developmental time of Q-flies. Storage at 10 °C was unsuitable, resulting in a low hatching rate for all exposure times. Hatching rate was also significantly reduced when eggs were exposed to 13 °C for 6 or 9 days, followed by a significant reduction in the number of pupae recovered. Storage at 16 °C yielded promising results, prolonging the preimaginal development of Q-flies up to 6.5 days without significantly affecting egg hatching or subsequent development. Cool storage of eggs shows promise as a tool for prolonging the development of Q-flies to manipulate schedules in mass rearing programs.

Outcome 2: Assess potential for cool storage of pupae

Benelli M[†], Ponton F[‡] & Taylor PW (2019) Cool storage of Queensland fruit fly pupae for improved management of mass production schedules. *Pest Management Science* 75: 3184-3192

Cool storage is a valuable means of manipulating insect development time. The Queensland fruit fly (Q-fly) is Australia's most economically significant pest of fruit crops. The present study investigates cool storage of Q-fly pupae for increasing production flexibility for sterile insect technique programs. Development time, survival and fly quality were assessed following continuous storage of 1-day-old pupae at temperatures ranging from 13 to 25 °C. Survival was reduced almost to zero by pupal storage at 13 and 15 °C, was greatly reduced by storage at 17 °C, and was modestly reduced by storage at 19 °C. Pupal development time was extended by 16 days at 17 °C and by 9 days at 19 °C. Cool storage negatively affected flight ability and depleted lipid reserves. Cool storage at 19 °C enhanced the ability of 3-day-old adults to recover from chill-coma compared to control flies, indicating cold acclimation. There is potential for use of cool storage in Q-fly mass rearing, especially to improve alignment between production and field releases. For the purpose of delaying the development time of Q-fly pupae with minimal quality reduction, storage at 23 °C is recommended for 1-day-old pupae.

Project 3: Adaptive potential of Q-fly to geographic distribution and climate change

Summary

It is becoming increasingly important for pest control programs to consider biological responses to climate change. Several projects of HG14033 offer great potential to furthering our understanding of whether the invasiveness of Qfly varies geographically or may be altered by climate change, and whether different geographic regions may become more, or less, vulnerable to Qfly outbreaks. This project especially considers the current and likely future vulnerability of southern growing regions to Qfly and other endemic and exotic fruit flies, informed by the detailed understanding of environmental tolerance that will be developed in the course of this overall research program.

Climate modelling indicates that under all the standard climate scenarios those areas historically affected by Qfly will continue to be affected at least to 2070, and that regions of Victoria and Tasmania that are currently free of Qfly will become increasingly vulnerable (Outcome 1). While SIT and other techniques are under development to manage Qfly pest populations, there is some risk that other Australian pest fruit flies might move into the void opened where populations of Qfly are reduced or eliminated. To better understand which species are likely to take advantage of reduced competition from Qfly, climate suitability and the potential changes of distribution of eleven endemic Australian pest fruit flies is assessed (Outcome 2). The climate suitability of major horticulture areas currently in eastern Queensland, southern-central New South Wales and southern Victoria to other endemic fruit flies is likely to increase as climate changes. Further, incursion can also be from exotic species. As preparedness, climate suitability and the potential distribution in Australia of the major exotic fruit fly threats is assessed, finding that *Bactrocera dorsalis* has the highest establishment likelihood under all climate scenarios, followed by *Zeugodacus cucurbitae* and *Bactrocera latifrons* (Outcome 3). Finally, a review of potential implications for horticulture of climate change related changes in fruit fly distribution is compiled (Outcome 4).

Conclusions & Recommendations

- Regions currently affected by Qfly will continue to be affected to at least 2070
- Some additional regions not currently occupied by Qfly are already suitable
- Additional regions are likely to become suitable for Qfly as climate changes
- Large regions of Australia are suitable for major exotic fruit fly pests, and will remain suitable to at least 2070

Achievements

Outcome 1: Predict impacts of climate change on Qfly distribution

Sultana S[†], Baumgartner JB[‡], Dominiak BC, Royer JE & Beaumont LJ (2017) Potential impacts of climate change on habitat suitability for the Queensland fruit fly. *Scientific Reports* 7: 13025.

Anthropogenic climate change is a major factor driving shifts in the distributions of pests and invasive species. The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Qfly), is the most economically damaging insect pest of Australia's horticultural industry, and its management is a key priority for plant protection and biosecurity. Identifying the extent to which climate change may alter the distribution of suitable habitat for Qfly is important for the development and continuation of effective monitoring programs, phytosanitary measures, and management strategies. We used Maxent, a species distribution model, to map suitable habitat for Qfly under current climate, and six climate scenarios for 2030, 2050 and 2070. Our results highlight that south-western Australia, northern regions of the Northern Territory, eastern Queensland, and much of south-eastern Australia are currently suitable for Qfly. This includes southern Victoria and eastern Tasmania, which are currently free of breeding populations. There is substantial agreement across future climate scenarios that most areas currently suitable will remain so until at least 2070. Our projections provide an initial estimate of the potential exposure of Australia's horticultural industry to Qfly as climate changes, highlighting the need for long-term vigilance across southern Australia to prevent further range expansion of this species.

Outcome 2: Impacts of climate change on distribution of Australian pest endemic fruit flies

Sultana S[†], Baumgartner JB[‡], Dominiak BC, Royer JE & Beaumont LJ (2020) Impacts of climate change on high priority fruit fly species in Australia. *PLoS ONE* 15 (2): e0213820

Tephritid fruit flies are among the most destructive horticultural pests posing risks to Australia's multi-billion-dollar horticulture industry. Currently, there are 11 pest fruit fly species of economic concern in Australia. Of these, nine are native to this continent (*Bactrocera aquilonis*, *B. bryoniae*, *B. halfordiae*, *B. jarvisi*, *B. kraussi*, *B. musae*, *B. neohumeralis*, *B. tryoni* and *Zeugodacus* cucumis), while *B. frauenfeldi* and *Ceratitis capitata* are introduced. To varying degrees these species are costly to Australia's horticulture through in-farm management, monitoring to demonstrate pest freedom, quarantine and trade restrictions, and crop losses. Here, we used a common species distribution model, Maxent, to assess climate suitability for these 11 species under baseline (1960–1990) and future climate scenarios for Australia. Projections indicate that the Wet Tropics is likely to be vulnerable to all 11 species until at least 2070, with the east coast of Australia also likely to remain vulnerable to multiple species. While the Cape York Peninsula and Northern Territory are projected to have suitable climate for numerous species, extrapolation to novel climates in these areas decreases confidence in model projections. The climate suitability of major horticulture areas currently in eastern Queensland, southern-central New South Wales and southern Victoria to these pests may increase as climate changes. By highlighting areas at risk of pest range expansion in the future our study may guide Australia's horticulture industry in developing effective monitoring and management strategies.

Outcome 3: Climate suitability in Australia for major exotic fruit fly threats

Sutana S[†] (manuscript) Estimating the current and future risk of exotic fruit fly species establishing in Australia

Of the 46 native and non-native tephritid fruit fly pests that have been identified as presenting an economic threat to the Australian horticultural industry, 19 are currently absent from this continent. However, their geographic proximity to Australia and/or their status elsewhere as pests of horticultural industries that are also present in Australia, have led to their identification as 'high priority pests'. To date, the likelihood of these species establishing in Australia under future climate change has not be explored. The goal of this chapter is to undertake climate matching for these 19 species and to assess how their relative establishment likelihoods (EL) may change due to shifts in climate. To do so, I combined maps of regions of Australia with a climate similar to species' known ranges, under current and future climates, with a key arrival pathway (i.e., the movement of people entering Australia from host countries) and the distribution of host plants, to estimate species relative ELs. I found that

Bactrocera dorsalis has the highest EL under all climate scenarios, followed by *Zeugodacus cucurbitae* and *B. latifrons*, while *B. occipitalis* and *Rhagoletis indifferens* consistently have the lowest EL. As the century progresses, the ranking of the species generally remains stable. However, the EL of *Anastrepha ludens*, *B. carambolae* and *Toxotrypana curvicauda* increases considerably. In contrast, EL of all three *Rhagoletis* species is projected to decline. My findings are valuable for the horticultural industry as well as pest managers, as they enable appropriate ongoing surveillance and management strategies to be planned and initiated.

Outcome 4: Review expected impacts of climate change on fruit fly impact

Sultana S[†] (manuscript) Review of potential impacts of climate change on tephritid pest species

Understanding the responses of pest species to climate change is imperative if monitoring programs and management strategies are to be effective in the future. Climate change will affect many insect species including those in the Tephritidae family, which include some of the world's most economically damaging horticultural pests. The goal of this review is to highlight how tephritid pests may respond to climate change. The evidence for direct responses – range shifts, responses to extreme events, changes to species' phenology, and adaptive capacity – and indirect responses, such as via host plants or natural enemies. is critically assessed. Few studies, beyond those using species distribution models to assess future range shifts, have been undertaken to explore the responses of tephritids to climate change. As such, the breath of responses must be inferred from studies on related taxa. Priority areas for future research, and the development of recent tools that could advance our understanding of the responses of tephritid species to climate change are highlighted.

Project 4: Local drivers of fruit fly behaviour

Summary

As populations of flies move and spread through the environment, they do so through a series of decisions of individual flies not as a collective. So, while we might discuss the movement and spread of populations as an issue, to understand the patterns of population movement and then how we might manipulate the environment in order to constrain the movement of populations we need to understand the behaviour of individual flies. For example, what light environments do flies seek out? How does variation in local conditions (e.g., ambient temperature, humidity, wind) affect fly movement? By understanding the environmental drivers of Qfly behaviour we can use this information to modify the environment in ways that assist in management and targeting of SIT releases. In addition to understanding dispersal patterns at a landscape level, we need to characterise how flies behave relative to resources at a local (i.e., fruit tree) level. While Theme 6 Project 1 indicates that sterile and wild flies co-locate in the same general areas of the landscape, do sterile flies respond at a finer scale to local habitat features in the same was as wild flies?

This project assessed microhabitat distribution of mass-reared fertile, sterile and wild Qfly males and females in tree canopies through the day (Outcome 1). Mass-reared fertile and sterile flies occupied different locations in the canopy from wild flies, especially at hotter times of day when the mass-reared flies stayed lower in the canopy where they apparently sought shelter. This pattern aligns with lower thermal tolerance and preferences of mass-reared Qflies (Theme 1 Project 1). Sterile and fertile mass-reared flies did not differ, indicating that the dominant effects are from mass-rearing and that there is little or not additional impact of irradiation. Next, this project assessed the spatial and temporal distribution of male mating activity at dusk (Outcome 2). Again, there were some differences between mass-reared (fertile and sterile) and wild flies; mass-reared flies called earlier in the evening and deeper in the canopy. Such temporal and spatial differences in mating activity could potentially lead to low levels of mating between wild and sterile flies even when they are co-located in the same general landscape locations (Theme 6 Project 1).

To some extent, local distribution of flies in plant canopies is mediated by aggressive interactions. Wild flies tend to enter agonistic interactions from greater distances than mass-reared flies (Outcome 3), and this likely reflects adaptation for crowded conditions. However, overall, wild and mass-reared flies are similar in their tendency to initiate agonistic interactions, and are also similar in their tendency to win (Outcome 4).

Irradiation used to sterilise Qflies is found to reduce general locomotor activity (Outcome 5) and this could potentially impact the rate of dispersal of the flies following release. General locomotor activity can be a good measure of general health, and the reduced locomotor activity of sterile flies likely reflects somatic damage. Irradiation also reduced the ability of flies to tolerate periods of food deprivation.

Conclusions & Recommendations

- Mass-reared (both fertile and sterile) males exhibit mating behaviours that differed spatially and temporally from wild conspecifics, which could lead to assortative mating.
- Although wild flies initiate agonistic interactions at greater distances, mass-reared flies are similarly likely to initiate and win overall
- Sterilization by irradiation at the pupal stage reduces Qfly locomotor activity and starvation tolerance

Achievements

Outcome 1: Assess microclimate preference of sterile and wild Qflies

Inskeep J[†], Allen AP, Taylor, PW, Rempoulakis C[‡] & Weldon CW (in press) Canopy distribution and microclimate preferences of sterile and wild Queensland fruit flies. *Scientific Reports*

Insects tend to live within well-defined habitats, and at smaller scales can have distinct microhabitat preferences. These preferences are important, but often overlooked, in applications of the sterile insect technique. Different microhabitat preferences of sterile and wild insects may reflect differences in environmental tolerance and may lead to spatial separation in the field, both of which may reduce the control program efficiency. In this study, we compared the diurnal microhabitat distributions of mass-reared (fertile and sterile) and wild Qfly. Flies were individually tagged and released into field cages containing citrus trees. We recorded their locations in the canopies (height from ground, distance from canopy center), behavior (resting, grooming, walking, feeding), and the abiotic conditions on occupied leaves (temperature, humidity, light intensity) throughout the day. Flies from all groups moved lower in the canopy when temperature and light intensity were high, and humidity was low; lower canopy regions provided shelter from these conditions. Fertile and sterile mass-reared flies of both sexes were generally lower in the canopies than wild flies. Flies generally fed from the top sides of leaves that were lower in the canopy, suggesting food sources in these locations. Our observations suggest that mass-reared and wild Qfly occupy different locations in tree canopies, which could indicate different tolerances to environmental extremes and may result in spatial separation of sterile and wild flies when assessed at a landscape scale.

Outcome 2: Assess effects of domestication and irradiation on temporal and spatial patterns of sexual activity

Inskeep J[†], Taylor PW, Mainali B[‡], Rempoulakis C[‡] & Weldon CW (2021) Spatio-temporal distribution of sexual calling behaviour in domesticated, sterile and wild Queensland fruit fly males under field cage conditions. *Pest Management Science* 77: 2522-2529

To assess the likely impact of SIT on wild Qfly populations it is important to assess the colocation and synchrony of male calling between sterile and wild flies. We observed the location and timing of calling behaviours of marked mass-reared (fertile and sterile) and wild Qfly males in walk-in field cages. We found that wild males called further from the canopy centre than mass-reared (fertile or sterile) males. Mass-reared (fertile or sterile) males called earlier in the evening than wild males and, consequently, mass-reared males called when temperature and light intensity were higher than when wild males called. Male calling is a prerequisite to mating among dacine fruit flies. Therefore, our observations of spatio-temporal divergence in male calling behaviour may lead to assortative mating between mass-reared and wild Qfly in SIT applications. The importance of these spatio-temporal differences warrants further inquiry, with particular focus on how environmental conditions modify calling behaviour and avenues to ameliorate differences between sterile and wild flies.

Outcome 3: Describe agonistic interactions of mass reared and wild Qfly

Inskeep J[†], Mainali B[‡] & Taylor PW (manuscript) Effect of prior conspecific interactions and age on the aggressive interactions of mass-reared and wild Queensland fruit flies.

Insects frequently engage in aggressive behaviors to secure resources, monopolize favourable microhabitats, acquire mates, or repel predators. Aggressive behaviors are common in tephritid fruit flies, and understanding of these behaviors may be insightful for applications of the sterile insect technique (SIT) used to manage some species. We observed aggressive interactions between male Queensland fruit flies, Qfly, (over 20-minute sessions. Flies were derived from wild or laboratory populations, at young (4-8 days) or old (12-16 days) ages, and held under solitary (1 individual per cage) or crowded (10 individuals per cage) conditions from emergence. Wild flies engaged in aggression at a further distance than mass-reared flies, older flies engaged in aggression for longer periods of time than younger flies, and more intense aggressive behaviors took place when flies were closer to each other. Flies held under crowded conditions engaged in fewer aggressive interactions than flies held singly from emergence. Our results suggest that mass-reared flies may engage in aggressive behaviors at a closer distance and may engage in fewer aggressions overall due to adaption to mass-rearing conditions.

Outcome 4: Assess effects of domestication and residency on agonistic interactions

Inskeep JR[†], Mainali B[‡], Adnan SM[†], Benelli M[†], Biswas J[†], Gaire S[†] & Taylor PW (manuscript) Aggression in the Queensland fruit fly: Effects of domestication and residency.

Aggressive encounters are frequent among Qfly adults, but there has been very little investigation of these interactions. We observed the aggressive encounters of marked Qfly from domesticated and wild populations in cages containing leaves from lemon trees. More aggressive encounters were observed at midday than at dusk, and domesticated and wild Qfly initiated aggression at similar rates. Residents and intruders won aggressive interactions at similar rates in domesticated and wild Qfly. This study adds to a very small body of literature describing the aggressive behaviours of Qfly. The role of intraspecific aggression in Qfly, as in many other tephritids, is unclear as while aggressive encounters between conspecifics are frequent their significance for fly fitness remains unknown.

Outcome 5: Assess effects of domestication and irradiation on locomotion and food deprivation tolerance

Inskeep JR[†], Adnan S[†] & Taylor PW (manuscript) Irradiation and desiccation stress affect the survival and activity of Queensland fruit flies.

Applications of the sterile insect technique (SIT) for management of Qfly may be improved by identifying, and ameliorating, the impact of stressors that constrain performance of released sterile insects. Irradiation used to sterilize insects induces somatic tissue damage that can directly reduce performance and can increase susceptibility to other sources of injury or stress. SIT is commonly deployed in hot, dry regions of Australia and desiccation can be a major source of stress and mortality for sterile Qfly; domestication tends to reduce desiccation tolerance in Qfly and this effect may be exacerbated by irradiation. The present study investigates the separate and combined effects of irradiation and desiccation stress on survival and locomotor activity of male and female Qfly. In survival under stress assays, males died faster than females and there was a significant interaction between effects of stress treatment (starvation vs. starvation and desiccation) and irradiation. Fertile flies exposed to starvation alone lived longer that those also exposed to desiccation, but for sterile flies there was no difference between stress treatments with flies exposed to both treatments having short survival that was similar to the fertile flies exposed to desiccation stress. Locomotor activity was higher in male flies, and this may contribute to their lower survivorship under stress; however, locomotor activity does not provide a general explanation, as fertile flies were more active than sterile flies but had similar or higher survivorship, depending on stress. Sterile males depleted more lipid stores than fertile males before death when exposed to starvation alone, but the pattern was reversed when flies were also exposed to desiccation. For females, there were no differences between stress treatments in lipid depletion before death. Our results indicate that irradiation of Qfly to induce sterility reduces activity levels and also ability to tolerate starvation, but does not reduce ability to tolerate the combined effects of starvation and desiccation.

Theme 7: Mating ability

Sex is the 'active ingredient' of SIT. If flies are successfully domesticated, reared, and released, and survive and mature in the field, but then fail to perform sexually, then SIT will be severely compromised or fail altogether. Numerous studies of overseas SIT programs have highlighted sexual deficiencies caused by mass rearing and irradiation of fruit flies. In order to understand where failures might occur or where there is potential for improvements, we first need to understand the mating system and what male traits confer high mating success. With this knowledge, we can then consider how mass-reared strains might be modified to produce males that are more likely to be accepted as mates by wild females.

Project 1: Variation and functions of pheromones

Summary

Pheromones - sexual odors - are central in fruit fly mating systems with each species producing a highly characteristic blend. Historically, Qflies have been thought to have five major components in their pheromone blend, and pheromone composition is almost certainly an important component of species recognition and mate assessment. Although pheromones have been described for numerous fruit fly species, including Qfly, little is known of the functional significance of pheromone components and blends. This is particularly striking given the relevance of pheromones to mating success and hence SIT.

This project investigated pheromone variation from multiple sources. Understanding the importance of each pheromone component and blend characteristics enables consideration of management implications, in particular opening the possibility of breeding of more attractive males.

To establish appropriate methods, this project started by comparing the available methods for collection and analysis of fruit fly pheromones, identifying preferred approaches and outlining the limitations of each approach (Outcome 1). This work also identified five previously unknown compounds in male pheromones, and three in females. Additional investigation of putative female pheromones was conducted, highlighting prominence of amides previously reported as the main components of the male pheromone blend (Outcome 2). Having quickly identified previously unreported compounds in male pheromones, and identified a complex putative pheromone blend emitted by females, a much more complex than expected pheromonal communication system was emerging. To document this more fully, the rectal gland secretions of males and females were subjected to even more refined analysis, identifying numerous additional compounds that potentially serve pheromonal functions (Outcome 3). With a high resolution of chemical analysis available, focus then turned to considering potential regional variation and effects of domestication. While no consistent effects of region were found, there were very consistent effects of domestication as male Qflies from older colonies produced larger amounts of pheromone in their mate-attracting emissions (Outcome 4). Rather than being a result of larger glands or greater production, the effects of domestication on pheromone emission were traced to changes in sexual effort as males from an old colony had higher calling rate (Outcome 5).

In the course of investigations in Theme 7 Project 1, a significant potential pheromonal role of chemicals associated with the Qfly cuticle was identified and explored. Cuticular chemistry is also an important mediator of desiccation resistance in insects, and so these studies also contribute to Theme 1 Project 1, Theme 5 Project 1 and Theme 6 Project 3. The cuticular chemistry of Qfly males and females is described in terms of compounds that are likely involved in environmental tolerance and compounds that are likely involved in communication (Outcome 6), and changes in chemical profile of the cuticle as males and females age is documented (Outcome 7). Comparing studies using wild flies (Outcome 6) and studies using domesticated flies (Outcome 7) revealed few qualitative differences, indicating that in a broad sense Qfly cuticular chemistry does not change much with domestication. However, there are some quantitative differences that are very likely important both for desiccation resistance and sexual communication.

Conclusions & Recommendations

- Both male and female pheromones are important in the Qfly mating system
- Previous studies have greatly underestimated the complexity of pheromonal communication
- Domestication increases pheromone emission, potentially improving sexual success
- Domestication increases sexual calling effort, potentially improving sexual success
- Cuticular chemistry is revealed as an important form of pheromonal communication

Achievements

Outcome 1: Develop suitable methods for sampling and analysis of Q-fly pheromones

Noushini S[†], Park SJ[‡], Jamie I, Jamie J & Taylor PW (2020) Sampling technique biases in the analysis of fruit fly pheromones: A case study of Queensland fruit fly. *Scientific Reports* 10: 19799

Diverse methods have been used to sample insect semiochemicals. Sampling methods can differ in efficiency and affinity and this can introduce significant biases when interpreting biological patterns. We compare common methods used to sample tephritid fruit fly rectal gland volatiles ('pheromones'), focusing on Queensland fruit fly, *Bactrocera tryoni*. Solvents of different polarity, n-hexane, dichloromethane and ethanol, were compared using intact and crushed glands. Polydimethylsiloxane, polydimethylsiloxane/divinylbenzene and polyacrylate were compared as adsorbents for solid phase microextraction. Tenax-GR and Porapak Q were compared as adsorbents for dynamic headspace sampling. Along with compounds previously reported for *B. tryoni*, we detected five previously unreported compounds in males, and three in females. Dichloromethane extracted more amides while there was no significant difference between the three solvents in extraction of spiroacetals except for (E,E)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane for which n-hexane extracted higher amount than both dichloromethane and ethanol. Ethanol failed to contain many of the more volatile compounds. Crushed rectal gland samples provided higher concentrations of extracted compounds than intact rectal gland samples, but no compounds were missed in intact samples. Of solid phase microextraction fibers, polyacrylate had low affinity for spiroacetals, ethyl isobutyrate and ethyl-2-methylbutanoate. Polydimethylsiloxane was more efficient for spiroacetals while type of fiber did not affect the amounts of amides and esters. In dynamic headspace sampling, Porapak was more efficient for ethyl isobutyrate and spiroacetals, while Tenax was more efficient for other esters and amides, and sampling time was a critical factor. Biases that can be introduced by sampling methods are important considerations when collecting and interpreting insect semiochemical profiles.

Outcome 2: Identify potential female pheromones

El Sayed AM, Venkatesham U, Unelius CR, Sporle A, Pérez J[‡], Taylor PW & Suckling DM (2019) Chemical composition of the rectal gland and volatiles released by female Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). *Environmental Entomology* 48: 807-814

The composition of the rectal gland secretion and volatiles emitted by female Queensland fruit fly, *Bactrocera tryoni* was investigated. Esters were found to be the main compounds in the gland extracts and headspace, while amides were the minor compounds in the gland extracts and headspace. Ethyl dodecanoate, ethyl tetradecanoate, ethyl (Z9)-hexadecenoate and ethyl palmitate were the main esters in the gland extracts, while ethyl dodecanoate and ethyl tetradecanoate were the main esters in the headspace. Four amides (N-(3-methylbutyl)acetamide), N-(2-methylbutyl)propanamide, N-(3-methylbutyl)propanamide, and N-(3-methylbutyl)-2-methylpropanamide were found in the gland extracts and the headspace. Among the amides, N-(3-methylbutyl)acetamide and N-(3-methylbutyl)propanamide were the main amides in the gland extracts and the headspace. Traces of three spiroacetals were found both in the gland extracts and in the headspace. (E,E)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane, (E,E)-2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane, (E,E)-2-propyl-8-methyl-1,7-dioxaspiro[5.5]undecane. All compounds found in the headspace were present in the extract of the rectal gland suggesting that the rectal gland is the main source of the headspace volatiles, whose function remains to be elucidated. This is the first comprehensive chemical analysis of the rectal gland secretions and volatiles of female *B. tryoni*, and further laboratory and field bioassays are required to determine the function of compounds

identified in this study. Discovery of the same amides previously identified in the male rectal gland in the female rectal gland raises questions about the pheromonal role previously suggested for these compounds.

Outcome 3: Increase resolution of potential pheromones in male and female Qfly

Castro-Vargas C[†], Pandey G, Yeap HL[‡], Lacey MJ, Lee SF[‡], Park SJ[‡], Taylor PW & Oakeshott JG (manuscript) Differences in rectal gland volatiles of Queensland fruit fly due to sex and mating status.

The Sterile Insect Techniques used against Queensland fruit fly (Qfly) relies on disrupting mating behaviour. We used solid-phase microextraction plus gas chromatography-mass spectrometry (SPME GC-MS) and gas chromatography-flame ionization detection (GC-FID) to characterise the chemistry of Qfly rectal glands, where pheromones contributing to these behaviours likely originate. A few amides and fatty esters dominate the chromatograms of males and females respectively, but we also find many other esters, several alcohols and ketones and over 150 unidentified volatiles in lesser amounts. The SPME GC-MS analysis identified 22 of the 29 compounds previously recorded from Qfly rectal glands, plus 26 other compounds that had not previously been reported from these glands. The GC-FID analyses showed 49 and 12 compounds were male- and female-specific, respectively, both before and after mating. Ten other compounds were male-specific among virgins but undetected in mated males, and 29 undetected in virgins were male-specific in mated samples. The corresponding figures for females were just four and zero. Most short retention time peaks (including an identified ketone and an ester) were male-specific, whereas in most female-biased peaks (including five identified fatty esters) had long retention times. Our results indicate previously unsuspected diversity of candidate pheromones for various mating-related behaviours in males, but far fewer in females.

Outcome 4: Assess impacts of domestication on pheromone emission

Pérez J[‡], Park SJ[‡] & Taylor PW (2018) Domestication modifies the volatile emissions produced by male Queensland fruit flies during sexual advertisement. *Scientific Reports* 8: 16503

Insects commonly undergo substantial changes during adaptation for laboratory or mass-rearing environments ('domestication') that may have significant implications for inferences from laboratory studies and utility for biological control. We assessed the effect of domestication on the amount and blend of volatiles released during sexual calling by laboratory-reared Bactrocera tryoni males using colonies from three regions of Australia: Brisbane, Cairns and Sydney. For each region, volatiles released by males from a young colony (five or fewer generations) and an old colony (20+ generations) during sexual calling was compared. Males from old colonies released more volatiles than males from young colonies. All components of the blend were more abundant in one or more of the older colonies, although differences varied by compound and by region. To assess changes over generations, the young and old colonies obtained from Brisbane were sampled at 5, 12 and 15 generations (young colony) and 25, 35 and 38 generations (old colony). While the old colony remained unchanged, flies from the young colony released more volatiles at each sequential sampling episode, and became increasingly similar to the old colony. Increased volatile production during domestication may be an adaptive response to crowded rearing conditions in which males need to overcome a chemically noisy environment to be sexually successful.

Outcome 5: Resolve links between acoustic calling and pheromone release

Pérez J[‡], Park SJ[‡], Cameron DNS & Taylor PW (manuscript) Increased calling tendency and calling rate explain increased pheromone release in domesticated Queensland fruit fly.

Sounds produced by fruit fly males through wing fanning are commonly known as "calling songs" and have been correlated with male mating success in some species. In addition to functioning as acoustic signals, wing fanning has been associated with the dispersion of sex pheromones. At dusk, males of Queensland fruit fly, *Bactrocera tryon*i ('Q-fly'), release a blend of volatiles and produce a series of buzzing to attract receptive females. In this study we compared the calling songs and the amount of volatiles released and produced by laboratory reared Q-fly males using a young and an old colony from the same region (8 and 28 generations, respectively). Calling probability, total calling time in an evening, pulse train duration, pulse train interval, and pulse train period did not differ between the colonies. However, males from the old colony had higher calling rate (pulses/minute) than

males from the young colony. Males from the old colony released a higher amount of volatiles than males from the young colony. Conversely, no significant difference was found in the total amount of volatiles present in the rectal glands between males from both colonies.

Outcome 6: Describe cuticular chemistry

Park SJ[‡], Pandey G, Castro-Vargas C[†], Oakeshott J, Taylor PW & Mendez V[‡] (2020) Cuticular chemistry of the Queensland fruit fly *Bactrocera tryoni* (Froggatt). *Molecules* 25: 4185

The cuticular layer of the insect exoskeleton contains diverse compounds that serve important biological functions, including the maintenance of homeostasis by protecting against water loss, protection from injury, pathogens and insecticides, and communication. *Bactrocera tryoni* (Froggatt) is the most destructive pest of fruit production in Australia, yet there are no published accounts of this species' cuticular chemistry. We here provide a comprehensive description of *B. tryoni* cuticular chemistry. We used gas chromatography-mass spectrometry to identify and characterize compounds in hexane extracts of *B. tryoni* adults reared from larvae in naturally infested fruits. The compounds found included spiroacetals, aliphatic amides, saturated/unsaturated and methyl branched C12 to C20 chain esters and C29 to C33 normal and methyl-branched alkanes. The spiroacetals and esters were found to be specific to mature females, while the amides were found in both sexes. Normal and methyl-branched alkanes were qualitatively the same in all age and sex groups but some of the alkanes differed in amounts (as estimated from internal standard-normalized peak areas) between mature males and females, as well as between mature and immature flies. This study provides essential foundations for studies investigating the functions of cuticular chemistry in this economically important species.

Outcome 7: Document variation in cuticular chemistry

Park SJ[‡], Taylor PW & Mendez V[‡] (manuscript) Cuticular chemistry of the Queensland fruit fly varies between the sexes, with age and across the body.

Chemicals excreted or deposited by insects on the cuticle are important for protection from desiccation and pathogens, and also for communication. Cuticular chemistry is of increasing interest in tephritid fruit flies, amongst the world's most destructive insect pests, in order to understand their environmental tolerances and reproductive biology. The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) is the most studied Australian fruit fly. Although rectal gland, headspace and cuticular chemistry of mature *B. tryoni* have been previously described, variation in cuticular chemistry through post-emergence development and aging, and across the body, have not been reported. Using gas chromatography-mass spectrometry (GC-MS) of n-hexane extracts, we here describe changes in cuticular chemistry of *B. tryoni* from emergence until 70 days of age and describe variation across the head, thorax, legs, abdomen, and wings of 14-day old (mature) flies. Cuticular chemistry of males did not vary with age. Females younger that 5 days post-emergence had cuticular chemistry that was very similar to that of males, but then diverged rapidly with most compounds increasing in abundance. In both males and females, cuticular chemistry varied across the body although in sex-specific patterns, this being the first report of such somatic variation in a tephritid fly. Our findings point to substantial unexplained biological variation in function of cuticular compounds between the sexes, across the body, and though the life of *B. tryoni*. Despite extensive research on environmental tolerance and its links to distribution, and sexual communication, in tephritid flies, and the knowledge that cuticular chemistry is a key mediator of these traits in other insects, the variation and function of cuticular chemistry in tephritid flies remains little known.

Project 2: Genetics/Genomics of Qfly reproductive fitness

Summary

This project characterises aspects of Qfly reproductive performance that are likely to affect the efficacy of SIT programs, to test how they differed between wild populations and then to investigate the genetic basis of the variation observed. We also investigated the species specificity of traits, using the sibling species *B. neohumeralis* as the major comparator. It was decided to focus on sex pheromones because of prior work suggesting they were

important in many fruit fly species and also given that another key trait, female remating inhibition was being analysed in Theme 8, Project 2.

Using state of the art gas chromatography and mass spectrometry, we analysed the cuticular compounds and rectal gland compounds in Qfly (Outcome 1). About 200 volatile secretions from Qfly rectal glands were detected with many qualitative and quantitative differences between the sexes and due to mating status. Prior work had suggested that six aliphatic amides may contribute to male sex pheromone functions, and those compounds were found to be abundant in male glands. Several smaller molecules were found to be male specific, while large numbers of fatty esters were found to be principally produced by females. More than thirty of the compounds, including some of the small male-specific compounds and one of the amides, showed significant heritable variation between isofemale lines established from different geographic source populations. Some but not all of the variation was correlated across peaks suggesting some genetic control. Additionally, a domesticated control line showed higher levels of many of the peaks than did the isofemale lines, raising the possibility that domestication could actually enhance the competitiveness of SIT males over their field counterparts for mating with field females (as also found in Theme 7 Project 1).

Conclusions & Recommendations

- Pheromone composition varies between individual males and is heritable
- Multiple genes are indicated in defining individual pheromone blend
- Many pheromone components increase in abundance through domestication
- Use a marker assisted breeding strategy to select colonies for mass rearing and to retain the desired specific pheromonal compounds or blends

Achievements

Outcome 1: Assess variation in reproductive fitness traits

Castro-Vargas C[†], Pandey G, Yeap HL[‡], Lacey M, Lee SF[‡], Park SJ[‡], Taylor PW & Oakeshott JG (manuscript) Rectal gland volatiles are polymorphic in Queensland fruit fly and change during domestication.

Genetic divergence in mating behaviour is hypothesised to be a major premating isolating mechanism between incipient species. However few cases of inherited intra-specific variation in sexual signalling have yet been reported. We have tested for such variation in a member of the tryoni complex of tephritid fruit flies, the Queensland fruit fly (Qfly). Aspects of Qfly mating behaviour depend on volatiles secreted from male rectal glands and we have previously identified 33 compounds among about 150 volatiles in these glands. Here we report inherited variation among 24 Qfly lines (23 isofemale lines from recent field collections and one domesticated line) in the abundance of two esters, two amides and 23 unidentified volatiles in male rectal glands, but none among volatiles in female rectal glands. The two amides have previously been implicated in female attraction. There was significant variation in the male volatiles among isofemale lines, indicating polymorphism both within and between geographic populations, and several volatiles were also more abundant in the domesticated line than in any of the isofemale lines. While some variation in different peaks was correlated across lines much of it was not, implicating multiple genes. These results indicate the existence of significant polymorphism in chemistry underlying a potential premating isolating mechanism. Qfly and related species are major horticultural pests, and our findings also have important implications for the Sterile Insect Technique used to control them.

Outcome 2: Assess genomic regions contributing to variation in reproductive fitness traits

Castro-Vargas C[†], Pandey G, Park SJ[‡], Lee SF[‡], Taylor PW & Oakeshott JG (manuscript) Sibling species differences in rectal gland volatiles and their diurnal profiles of emissions.

Our previous work has shown an unexpectedly large number of rectal gland volatiles in the Queensland fruit fly, *Bactrocera tryoni* (Qfly) which could be candidates for sex pheromone functions. As a first step towards identifying

those that are directly involved in reproductive behaviour, this experiment investigated the association between their release and the wing vibrations, aka 'calling' behaviour, exhibited by pre-courtship males. We did this by characterising the gland contents of virgin and mated males and females and then the emissions of volatiles of reproductively mature males across 24-hour periods, including one at dusk while they were calling. To further interrogate any associations found, we repeated the experiment on the sibling species, the lesser Queensland fruit fly, *Bactrocera neohumeralis* (LQfly), which calls in the middle of the day rather than dusk. We tested two strains of each species because our earlier work had shown some quantitative differences between Qfly strains in their rectal gland volatiles. Samples were analysed by solid-phase microextraction and gas chromatography-mass spectrometry (SPME GC-MS) as per our previous work. We found qualitative and quantitative differences between the rectal gland volatiles of males and females of each species dissected three hours before their respective calling times. The Qfly results concur with our previous work. The LQfly profiles in each sex were qualitatively similar to those for Qflies of the same sex but, as with the Qflies, there were several qualitative and quantitative differences between the sexes. The emissions we detected from the males of each species were significantly smaller subsets of their rectal gland profiles. Both species showed qualitative and quantitative changes in their emissions profiles across their diurnal cycles and total emissions in each species peaked at their respective calling times. Certain amides previously implicated in short range attraction of females contributed to the respective calling time peaks of the two species but so did some other, as yet uncharacterised, compounds. The latter compounds are also now prime candidates for sex pheromone functions, including longer range female attraction. These findings form the basis for future characterisation of the new candidate pheromones chemically and in their effects on female behaviour. As many of the volatiles change in abundance during domestication, they also provide chemical markers for maintaining male attractiveness during the development of mass release strains for SIT programs.

Theme 8: Protecting sterile matings

While mating between released sterile males and wild females is an essential step on the path to successful SIT, it is not the last. Female fruit flies store sperm and then use these sperm to fertilize eggs through their life. If a wild female mates with a released sterile male but then later remates with a fertile wild male then she will be able to produce viable offspring and SIT will be compromised.

Project 1: Prevalence and predictors of multiple mating

Summary

SIT depends on low levels of remating by wild females. Remating of wild females can severely restrain the effectiveness of SIT, as females that remate can acquire viable sperm from fertile wild males, nullifying the effect of a first mating with a sterile male. Understanding sperm storage and usage can aid in understanding the consequences of remating, which can in turn guide decisions of how to respond operationally.

Techniques historically used to quantify sperm storage in Qfly and other fruit flies have been difficult to implement and prone to error. The first task of this project was to develop a new kind of assay for assessing sperm number that would enable more accurate understanding of post-copulatory interactions. A new PCR-based technique was developed and demonstrated to be highly effective and accurate (Outcome 1). Of note, this method demonstrated that previous studies likely substantially underestimated the number of sperm transferred and stored in Qfly copulations. Overall, though, the general patterns of sperm distribution detected using the new method broadly resembled the patterns detected using the previous less accurate methods, with a high degree of asymmetry of sperm storage between the spermathecae that sets the stage for differential storage of sperm of different males in different storage organs by females. Such patterns could increase the prevalence of female fertility in cases where females mate with both a sterile and a fertile male.

Subsequent studies developed Qfly strains with distinct microsatellite profiles and then used the PCR method developed in Outcome 1 to quantify the sperm of different males in the two spermathecae of twice-mated females. These studies found that females tended to store fewer sperm from a second mate, and that asymmetry of storage of the first male's sperm had a direct effect on sperm storage patterns of the second mate (Outcome 2). That is, asymmetry of storage by the first mates does result in differential storage of sperm from multiple males in different spermathecae, and would very likely mean that if females mate with both a sterile and a wild male then

she is likely to express some fertility through most of her reproductive life.

The next series of experiments moved on to quantify sperm usage patterns of female Qflies that mated with two different males, using flies from a CRISPR-modified 'yellow' line for which paternity is readily identifiable from unmodified flies. Initially the paternity patterns indicated that females drew equally from the sperm of their first and second mate to fertilize eggs, but this gradually shifted in favour of the first male through the female's reproductive life (Outcome 3). This pattern indicates partial stratification of sperm as the sperm from the second male somewhat overlays that of the first mate. However, the impact of stratification is offset by asymmetry of sperm storage, which means that sperm of the first male tend to be close to the top from the outset of female reproduction in one of the two spermathecae. Impact of stratification is also offset by the numerical advantage of the first male's sperm. This has implications for SIT, as some previous studies have reported low numbers of sperm stored by mates of sterile males (Collins et al. 2012, *Journal of Insect Physiology*), and that sterile males are unable to replenish sperm supplies after mating (Radhakrishnan et al. 2009, *Animal Behaviour*).

While there have been some studies investigating multiple paternity in wild populations of other fruit flies, including medflies, olive fruit flies, and tobacco flies, there had been no studies of paternity (an estimate of remating tendency) in wild female Qflies. This information is important for understanding Qfly reproductive biology, and also for its applied benefits in SIT. Published laboratory studies of domesticated, mass-reared, Qflies indicate that 20-40% do remate at least once. While these studies have raised some concerns about remating rates of wild female Qflies, it is important to be careful not to interpret these findings directly as estimates of remating levels of wild females in the field. Data from wild type flies in the field were needed as a baseline for estimation of natural remating rates. If many wild females remate, then it is important for sterile releases to be maintained at higher levels and closer intervals than if few wild females remate. A set of highly polymorphic microsatellite markers previously used in population genetic analyses was used to for assess the prevalence of remating in two wild Q-fly populations (Sydney, NSW and Maroochydore, QLD). The number of mates in wild females was estimated by comparing the alleles detected in the sperm DNA from their spermathecae against their respective population frequencies. Our results showed that remating of female Qflies is common in nature (Outcome 4). Remating patterns are particularly important during the period when only a bisex strain is available for SIT as it is important for estimation of the extent to which the released sterile females will distract released males. If released females tended to mate only once, then the effect will be far less than if they mate more often. The prevalence of remating in wild populations may require increased numbers and frequency of release to ensure sustained SIT efficacy in the field.

Conclusions & Recommendations

- Remating by female Qflies is common in the field
- A high abundance of sterile flies in the field is important not only to increase sterile matings of virgin females but to ensure that a large proportion of rematings are also with sterile males
- Females commonly store sperm from more than one male
- Paternity of first and second mates changes as females age, with increasing use of first mate sperm

Achievements

Outcome 1: Develop an accurate sperm quantification assay

Shadmany J[†], Lee R[‡] & Taylor PW (2021) Real-time PCR-based Y-specific sperm quantification assay in Queensland fruit fly: Insights to patterns of sperm storage. *Insect Molecular Biology* 30: 315-324

Studies of reproductive biology in insects often require quantification of sperm production, transfer or storage. Here, we develop a quantitative real-time PCR-based assay using a Y-specific marker for quantification of sperm from spermathecae of female Queensland fruit fly ('Q-fly'), overcoming constraints typical of traditional sperm quantification methods. The assay enables accurate and reliable quantification of as few as 50 sperm and provides a means to analyse large numbers of samples with flexible timing. The real-time PCR method enables revised understanding of how many sperm are stored by female Q-flies, the distribution of storage between the two spermathecae, and the relationship between copula duration and sperm storage. Real-time PCR assays based on Y-

specific markers provide an effective solution for sperm quantification in tephritid flies, as well as in other insects and potentially other animals with sperm storage organs.

Outcome 2: Determine sperm storage patterns

Shadmany J[†], Lee R[‡] & Taylor PW (in press) Patterns of sperm storage in twice-mated Queensland fruit flies. *Journal of Insect Physiology*

Polyandry, whereby females mate with more than one male in a reproductive cycle, can result in sperm competition or cryptic female choice, and can influence on both male and female fitness. Understanding patterns of sperm storage in twice-mated females can provide valuable insights to mechanisms that mediate sperm use and paternity. In the Queensland fruit fly, *Bactrocera tryoni* (Qfly), and other insects that are managed by the Sterile Insect Technique (SIT), polyandry can reduce the extent to which released sterile males are able to reduce fertility of pest populations. Patterns of sperm storage in twice-mated Qflies were studied by developing three fly lines that are homozygous for different alleles of a microsatellite marker (Bt32) and using a combination of quantitative real time polymerase chain reaction (qPCR) and capillary electrophoresis-based techniques to quantify and genotype sperm in each spermatheca. Female Qflies consistently stored fewer sperm from their second mate than from their first mate. Further, asymmetry between the spermathecae in the distribution of sperm stored from the first mate appears to in part determine the distribution of sperm stored from the second mate, likely because of constraints in storage capacity in the two spermathecae. Implications of these findings for elucidating pattern of sperm competition in this species, and for SIT, are discussed.

Outcome 3: Determine sperm usage patterns

Shadmany J[†], Lee R[‡], Nguyen TNM & Taylor PW (manuscript) Patterns of sperm use in twice-mated Queensland fruit flies

Multiple mating by females, polyandry, is common in insects, including in tephritid fruit flies. Female insects that remate commonly store sperm of multiple males. How the sperm of different males contributes to paternity is an important element of sexual selection. Sexual behaviour and reproduction of the Queensland fruit fly (Qfly), *Bactrocera tryoni*, has been extensively investigated both in relation to understanding this economically important species' reproductive biology and in relation to implications for sterile insect technique, whereby sterile flies are released to constrain reproduction of pest populations. Despite numerous studies of pre- and post-copulatory sexual selection in Qfly, there have been no direct studies of paternity patterns in polyandrous female Qflies. We used two morphologically distinguishable lines to investigate patterns of sperm use in Qfly. The two lines showed comparable mating performance evidenced by similar mating and remating frequency, copula duration, and proportion of second mate paternity (P2) between reciprocal crosses. The mechanism of sperm usage, with P2 close to 0.5 immediately after the second mating followed by gradual decrease of P2 as females aged, is most consistent with stratification or repositioning of sperm. Patterns observed in the present study are compared with the available information from other tephritid fruit flies, and are discussed in relation to this species' reproductive biology, known patterns of sperm storage, and sterile insect technique.

Outcome 4: Quantify incidence of remating in field populations

Shadmany J[†], Lee R[‡] & Taylor PW (manuscript) Polyandry in wild populations of the Queensland fruit fly.

Female insects commonly have more than one mate during a breeding period ('polyandry'), storing and using sperm from multiple mates. Polyandry has substantial evolutionary significance, and also has practical implications for pest management using the sterile insect technique (SIT). In SIT large numbers of sterile males are released to mate with wild females, reducing reproduction and hence reducing numbers in the next generation. High remating rates in wild females can reduce SIT efficiency. The Queensland fruit fly, *Bactrocera tryoni* (Froggatt), is one of Australia's major horticultural pests and is managed by SIT in some regions. The present study assesses polyandry rate of female *B. tryoni* in two field populations from New South Wales (NSW) and Queensland (QLD) through microsatellite genotyping of the stored sperm. Conservative and probabilistic estimates of average number of mates per wild female were 1.27 and 1.54 for the NSW population and 1.8 and 2.64 for the QLD population

respectively. These values for wild populations are in general agreement with remating propensity as reported in laboratory studies of domesticated *B. tryoni*. Implications of these findings for SIT operations are discussed.

Project 2: Mechanisms of sexual inhibition

Summary

Diverse insect species, including Qfly (Theme 8 Project 1), are known to utilise polyandrous mating systems, in which females mate with multiple males. Apart from its importance to Darwinian fitness in natural populations, female remating propensity is also an important issue for SIT. The success of SIT depends on mated females not remating to any significant extent; otherwise, wild females initially mated to sterile SIT males can still produce fertile eggs with any wild male with which they subsequently remate. If minimal remating can be achieved, the size of the pest population will be reduced drastically in the next generation.

Females of many flies become sexually unreceptive to later suitors for varying lengths of time after their first mating. Most of the evidence to date indicates that certain small seminal fluid proteins play a major role in inhibiting females from remating. Male seminal fluid is a complex mixture of sperm, seminal fluid proteins, salts, sugars, lipids, hormones, water, immune regulators and vesicles from male reproductive tissues. However, in the relatively few systems which have been analysed in any depth it is the proteins which are generally found to modulate the post-mating behaviours of females.

Remating inhibition has been most extensively studied in *Drosophila melanogaster*, where the Sex Peptide (SP) is the primary male factor that regulates remating inhibition and other post-mating responses in mated females. Although the *D. melanogaster* SP is a useful paradigm for understanding the mechanism of sexual inhibition in some *Drosophila* species, it is probably not the only inhibitory pathway in insects. This is because SP is only found in the *Drosophila* genus and heterologous injection experiments have also demonstrated clear phylogenetic constraints on SP function. It is therefore unclear to what extent the *Drosophila* SP-mediated remating inhibition mechanism can be extrapolated to the bactroceran fruit flies.

Understanding the behavioural, genetic and biochemical mechanisms involved in sexual inhibition would allow more effective monitoring of this trait during the rearing and sterilisation process, and could identify strategies to manipulate the process to achieve greater levels of suppression, thereby improving the efficacy of SIT releases. This project started by investigating behavioural mechanisms that might underpin the expression of sexual inhibition and indicate a mating-induced change in olfactory preferences as key (Outcome 1). While virgin females are more attracted to pheromones emitted by males than to fruit odors, mated females are more attracted to fruit odors. This makes biological sense given the need for virgin females to seek males for sperm to fertilize their eggs and the need for mated females to seek fruit to oviposit. This behavioural pattern also provides valuable clues about the behavioural mechanisms mediating sexual inhibition. While physical resistance has a role, in many cases mated females would be able to maintain a high level of control over both mating and remating through their responses to male pheromones.

Understanding natural genetic variation in sexual inhibition would support either the ability to identify and choose lines of insects with ample ability in this trait for SIT, or to artificially select lines for increased ability to induce sexual inhibition (Outcome 2). In experiments using colonies established from different collections, and followed through the domestication process, massive differences amongst colonies were found. Through the domestication process females became increasingly likely to accept remating, but there was no evidence for changes in male ability. The positive effects of domestication on female remating propensity is a potential risk for SIT under the current bisexual system because this means that the released sterile females will absorb a disproportionate fraction of male mating effort leaving fewer males on any evening to mate with wild females.

Investigation then turned to focus on the characteristic of male ejaculate components that are responsible for inducing sexual inhibition in females. Numerous seminal fluid proteins and other candidate compounds for mediation of sexual inhibition were identified (Outcome 3). In a parallel approach, stable isotope marking of male ejaculate was used to track the movement of ejaculate molecules after transfer to females (Outcome 4). This approach yielded a shortlist of candidate male ejaculate proteins to be targeted as prospective mediators of sexual inhibition in Qfly.

Conclusions & Recommendations

- There is substantial regional variation in female remating tendency
- Regional variation is heritable
- Include male ability to induce sexual inhibition in mates as a criterion for choice of mass-reared colony
- Females become increasingly prone to remate through domestication
- Increased female remating tendency through domestication could impede SIT by absorbing a disproportionate amount of sterile male mating effort
- Domestication-induced changes in female remating should be considered in decision of when to replace domesticated colonies
- Female responsiveness to male pheromones is an important behavioural mechanisms of sexual inhibition
- Seminal fluid proteins potentially involved in induction of sexual inhibition of females are identified

Achievements

Outcome 1: Identify behavioural mechanisms that mediate sexual inhibition

Devescovi F[‡], Hurtado J. & Taylor P.W. (2021) Mating-induced changes in responses of female Queensland fruit fly to male pheromones and fruit: A mechanism for mating-induced sexual inhibition. *Journal of Insect Physiology* 129: 104195

In order to reproduce, female tephritid fruit flies need both mates for fertilization and fruit for oviposition. Virgin females are prone to mating and approach males, attracted by their pheromones. Mated females, however, may experience an abrupt reduction of mating propensity and prioritise the search for suitable fruit rather than additional mates. Accordingly, mating in fruit flies may induce a switch in olfactory preferences of females from pheromones to fruit stimuli, and this switch may also be an important mediator of mating-induced sexual inhibition. To test for mating-induced switches in olfactory preference of female Queensland fruit fly, *Bactrocera tryoni*, we used wind tunnel assays to assess attraction of mated and virgin females to (1) male sex pheromone delivered through a perforated glass sphere or (2) an entire fruit. Electroantennogram (EAG) responses were also used to test for mating-induced changes in olfactory sensitivity to pheromones and fruit odours. Pheromones elicited quicker upwind responses in virgin females than in mated females; during the first six minutes of trials more virgin females than mated females were observed in the upwind end of the wind tunnel where pheromone odours were released. Fruit cues, in contrast, elicited stronger association with the upwind end of the wind tunnel in mated females than in virgin females from the fifth minute onwards. Also, mated females were observed on the fruit for longer periods than virgin females. EAG responses to pheromones and fruit odours were similar in virgin and mated females, indicating that changes in preferences are not a consequence of changes in peripheral sensitivity of antennae to odours but instead appear to be mediated by post-receptor processing. Our results show that mating reduces attraction to male-produced pheromones and increases attraction to fruit stimuli in *B. tryoni* females. We propose that this behavioural switch from mating stimuli to oviposition stimuli is an important mediator of mating-induced sexual inhibition in this species.

Outcome 2: Quantitative characterization of phenotype

Ahmed KA[†], Yeap HL[‡], Pandey G, Lee SF[‡], Taylor PW & Oakeshott JG (manuscript) Population differences and domestication effects on mating and remating frequencies in Queensland fruit fly.

Fundamental to the Sterile Insect Technique (SIT) is the ability of the sterile mass-released males to inhibit their wild female partners from remating with wild males. The available evidence indicates that this effect is a largely sperm-independent but little else is known about it. As a first step in elucidating its basis, and therefore potentially increasing its strength and the efficiency of SIT, we have investigated whether there is genetic variation for its strength in wild populations and how that variation might change during domestication.

In our first experiment we measured intra-strain mating and remating frequencies among three recently collected caught populations from different Qfly populations (Canberra, Sydney and Cape Tribulation) and how that changed

in the first ~20 generations of laboratory rearing. We found up to three-fold inherited differences between strains from different localities in the level of intra-strain remating inhibition. This ties in with evidence from work on other traits and various genetic and genomic studies showing ecotypic variation between wild Qfly populations, including those studies here. We also found that the level of inhibition declined significantly during domestication, which implied the existence of genetic variation for the trait within the starting populations as well. Notably about half the variation in remating frequencies of the females from the different strains was correlated with their frequencies of first matings, but the other half was independent of first mating frequencies.

To understand the basis for patterns in remating frequencies we carried out two further experiments, in this case including inter-strain first and second pairings. These trials showed that the differences due to source population and domestication seen in the first experiment were mainly due to female genotype and the second male, with only a minor effect of initial male genotype. The major roles of both the female and second male genotypes were in effect both first mating effects since trials with virgin second males gave the same result. The small role of first male genotype does not indicate that the first male does not contribute to remating inhibition, but it does indicate that there was little genetic variation for the first male contribution, within or between the populations studied.

Outcome 3: Transcriptomic / proteomic analysis of male tissues involved in sexual inhibition

Ahmed KA[†], Lee SF[‡], Pandey G, Taylor PW & Oakeshott JG (manuscript) Proteomic analysis of remating inhibition in Queensland fruit fly.

The Queensland fruit fly (Qfly) *Bactrocera tryoni* is a major pest of Australian horticulture. Sterile Insect Technique is being developed to control Qfly but remating of females with wild males could undermine its success. In Qfly, the transfer of seminal fluid during mating influences the remating propensity of females. However, the protein and peptide composition of Qfly seminal fluid has not been characterised. Here we present a proteomic analysis of Qfly seminal fluid to identify candidate peptides for sexual inhibition. We characterised the reproductive gland proteomes of sexually mature virgin males, which possess full gland contents, just-mated males of the same age whose gland contents have been transferred to their mates, and males dissected 13–16.5 hours after mating whose gland contents should be substantially replenished. We identified 63 seminal fluid proteins, 42 of which could not be functionally unannotated. However, we did not find homologues of the Sex Peptide responsible for remating inhibition in *Drosophila melanogaster* but matches to three other peptide hormones were found. Several proteases, post-translational protein modifying enzymes, and ligand-binding proteins were also identified and some number of which could be responsible for activation and transport of prohormones to their receptors in the female.

Outcome 4: Transcriptomic / proteomic / metabolomic analysis of female tissue completed and the fate and physiological effects of male-donated proteins in mated females determined

Ahmed KA[†], Lee SF[‡], Pandey G, Kamath K, Song SM, Taylor PW & Oakeshott JG (manuscript) Stable isotope proteomic analysis on mated female reproductive organs to identify male donated molecules during mating in the Queensland fruit fly.

Sexual inhibition of mated females in many insects is induced by male seminal fluid contents. We present here a stable isotope (N^{15}) workflow to track movement of male-donated molecules in mated females of the Queensland fruit fly (Qfly), *Bactrocera tryoni*. To minimise confounding effects from dietary nitrogen sources other than the ammonium sulphate supplement, we systematically altered each nitrogen-containing component in the diets and ensured that such changes did not affect mating. Substitutions of agar with agarose and brewer's yeast with baker's yeast in either the larval or adult diet did not abolish mating, despite reduced productivity. To produce N^{15}-labelled flies, we replaced the N^{14}ammonium sulphate in the baker's yeast growth medium with N^{15} ammonium sulphate and incorporated the N^{15} yeast in the insect diets. Reciprocal mating pairs were set up between the N^{15} and N^{14} flies and the reproductive organs were harvested from mated females. Proteomic analysis was then performed on these female reproductive tissues. N^{14}-labelled, male-donated molecules were identified in mated female reproductive tissues, which were N^{15}-labelled. Using the Proteome Discoverer software, we identified 95-108 hits when matched against the *B. dorsalis* reference protein database, and 89-179 hits against the Qfly male reproductive tissue transcriptome database. Further, we also used Open-sourced Trans-proteomic pipeline, which

enhance the number of proteins matches against Qfly male reproductive tissue transcriptome to 412. One hundred and thirty-nine of these proteins have a signal peptide, indicating that they might be secreted or membrane-bound proteins, some of which whose homologues in *Drosophila melanogaster* are seminal fluid proteins or have also been detected in our male reproductive organ proteomes (accessory glands, ejaculatory bulbs, testes). The present study therefore validates the transfer of these candidate male-donated proteins and provides a shortlist of targets for detailed experimentation in the future.

Theme 9: Compatible control technologies

Project 1: Predators as control agents

Summary

As fruit fly management moves to a more environmentally soft approach, there will be a substantial increase in the potential for natural enemies to contribute to control. There is a need to understand how to better harness this resurgent pest management resource. Very little is currently known about sources of mortality of released Qflies, and in particular the role of predators. While abiotic factors such as heat, desiccation and nutrition are surely important, abiotic factors such as pathogens, parasites and predators are also important.

Predators are not only enemies of wild flies, but they are also enemies of released sterile flies and may contribute to the decline in numbers after release. Initial studies used enclosure methods to investigate the performance of sterile flies at inducing sterility in females in the presence and absence of predators (Outcome 1). Males were more vulnerable to predation than females, but sterile and fertile males were similar in risk. Females had greatly reduced fertility in the presence of sterile males, regardless of the presence of predators. If predators are an important source of mortality in the field, then it is important that the released sterile flies be competent in anti-predator responses. Domestication and irradiation affect many aspects of Qfly performance, and this project considered whether this extends to anti-predator responses. While domestication and irradiation had little impact on the behavioural responses of flies to predators, both domestication and irradiation diminished the ability of male Qflies to evade predation (Outcome 2). This likely reflects a general reduction in activity of domesticated and irradiated flies (Theme 2 Project 2).

While killing is the most obvious and well-studied aspect of predator-prey interactions, predators can also have substantial 'non-consumptive' effects on prey species that can be of importance in pest management contexts. Mating is key to SIT, and interference of mating by predators could potentially impact SIT efficacy. Mantids are common fruit fly predators, but in this study no evidence was found of mating interference in the presence of mantids (Outcome 3). In contrast, odors emitted by spiders and ants were found to have very strong impacts on fly behaviour, significantly inhibiting foraging, oviposition and mating and in some cases inducing increased movement that reflects an escape response (Outcome 4). This finding indicates that, in addition to contributing to mortality of released sterile Qflies, predators could interfere significantly with diverse aspects of fruit fly biology. While hunting and volatile emissions of predators may interfere with matings by sterile flies in SIT programs, this would be balanced by comparable interference of matings by wild flies. In areas where SIT is not conducted, if predators are maintained at adequate levels then the combined consumptive an non-consumptive impact of predators on fruit fly numbers could be significant.

Ultimately while it is highly desirable to have direct estimates of the impact of predators in the field, this is also technically very challenging. In order to estimate the real-world impact of predators on Qflies in the field, a real-time PCR protocol was developed to identify the presence of Qfly DNA in the gut of predators (Outcome 5). This tool can be used to survey the prevalence of Qfly feeding by predators to estimate the impact of predation on fly numbers.

Conclusions & Recommendations

- Sterile Qflies have diminished ability to evade predators, likely owing to reduced motility
- Incorporate testing of anti-predator performance in periodic QC assays
- Predators odors can induce avoidance or stasis, depending on predator species
- Odors from diverse predators inhibit foraging, mating and oviposition in Qfly
- A PCR assay is developed to monitor and quantify predation of Qfly

Achievements

Outcome 1: Impact of predators on efficacy of sterile Qflies

Rathnayake DN[†], Lowe EC[‡], Rempoulakis P[‡] & Herberstein ME (2019) Effect of natural predators on Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) control by sterile insect technique (SIT). *Pest Management Science* 75: 3356-3362

Queensland fruit fly (Q-fly) is a destructive insect pest that infests a wide variety of agricultural plants in Australia. The sterile insect technique (SIT) is used to manage Q-flies, but the effectiveness of SIT has not been tested in the presence of natural predators. The objective of this study was to investigate the effect of natural predators and SIT on the survival and reproduction of laboratory reared Q-flies under semi-natural conditions. We altered the presence of predators and irradiated Q-fly males, and measured survival, number of eggs laid and egg-hatching rate. The presence of natural predators significantly affected the survival of Q-flies and appeared to decrease the number of eggs laid. Interestingly, we found that both sterile and fertile males were more prone to predation than females, but we found no difference among males. The presence of sterile males significantly reduced Q-fly fertility, but the interaction of natural predators and sterile males did not significantly reduce the number of fertile eggs. Our findings highlight the important role of natural predators in controlling Q-flies together with SIT and provide a solid foundation for similar large-scale field trials using wild counterparts.

Outcome 2: Assess impact of domestication and irradiation on vulnerability to predators

Rathnayake DN[†], Rendon D[†], Lowe EC[‡], Taylor PW & Herberstein ME (manuscript) Effect of irradiation and mass rearing on predator evasion in Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae).

Queensland fruit fly is a serious horticultural pest in Australia and is currently controlled using Sterile Insect Technique (SIT). Domestication and mass rearing are key requirements in SIT, but theses processed are likely to negatively affect the performance of the SIT flies. High-density mass rearing is expected to reduce the fly's awareness of the surrounding movements, and the irradiation lowers the fly activity, emergence, flight ability and survival. Combined, these treatments are expected to make flies more vulnerable to their predators once released. Therefore, in this study we aimed to compare predator evasion of domesticated, irradiated and wild Q-flies using two potential invertebrate predators; a praying mantid and a jumping spider. Contrary to our predictions, we found that the mass reared irradiated male flies responded to both predators in a similar way to wild flies. However, we discovered that the wild female flies are most capable at evading predators and the irradiated, domesticated male flies were the least capable of predator evasion. The time taken to first attack, to kill and the number of attacks by both predators were similar for domesticated, irradiated and wild male flies. These results encourage monitoring of the quality of released flies against their natural predators for better Q-fly SIT control in Australia.

Outcome 3: Assess non-consumptive effects of predators on Qfly mating activity

Rathnayake DN[†] (manuscript) Effect of predation risk on the mating behaviour of Queensland Fruit Fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae).

Queensland fruit fly (Q-fly) is a serious horticultural pest that infests over one hundred economically important fruits and vegetables in Australia. Sterile insect technique (SIT) is currently used to control Q-flies. Natural

predators may help in keeping the Q-fly population in balance, but, at the same time may negatively interfere with SIT by manipulating mating opportunities/behaviours of SIT released flies. However, very little is known about the effect of natural predators on the mating behaviour of Q-flies and thereby on the success of SIT released males. Therefore, in the current study, I compared the mating behaviour of wild, domesticated and irradiated male Q-flies in a laboratory setting in the presence and absence of a predation risk. The flies were scored for mating frequency, copulation latency and copulation duration in the presence and absence of a mantid predator. Surprisingly, results suggest that the presence of a predator did not affect Q-fly mating behaviour. However, the origin of the male (whether it was wild or domesticated) seemed to have a significant effect on their mating behaviour. The frequency of mating in wild males was significantly lower, and, they took longer to copulate but copulated for a longer time compared to the domesticated flies, irrespective of predator presence. While this study does not indicate that predators will negatively impact the current SIT, future studies that test different predator species and in large scale semi-natural/natural set up are crucial before making broad conclusions.

Outcome 4: Assess effects of predator odors of Q-fly locomotion, mating, oviposition and foraging

Kempraj V[†], Park SJ[‡] & Taylor PW (2020) Forewarned is forearmed: Fruit fly detects olfactory cues from predators and responds with predator-specific behaviour. *Scientific Reports* 10: 7297

Animals can gain significant advantages from abilities to detect cues from predators, assess risks, and respond adaptively to reduce the likelihood of injurious interactions. In contrast, predator cue-induced changes in behaviour may interfere with fitness-associated activities such as exploration, foraging and reproduction. Despite the ecological importance of predator-prey interactions in insects, remarkably little is known about the abilities of insects to detect and respond to olfactory cues from predators, or the potential costs of such responses. We here demonstrate that a tephritid fruit fly, the Queensland fruit fly *Bactrocera tryoni*, is able to detect and respond differentially to volatile olfactory cues from four potential predators (three spiders and an ant) that vary in prevalence and diurnal activity. Male and female flies increased or decreased motility (velocity, active time, distance moved), or exhibited no change in motility, depending on which predator volatiles they encountered. Further, flies significantly reduced foraging, oviposition and mating propensity in the presence of volatiles from any of the predators. This study is the first report of predator-specific responses to olfactory cues in a tephritid fruit fly, and highlights that such anti-predator responses can impose costs on general activity and reproductive behaviour.

Outcome 5: Develop an assay to test for Q-fly predation in field-caught predators

Rathnayake DN[†], Power ML, Ponton F[‡] & Herberstein ME (manuscript) Real-time PCR as a tool to identify Queensland fruit fly [*Bactrocera tryoni,* (Froggatt) (Diptera: Tephritidae)] predators.

Queensland fruit fly, *Bactrocera tryoni* (Q-fly) is one of Australia's most costly horticultural pests infesting over hundreds of economically important fruits and vegetables. The few available biocontrol tools have limited action as they only disrupt the Q-fly eggs, larvae and pupae. An option for an additional biocontrol approach is natural predation on adult Q- fly, although predator identity and suitability as a biological control has not been studied in detail. Detecting adult fly predation using direct observations is problematic for any predator – prey system because of the infrequent occurrence of predatory events. Molecular based methods offer greater specificity for targeted prey detection and high sensitivity. Here, we used a molecular approach to detect Q-fly DNA from experimentally fed, lab reared predator gut samples (St Andrew's cross spiders; *Argiope keyserlingi*) to measure the detectability and the persistence of Q-fly DNA in the gut. Our method successfully amplified Q-fly DNA from 63% (out of 60 samples) of spiders fed with Q-fly. Detection was higher (80- 100%) within the first 48 hours after feeding the spider, but reduced to 50-30% after 72 hours. Further, Probit Analysis suggested the half-life detectability of Q-fly DNA in the spider gut is 83.8 hours or approximately 3.5 days. The half-life of Q-fly DNA was similar to that of most of the other spider prey detection studied. Suitability of the method for field trials was demonstrated by using field collected predators (spiders). We believe that the method will provide high potential for identifying Q-fly predators in ecosystems where Q-fly presents economic issues with view of identifying and deploying suitable predators for Q-fly bio-control in Australia.

Project 2: The importance of crop hygiene and host management

Summary

Crop hygiene (sanitation) involves collecting and disposing of fallen fruits and infested fruits on trees, and in an area wide context also includes removal of host plants both in urban areas and in untended orchards. Crop hygiene has been found to be very important to reduce abundance of pest fruit flies although there is little formal literature on this issue, and little in the way of a compelling evidence base to support associated practices. Where do all the flies come from? In the very successful Hawaii AWM program, poor crop hygiene and untended hosts were found to be major sources of pest fruit flies. Very significant improvements in crop protection were achieved by simply maintaining basic crop hygiene, not allowing fallen fruit to remain long enough for larvae to exit and pupate in the soil, and removing untended hosts. In Australia, there has been no parallel investigation of the extent to which crop hygiene practices could contribute to reducing pest pressure from Qflies and other fruit flies.

Field studies were conducted to establish the extent to which infested fruit on unmanaged host trees might exacerbate the fruit fly problem. The infestation rates and number of flies produced varied significantly amongst fruit types (Outcome 1). By extrapolating typical fruit fly production per fruit to number of fruit per tree it is possible to make a upper bound estimate of the potential contribution of a host tree to pest populations.

Recommendations & Conclusions

- Untended hosts and unharvested fruit can be a serious source of Qflies
- Fallen and infested fruits should be removed wherever possible and economical
- Untended fruit trees should be removed where possible as a long-term solution

Achievements

Outcome 1: Establishing infestation rate of fruits in backyards and untended orchards

Adnan SM[‡], Mendez V[‡], Rempoulakis P[‡], Taylor PW & Mainali BP[‡] (manuscript) Queensland fruit fly carrying capacity by fruit types: Infestation rates of different fruits in untended orchards and backyards

Untended fruit trees, with mature fruits, for each fruit type including loquat, pear, apple (green apple and Royal Gala) were sampled from backyards and untended orchards in and around Sydney. Fruits on the ground as well as from the sampled trees were collected. Samples were brought to the laboratory and weighed before they were held inside a container with vermiculite on the bottom. Pupal yield from each fruit type was counted and pupal weight was recorded. Collected pupae were then held in an emergence container and percentage of emergence of adult flies recorded. The study revealed no correlation between fruit weight and pupal number in all the fruit types. Pears had the highest number of pupae per fruit (~12) followed by loquats (10) whereas orange (~4), green apple (~3) and royal gala apple (~5) had low numbers. There was no difference in average pupal weight among the flies exiting different fruit types. The average pupal weight ranged from 6 to 8 mg. However adult emergence rate was higher from the pupae from green apple and royal gala apple (80%) while those from loquats was the least (70%). Although, sanitation has long been recommended as a cultural method to control of fruit fly species, the practice has not been widely implemented in because it is labor intensive and costly. However, it is recommended that fallen and infested fruits be removed and ripened fruits not to be left on trees unprotected from infestation.

Theme 10: Combining and implementing control technologies

Project 1: Enclosure testing of SIT and AWM practices

Summary

To date, AWM practices in Australia have been developed largely in the absence of robust empirical support for many of the tools and in particular for the combinations of tools. As we move toward softer, more complex, systems approach for fruit fly management, there is a need for greater confidence in tool selection that can only come from direct investigation. Current approaches tend to rely on establishing area wide programs, and then conducting very constrained experimentation in this operational setting. But such approaches are extremely limited in the options they can explore.

An alternative low-cost and tractable approach entails simulated management practices in field cage settings where sufficient control over key variables can be maintained while at the same time exposing the system to field-like environmental conditions. This project presents a field cage simulation of SIT, releasing and maintaining populations of sterile and wild flies and then assessing number and fertility of eggs.

Recommendations

- Simulated SIT operation under field cage conditions support SIT viability, reducing populations of wild flies
- Field cage SIT simulations could be developed as a periodic QC assay, going a step beyond the single generation FRIED test

Achievements

Outcome 1: Demonstrate population suppression with the release of sterile Qfly in field cage studies

Adnan SM[‡], Taylor PW & Mainali BP[‡] (manuscript) Releases of sterile Queensland fruit fly to suppress population of established fertile conspecifics: A field cage study

An experiment was conducted to assess population suppression with the release of sterile flies that had been provided yeast hydrolysate for a five-day pre-release holding period, as is the practise in current trial field SIT operations. The experiment was run in in four large field cages with established Qfly populations. Two cages were assigned as 'treatment' cages in which, in addition to the normal number of pupae added to the cages, sterile flies were released repeatedly. The remaining two cages did not receive any sterile Qfly (control cages). Oviposition devices were placed in the cages and fecundity of the eggs from the treatment and control cages and their hatchability across the experimental period were observed. In total five releases of sterile males were made in the treatment cages.

Overall, egg production by Qfly females differed significantly across the generations and this may be in part owing to changes in season. However, a significant interaction between treatment and release revealed that the reduction in fecundity and fertility became more evident at later generations in the cages where sterile flies were released. Also, significant interaction between treatment and release period demonstrated that while percent egg hatching of the control population did not change significantly across the generations there was a significant reduction in egg hatching in the treatment cages from the second generations onwards. These findings confirm the viability of sterile releases for reducing reproduction of pest populations in a setting that is manageable and tractable for experimentation.

Project 2: Field testing of SIT and AWM practices

Summary

There will be a need to bring practises developed during HG14033 into operational settings through trial AWM and SIT programs. At the commencement of HG14033 there were not operational programs. Accordingly, while highlighting the need for field testing and adoption this was excluded from the scope of required work for HG14033 which focuses on the underlying development of knowledge and technology to support SIT and AWM through the SITPlus group.

However, throughout HG14033 it was possible to run small-scale experimental releases in Somersby and Menagle (both NSW) to test a variety of SIT practices, such as age of release (Theme 6 Project 1), pre-release raspberry ketone and methoprene treatments (Theme 3 Project 1), post-release identification (Theme 4 Project 3), to assess dispersal of sterile flies and to compare the landscape-scale distribution of sterile and wild flies (Theme 3 Project 1, Theme 6 Project 1). Vast amounts of data have been collected on fly movement and survival, and we have learned much about how these practises impact on field performance of released sterile flies. Information from HG14033 experimental trials provided the foundations for operational SIT practices adopted in the trial operational releases of FF17001. For example, based on the findings of HG14033, for the first time an extended pre-release holding period has been adopted in SIT releases. Pre-release yeast hydrolysate has been confirmed as highly effective and is adopted in FF17001. Transportation of pupae from factory to rear-out facilities is based on HG14033 assessment of quality and development of logistics models. Quality control assessment if flies at factory and rear-out are based on protocols delineated in HG14033. Also, the fluorescent markers tested in HG14033 (Theme 4, Project 1) are used to reliably identify released flies in FF17001. As FF17001 continues there will be ongoing transfer to technology and support from HG14033, which has provided a substantial knowledge base to use in troubleshooting of issues and for medium to longer term improvement of mass-reared Qflies and SIT practises.

Monitoring and evaluation

(1) Essential R&D for immediate application to SIT

The primary outcome of HG14033 is the attainment of essential R&D for the development of effective, economical and sustainable Qfly SIT. The greatest impediments to the establishment of effective SIT at the commencement of HG14033 are a paucity of knowledge and time. SIT entails a vast array of biological and management processes, and the available SIT programs have been well short of the required standard of knowledge. HG14033 brought together a very large group of researchers from across the SITPlus consortium, and beyond, to address a common goal of developing the knowledge needed to provide an SIT solution for Australian growers, and then implementing this knowledge in the operations of the new SITplus factory and trial operations.

The primary output of HG14033 includes clearly defined technical procedures for the establishment, maintenance and implementation of Qfly SIT to international standards. For example, HG14033 has defined quality control procedures for use in the SITplus factory and at rear-out facilities (Theme 2 Project 1), confirmed x-irradiation procedures as equivalent to the previous gamma-irradiation procedures in terms of induced sterility and fly quality (Theme 2 Project 2), Established a logistics framework for the delivery of pupae from the SITplus factory to rear-out facilities (Theme 2 Project 4), demonstrated efficacy of several potent pre-release treatments (Theme 3 Project 1), refined chilling and release protocols (Theme 3 Project 2), established suitable dyes and application rates for post-release identification (Theme 4 Project 1), developed isotope ratio analysis as a ready-to-use back up marking method (Theme 4, Project 3), identified regional genetic features that have enabled identification of outbreak origins (Theme 5 Project), established 6 – 8 fold increases in field abundance of mature sterile males by modifying releases to after 5 days, rather than 2 – 3 days, of post-emergence holding (Theme 6 Project 1), demonstrated landscape-scale co-location of sterile flies with the wild population (Theme 6 Project 1), demonstrated the risk presented by unmanaged host fruit (Theme 9 Project 2), and demonstrated the ability of sterile males to induce sterility in wild populations (Theme 9 Project 1, Theme 10 Project 1).

(2) Deep foundations for SIT

SIT is a form of biological control whereby a large proportion of the present pest generation is rendered infertile through matings with released sterile males. Numerous biological and management processes are involved in each step of the SIT process, including domestication, mass rearing, transport and release, dispersal, survival, and mating. Problems can arise at any of these steps and preparedness to anticipate and respond to challenges that threaten effective delivery of SIT is important for long term delivery of an effective Qfly solution. The vast body of knowledge developed in the course of this project will provide an invaluable resource for future researchers and managers to maintenance of desirable traits in domesticated mass-reared flies (Theme 1 Project 1, Theme 5 Project 1, Theme 7 Project 2, Theme 8 Project 2), develop improved transportation protocols (Theme 2 Project 3), maintain healthy Qfly colonies (Theme 2 Project 5), develop techniques for simultaneous application of MAT and SIT (which would reduce the number of flies needed for a given effect level by as much as 95%) (Theme 3 Project 1), develop a simple field assay to detect irradiation-induced changes (Theme 4 Project 2), develop genetic biomarkers (Theme 4 Project 4), prepare for expected future distribution of Qflty and other pest fruit flies (Theme 6 project 3), improve sexual performance in mating (Theme 7) and induction of sexual inhibition (Theme 8).

(3) Transforming SITplus from distinct projects to a coherent program

SITPlus was developed by a weaving together of diverse projects carried out by the member organizations to yield a greater whole. Through HG14033, the SITPlus initiative was transformed from a series of distinct projects carried out by distinct organizations to a coherent program characterised by greatly increased collaboration and cross-institutional collective activity. HG14033 took the SITPlus program to first principles, considering what new knowledge was needed to attain the goal of effective, economical and sustainable Qfly SIT, and to support potential future improvements. HG14033 developed a series of research activities that not only linked together but also linked to other ongoing research activities to yield a far greater coherency than has ever been seen in Australian fruit fly research. For example, activities under Theme 2 Project 5 (Microbial symbionts) relied heavily on collaboration between Macquarie University and NSW DPI researchers who had not collaborated previously.

This included research fellows and PhD students working fluidly between organisations. Similarly, activities of Theme 1 (Preserving genetic quality in domestication and mass rearing), Theme 5 (Ecological competence) and Theme 7 (Mating ability) and Theme 8 (Protecting sterile matings) all relied heavily on new collaboration between Macquarie University and CSIRO, and included several research fellows and numerous PhD students embedded full time with CSIRO. Field studies testing co-location of sterile and wild flies and sterile flies, assessing efficacy of isotopic markers, and testing effects of pre-release holding conditions were carried out by Macquarie University staff and PhD students on NSW DPI sites. Studies of γH2AX as a biomarker for irradiation, and as a post-release marker of sterile flies were carried out by Macquarie University staff embedded full time with SARDI

(4) Capacity for the future

Peer-reviewed publications

HG14033 has generated a very large increase in the peer-reviewed Qfly literature through the substantial research activity of this project, such outputs being in line with the strategic objectives of all of the SITPlus partners. To date, 50 articles have been published, or are 'in press', in the peer reviewed literature. An additional 49 manuscripts have either been submitted for publishing or are at an advanced state of preparation. Most of the unpublished work is already publicly available in the 19 completed MRes and PhD theses.

Overall, we expect at least 93 peer-reviewed publications to arise directly from the work conducted in the course of HG14033. This is a very substantial increase in foundation knowledge that will guide Qfly management for decades to come. Future scientists and managers will benefit greatly from this valuable research resource. Scientific progress is a compounding process and, just like compounding interest in a bank, the earlier an accumulation is made the greater the benefits in the long term. By generating a significant increase in the rate of Qfly research productivity and publication, and focus, over the next five years, we aim to advance the field by 15 years. In doing so, we not only address today's problems but also provide the knowledge base from which the solutions to tomorrow's problems will be drawn.

Research Training

Training the next generation of biosecurity scientists and managers figured prominently in the overall program. HG14033 supported 2 MRes and 21 PhD theses, and numerous additional PhD students were indirectly involved on related projects. Each of these students was enrolled at Macquarie University where they benefited from one of Australia's most rigorous, and best supported, higher degree research training programs. In addition, each of these students interacted extensively with joint supervisors and experts in CSIRO, NSW DPI, SARDI and PFR. Some students spent very significant portions of the time in these governmental research agencies where they benefited from working more closely with industry-oriented institutions. While the immediate challenges and projects undertaken by the supported students focused on Qfly, each student was trained in approaches and techniques that are much more broadly applicable. As mature scientists, these students will later be well equipped to confront a vast diversity of other biosecurity threats facing Australia and the broader region. Additionally, 24 research fellows were directly involved. These research fellows worked fluidly between the collaborating agencies, and took primary responsibility for some elements of the research program, and in doing so gained valuable research leadership and management experience under the mentorship of senior investigators. These research training and mentoring activities are integral to our overall program; PhD graduates and trained research fellows are important outputs, contributing biosecurity research capacity for the coming decades.

Refereed scientific publications

Peer-Reviewed Journal Articles

2017

Akter, H., Adnan, S., Morelli, R., Rempoulakis, P., & Taylor, P. W. (2017). Suppression of cuelure attraction in male Queensland fruit flies provided raspberry ketone supplements as immature adults. *PLoS One*, 12(8), e0184086

Akter, H., Mendez, V., Morelli, R., Pérez, J., & Taylor, P. W. (2017). Raspberry ketone supplement promotes early sexual maturation in male Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). *Pest Management Science*, 73, 1764-1770.

Sultana, S., Baumgartner, J. B., Dominiak, B. C., Royer, J. E., & Beaumont, L. J. (2017). Potential impacts of climate change on habitat suitability for the Queensland fruit fly. *Scientific Reports*, 7, 13025.

2018

Adnan, S. M., Mendez, V., Morelli, R., Akter, H., Farhana, I., & Taylor, P. W. (2018). Dietary methoprene supplement promotes early sexual maturation of male Queensland fruit fly *Bactrocera tryoni*. *Journal of Pest Science*, 91, 1441-1454.

Akter, H., & Taylor, P. W. (2018). Sexual inhibition of female Queensland fruit flies mated by males treated with raspberry ketone supplements as immature adults. *Journal of Applied Entomology*, 142, 380-387.

Lynch, K. E., White, T. E., & Kemp, D. J. (2018). The effect of captive breeding upon adult thermal preference in the Queensland fruit fly (*Bactrocera tryoni*). *Journal of Thermal Biology*, 78, 290-297.

Morimoto, J., Nguyen, B., Tabrizi, S. T., Ponton, F., & Taylor, P. (2018). Social and nutritional factors shape larval aggregation, foraging, and body mass in a polyphagous fly. *Scientific Reports*, 8, 14750.

Pérez, J., Park, S. J., & Taylor, P. W. (2018). Domestication modifies the volatile emissions produced by male Queensland fruit flies during sexual advertisement. *Scientific Reports*, 8, 16503.

2019

Adnan, S. M., Farhana, I., Inskeep, J. R., Rempoulakis, P., & Taylor, P. W. (2019). Accelerated sexual maturation in methoprene-treated sterile and fertile male Queensland fruit flies (Diptera: Tephritidae), and mosquito larvicide as an economical and effective source of methoprene. *Journal of Economic Entomology*, 112, 2842-2849.

Benelli, M., Ponton, F., & Taylor, P. W. (2019). Cool storage of Queensland fruit fly eggs for increased flexibility in rearing programs. *Pest Management Science*, 75, 1056-1064.

Benelli, M., Ponton, F., Lallu, U., Mitchell, K. A., & Taylor, P. W. (2019). Cool storage of Queensland fruit fly pupae for improved management of mass production schedules. *Pest Management Science*, 75, 3184-3192.

Dinh, H., Mendez, V., Tabrizi, S. T., & Ponton, F. (2019). Macronutrients and infection in fruit flies. *Insect Biochemistry and Molecular Biology*, 110, 98-104.

El-Sayed, A. M., Venkatesham, U., Unelius, C. R., Sporle, A., Pérez, J., Taylor, P. W., & Suckling, D. M. (2019). Chemical composition of the rectal gland and volatiles released by female Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). *Environmental Entomology*, 48, 807-814.

Kempraj, V., Park, S. J., & Taylor, P. W. (2019). γ-Octalactone, an effective oviposition stimulant of *Bactrocera tryoni*. *Journal of Applied Entomology*, *143*, 1205-1209.

Majumder, R., Sutcliffe, B., Taylor, P. W., & Chapman, T. A. (2019). Next-Generation Sequencing reveals relationship between the larval microbiome and food substrate in the polyphagous Queensland fruit fly. *Scientific Reports*, 9, 14292.

Morimoto, J., Nguyen, B., Tabrizi, S. T., Lundbäck, I., Taylor, P. W., Ponton, F., & Chapman, T. A. (2019). Commensal microbiota modulates larval foraging behaviour, development rate and pupal production in *Bactrocera tryoni*. *BMC Microbiology*, 19, 286.

Morimoto, J., Nguyen, B., Dinh, H., Taylor, P. W., & Ponton, F. (2019). Crowded developmental environment promotes adult sex-specific nutrient consumption in a polyphagous fly. *Frontiers in Zoology*, 16, 4.

Morimoto, J., Tabrizi, S. T., Lundbäck, I., Mainali, B., Taylor, P. W., & Ponton, F. (2019). Larval foraging decisions in competitive heterogeneous environments accommodate diets that support egg-to-adult development in a polyphagous fly. *Royal Society Open Science*, 6, 190090.

Nguyen, B., Ponton, F., Than, A., Taylor, P. W., Chapman, T., & Morimoto, J. (2019). Interactions between ecological factors in the developmental environment modulate pupal and adult traits in a polyphagous fly. *Ecology and Evolution*, 9, 6342-6352.

Rathnayake, D. N., Lowe, E. C., Rempoulakis, P., & Herberstein, M. E. (2019). Effect of natural predators on Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) control by sterile insect technique (SIT). *Pest Management Science*, 75, 3356-3362.

2020

Adnan, S. M., Pérez-Staples, D., & Taylor, P. W. (2020). Dietary methoprene treatment promotes rapid development of reproductive organs in male Queensland fruit fly. *Journal of Insect Physiology*, 126, 104094.

Adnan, S. M., Farhana, I., Inskeep, J., Rempoulakis, P., & Taylor, P. W. (2020). Dietary methoprene enhances sexual competitiveness of sterile male Queensland fruit flies in field cages. *Journal of Pest Science*, 93, 477-489.

Adnan, S. M., Farhana, I., Park, S. J., Rempoulakis, P., & Taylor, P. W. (2020). Caffeine as a promotor of sexual development in sterile Queensland fruit fly males. *Scientific Reports*, 10, 14743.

Akter, H., Pérez, J., & Park, S. J. (2021). Raspberry ketone supplements provided to immature male Queensland fruit fly, *Bactrocera tryoni* (Froggatt), increase the amount of volatiles in rectal glands. *Chemoecology*, 31, 89-99.

Akter, H., Taylor, P. W., & Crisp, P. (2020). Visibility and persistence of fluorescent dyes, and impacts on emergence, quality, and survival of sterile Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae). *Journal of Economic Entomology*, 113, 2800-2807.

Biswas, M. J. H., Mainali, B., Park, S. J., Taylor, P., & Rempoulakis, P. (2020). Electrophysiological responses to Cuelure of raspberry ketone-fed Queensland fruit flies. *Journal of Economic Entomology*, 113, 2832-2839.

Kempraj, V., Park, S. J., & Taylor, P. W. (2020). Forewarned is forearmed: Queensland fruit flies detect olfactory cues from predators and respond with predator-specific behaviour. *Scientific Reports*, 10, 7297.

Majumder, R., Sutcliffe, B., Adnan, S. M., Mainali, B., Dominiak, B. C., Taylor, P. W., & Chapman, T. A. (2020). Artificial larval diet mediates the microbiome of Queensland fruit fly. *Frontiers in Microbiology*, 11, 576156.

Majumder, R., Sutcliffe, B., Taylor, P. W., & Chapman, T. A. (2020). Microbiome of the Queensland fruit fly through metamorphosis. *Microorganisms*, 8 (6), 795.

Majumder, R., Sutcliffe, B., Taylor, P. W., & Chapman, T. A. (2020). Fruit host-dependent fungal communities in the microbiome of wild Queensland fruit fly larvae. *Scientific Reports*, 10, 16550.

Morimoto, J., Nguyen, B., Lundbäck, I., Than, A. T., Tabrizi, S. T., Ponton, F., & Taylor, P. W. (2020). Effects of carbohydrate types on larval development and adult traits in a polyphagous fruit fly. *Journal of Insect Physiology*, 120, 103969.

Nguyen, B., Than, A., Dinh, H., Morimoto, J., & Ponton, F. (2020). Parental microbiota modulates offspring development, body mass and fecundity in a polyphagous fruit fly. *Microorganisms*, 8(9), 1289.

Popa-Báez, Á. D., Lee, S. F., Yeap, H. L., Westmore, G., Crisp, P., Li, D., Catullo, R., Cameron, E. C., Edwards, O. R., Taylor, P. W., & Oakeshott, J. G. (2021). Tracing the origins of recent Queensland fruit fly incursions into South Australia, Tasmania and New Zealand. *Biological Invasions*, 23, 1117-1130.

Popa-Báez, Á. D., Lee, S. F., Yeap, H. L., Prasad, S. S., Schiffer, M., Mourant, R. G., Castro Vargas, C., Edwards, O. R., Taylor, P. W., & Oakeshott, J. G. (2020). Climate stress resistance in male Queensland fruit fly varies among populations of diverse geographic origins and changes during domestication. *BMC Genetics*, 21, 135.

Siddiqui, M. S., Taylor, P., & Crisp, P. (2019). Gamma-H2AX: A Promising Biomarker for Fruit Fly Phytosanitary Irradiation Exposure. In *Area-Wide Management of Fruit Fly Pests* (pp. 169-173). CRC Press.

Sultana, S., Baumgartner, J. B., Dominiak, B. C., Royer, J. E., & Beaumont, L. J. (2020). Impacts of climate change on high priority fruit fly species in Australia. *PLoS One*, *15*(2), e0213820.

2021 & In Press

Benelli, M., Mainali, B., Taylor, P. W., & Rempoulakis, P. (2021). Reduced quality of sterile Queensland fruit fly following post-production stress from hypoxia, irradiation and vibration. *Journal of Pest Science*, 94, 473-485.

Devescovi, F., Hurtado, J., & Taylor, P. W. (2021). Mating-induced changes in responses of female Queensland fruit fly to male pheromones and fruit: A mechanism for mating-induced sexual inhibition. *Journal of Insect Physiology* 129: 104195

Gaire, S. K., Biswas, M. J. H., Benelli, M., Rempoulakis, P., Taylor, P. W., & Mainali, B. P. (in press). Effect of chilling on quality control parameters of sterile Queensland fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology*.

Biswas, M. J. H., Mainali, B., Inskeep, J. R., Cross, D., Benelli, M., Allen, A. P., Taylor, P. W., & Rempoulakis, P. (in press). Pre-release dietary supplements of methoprene and raspberry ketone increase field abundance of sterile Queensland fruit flies. *Journal of Economic Entomology*

Biswas, M. J. H., Mainali, B. P., Inskeep, J. R., Gaire, S. K., Cross, D., Stringer, L. D., Taylor, P. W. & Rempoulakis, P. (in press). Extended holding period and yeast hydrolysate in pre-release diet increase abundance of mature sterile Queensland fruit fly males in the field. *Journal of Pest Science*.

Inskeep, J. R., Allen, A. P., Taylor, P. W., Rempoulakis, C., & Weldon, C. W. (in press). Canopy distribution and microclimate preferences of sterile and wild Queensland fruit flies. *Scientific Reports*

Inskeep, J. R., Taylor, P. W., Mainali, B., Rempoulakis, P., & Weldon, C. W. (2021). Spatio-temporal distribution of sexual calling behaviour in domesticated, sterile and wild Queensland fruit fly males under field cage conditions. *Pest Management Science*, 77, 2522-2529.

Mainali, B., Andrew, A. S., Taylor, P. W., & Rempoulakis, P. (in press). Stable isotopes for reliable identification of wild and mass-reared Queensland fruit flies in sterile insect technique programs. *Journal of Pest Science*.

Pereira, R., Yuval, B., Liedo, P., Teal, P., Shelly, T., Mcinnis, Haq, I., Taylor, P.W., & Hendrichs, J. (2021). Improving post-factory performance of sterile male fruit flies in support of the Sterile Insect Technique. In*: Sterile Insect Technique* (pp. 631-656). CRC Press.

Pérez, J., Mendez, V., Yuval, B., & Taylor, P. W. (in press). Domestication-related changes in sexual performance of

Queensland fruit fly. *Insect Science*.

Popa-Baez, A., Lee, S. F., Yeap, H. L., Westmore, G., Crisp, P., Li, D., Catullo, R., Cameron, E. C., Edwards, O. R., Taylor, P. W. & Oakeshott, J. G. (in press). Tracing the origins of recent Queensland fruit fly incursions into South Australia, Tasmania and New Zealand. *Biological Invasions*

Shadmany, J., Lee, R., Nguyen, T. N. M., & Taylor, P. W. (in press) Patterns of sperm use in twice-mated Queensland fruit fly. *Insect Science*

Shadmany, J., Lee, S. F., & Taylor, P. W. (2021). Real-time PCR-based Y-specific sperm quantification assay in Queensland fruit fly: Insights to patterns of sperm storage. *Insect Molecular Biology*, 30, 315-324.

Shadmany, J., Lee, R., & Taylor, P. W. (in press). Patterns of sperm storage in twice-mated Queensland fruit fly. *Journal of Insect Physiology*

Theses

2017

Akter, H. (2017). Raspberry ketone as a promising pre-release supplement for the Sterile Insect Technique (SIT) of Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). PhD thesis, Macquarie University.

2018

Ahmed, A. (2018). Proteomic analysis of remating inhibition in Queensland fruit fly. MRes thesis, Macquarie University.

Benelli, M. (2018). Effect of short-term suboptimal temperature storage to assist large-scale production of two dipterans: *Exorista larvarum* (L.) and *Bactrocera tryoni* (Froggatt). Cotutelle PhD thesis, Macquarie University and University of Bologna.

2019

Rathnayake, D. (2019). Effect of the Sterile Insect Technique (SIT) on Predator-Prey Interactions in Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). PhD thesis, Macquarie University.

Than, A. (2019) Functional significance of ecological factors during larval development. MRes thesis, Macquarie University.

2020

Adnan, S. (2020). Dietary methoprene and caffeine as pre-release supplements for Queensland fruit fly *Bactrocera tryoni* (Froggatt) Sterile Insect Technique. PhD thesis, Macquarie University.

Dinh, H. (2020). An integrated approach of the interactions between nutrition and resistance to infection in fruit flies. PhD thesis, Macquarie University.

Inskeep, J. (2020). The behaviour of the Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae), and its implications for improving integrated pest management using the sterile insect technique. PhD thesis, Macquarie University.

Majumder, R. (2020). Dynamics of the Queensland fruit Fly microbiome under changes in host environment. PhD thesis, Macquarie University.

Moadeli, T. (2019). Improved larval diets for mass-rearing of Queensland fruit fly. PhD thesis, Macquarie University.

Noushini, S. (2020). Studying volatile emissions of fruit flies as chemical lures. PhD thesis, Macquarie University.

Nguyen, B. (2020). Impact of microbiota on the life-history traits of a polyphagous fly. PhD thesis, Macquarie University.

Popa, A. (2020). Ecotypic and genetic differentiation among native range and invasive populations of Queensland fruit fly. PhD thesis, Macquarie University.

Shadmany, J. (2020). Polyandry and paternity in the Queensland fruit fly (Qfly), *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). PhD thesis, Macquarie University.

Sultana, S. (2020). What does climate change mean for the ecology, invasiveness and management of fruit flies in Australia?. PhD thesis, Macquarie University.

2021

Ahmed, A. (2021). Genetics and biochemistry of sexual inhibition in Queensland fruit fly. PhD thesis, Macquarie University.

Biswas, J. (2021). Improved pre-release management to enhance the efficacy of the Queensland fruit fly *Bactrocera tryoni* (Froggatt) sterile insect technique. PhD thesis, Macquarie University.

Castro-Vargas, C. (2021). Rectal gland volatiles of *Bactrocera tryoni* and *Bactrocera neohumeralis*. PhD thesis, Macquarie University.

Farhana, I. (2021). Cuticular compounds of the Queensland fruit fly (*Bactrocera tryoni*) and their role as chemical footprints. MRes thesis, Macquarie University.

Gaire, S. (2021). Refinement of production and delivery operations to improve quality of sterile Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). PhD thesis, Macquarie University.

Kemparaju, V. (2021). Queensland fruit fly-predator interaction: a chemical ecology perspective. PhD thesis, Macquarie University.

Prasad, S. (2021). Genetics and biochemistry of desiccation resistance in *Bactrocera tryoni*. PhD thesis, Macquarie University.

Southwood, D. (2021). Advanced bioinformatics approaches for hybrid de novo whole-genome assembly. PhD thesis, Macquarie University.

Presentations

2015

Akter, H. (2015). Prelease treatments to accelerate development and increase mating activity of sexually immature adult males of Queensland fruit fly, *Bactrocera tryoni* (Froggatt). HDR Conference, Macquarie University, Sydney (Australia), June.

Akter, H. (2015). Raspberry ketone supplement as pre-release treatment to accelerate mating activity of male Queensland fruit fly, *Bactrocera tryoni* (Froggatt) for Sterile Insect Technique (SIT). 46th AGM and Scientific Conference of the Australian Entomological Society (AES), Cairns, 27 - 30 September.

Perez, J. (2015). Quantitative changes in sex pheromone release of laboratory reared *Bactrocera tryoni* males. Australian Entomological Society (AES). 46th AGM and Scientific Conference of the Australian Entomological Society (AES), Cairns, 27 - 30 September.

2016

Adnan, S. (2016). Methoprene and dietary yeast as pre-release supplements for Queensland fruit fly SIT. HDR Conference, Macquarie University, Sydney (Australia), 15th-16th June.

Akter, H. (2016). Raspberry ketone supplements as pre-release treatment to accelerate mating activity of male Queensland fruit fly, *Bactrocera tryoni* (Froggatt) for Sterile Insect Technique (SIT). HDR Conference, Macquarie University, Sydney (Australia), 15th-16th June.

Akter, H. (2016). Raspberry ketone pharmacophagy promotes early sexual maturation in male Queensland fruit fly *Bactrocera tryoni*. 1st Symposium of Tephritid Workers of Asia, Australia and Oceania (TAAO), Putrajaya (Malaysia), 15th-18th August.

Akter, H. (2016). Raspberry ketone as a promising pre-release supplement for the Sterile Insect Technique (SIT) of Queensland fruit fly, *Bactrocera tryoni*. XXV International Congress of Entomology, Orlando, Florida (USA), 25th-30th September.

Akter, H. (2016). Effect of raspberry ketone treatment of immature Queensland fruit fly males on ability to induce sexual inhibition in mates. The 4th combined Australian and New Zealand Entomological Societies Conference, Melbourne (Australia), 27th-30th November.

Akter, H. (2016). Raspberry ketone as a promising pre-release supplement for the Sterile Insect Technique (SIT) of Queensland fruit fly, *Bactrocera tryoni* (Froggatt). Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

Akter, H. (2016). Effect of raspberry ketone treatment of immature Queensland fruit fly on reproductive organs development and ability of males to induce sexual inhibition in mates. 4th Australian Biology of Tephritid Fruit Flies Meeting, Melbourne (Australia), 1st December.

Benelli, M. (2016). Using cold storage technology to assist mass production of *Bactrocera tryoni* (Diptera: Tephritidae). HDR Conference, Macquarie University, Sydney (Australia), 15th-16th June.

Benelli, M. (2016). Cold storage of Queensland fruit fly eggs for mass-rearing programs. 1st Symposium of Tephritid Workers of Asia, Australia and Oceania (TAAO), Putrajaya (Malaysia), 15th-18th August.

Benelli, M. (2016). Cold storage of Queensland fruit fly for mass-rearing programs. 4th Australian Biology of Tephritid Fruit Flies Meeting, Melbourne (Australia), 1st December.

Benelli, M. (2016). Cold storage of Queensland fruit fly eggs and pupae for mass-rearing programs. Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

Farhana, I. (2016). Caffeine and dietary yeast as pre-release supplements for Queensland fruit fly. Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

Lee, R. (2016). Maximising field vigour of mass reared Qfly. Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

Lynch, K. (2016). Estimating complex heritabilities. ECR Biology Research Showcase, Macquarie University, Sydney (Australia), May.

Majumder, R. (2016). Changes in Q-Fly Gut Microbiome During Domestication. Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

Perez, J. (2016). Effects of domestication on pheromone production and release by male Queensland fruit fly. 1st Symposium of Tephritid Workers of Asia, Australia and Oceania (TAAO), Putrajaya (Malaysia), 15th-18th August.

Perez, J. (2016). Quantitative changes in sex pheromone released by laboratory reared *Bactrocera tryoni* males. Lab Chat, Macquarie University, Sydney (Australia).

Rathnayake, D. (2016). Predator-prey interactions in Queensland fruit flies. HDR Conference, Macquarie University, Sydney (Australia), 15th-16th June.

Rempoulakis, P. (2016). Stable isotopes for reliable identification of Q-flies released in SIT programs: Wild and Laboratory flies. Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

Rempoulakis, P. (2016). Stable isotopes for reliable identification of Q-flies released in SIT programs: 13C isotopic enrichment of larval diet. Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

Siddiqui, M. (2016). γH2AX : a dose-dependent marker to detect prior irradiation of Queensland Fruit Fly. 1st Symposium of Tephritid Workers of Asia, Australia and Oceania (TAAO), Putrajaya (Malaysia), 15th-18th August.

Sultana, S. (2016). Impacts of climate change on habitat suitability for the Queensland fruit fly. 4th Australian Biology of Tephritid Fruit Flies Meeting, Melbourne (Australia), 1st December.

Taylor, P. W. (2016). Co-investment and collaboration for delivery of sustainable Q-fly management tools. 3rd Australian Biology of Tephritid Fruit Flies Meeting, Brisbane (Australia), May.

Taylor, P. W. (2016). Recent advances in Australian research and research partnership to combat the Queensland fruit fly. 1st Symposium of Tephritid Workers of Asia, Australia and Oceania (TAAO), Putrajaya (Malaysia), 15th-18th August.

Taylor, P. W. (2016). Raising Q-fly Sterile Insect Technique to a world standard. Fruit Fly Workshop, Auckland (New Zealand), 15th September.

Yeap, H. L. (2016). Maximising field vigour of mass reared Q-fly. Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

2017

Adnan, S. (2017). Methoprene and dietary yeast as pre-release supplements for Queensland fruit fly SIT. HDR Conference, Macquarie University, Sydney (Australia), 13th-15th June.

Adnan, S. (2017). Methoprene and dietary yeast as pre-release supplements for Queensland fruit fly SIT. 5th Australian Biology of Tephritid Fruit Flies Meeting, Macquarie University, Sydney (Australia).

Adnan, S. (2017). Juvenile hormone analogue increases activity rate but also increases starvation and desiccation risk for polyphagous Queensland fruit fly. Australian Entomological Society (AES) Conference, Terrigal (Australia), 17th-20th September.

Ahmed, A. (2017). What's in the Seminal Fluid of Male Qfly? A Molecular Approach to Fine Tune Sterile Insect Technique. MRes Introductory Poster, Macquarie University, Sydney (Australia), September.

Ahmed, A. (2017). What's in the Seminal Fluid of Male Qfly? A Molecular Approach to Fine Tune Sterile Insect Technique. MRes Introductory Presentation, Macquarie University, Sydney (Australia), September.

Akter, H. (2017). Raspberry ketone feeding affects the tolerance of Queensland fruit fly *Bactrocera tryoni* (Froggatt) to nutritional and desiccation stress. Third FAO–IAEA International Conference on Area-wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques, Vienna (Austria), 22nd-26th May.

Benelli, M. (2017). Cold storage of Queensland fruit fly pupae for mass-rearing programs. Third FAO–IAEA International Conference on Area-wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques, Vienna (Austria), 22nd-26th May.

Benelli, M. (2017). Effect of short-term suboptimal temperature storage to assist large scale production of two dipterans. European PhD Network "Insect Science" VIII Annual Meeting, Centro Congressi Federico II, Naples (Italy), 15th-16th November.

Biswas, J. (2017). Integration of Queensland fruit fly, Bactrocera tryoni Froggatt sterile insect techniques (SIT) into an IPM scheme. HDR Conference, Macquarie University, Sydney (Australia), 13th-15th June.

Dinh, H. (2017). Nutrition and infection in Queensland fruit flies. 5th Australian Biology of Tephritid Fruit Flies Meeting, Macquarie University, Sydney (Australia).

Dinh, H. (2017). Studies on nutritional immunology in Queensland fruit flies. Australian Entomological Society (AES) Conference, Terrigal (Australia), 17th-20th September.

Farhana, I. (2017). Dietary caffeine promotes sexual maturation of male Queensland fruit fly. Australian Entomological Society (AES) Conference, Terrigal (Australia), 17th-20th September.

Gaire, S. (2017). Quality control of Queensland fruit fly (Q-fly) strains reared for Sterile Insect Technique (SIT). HDR Conference, Macquarie University, Sydney (Australia), 13th-15th June.

Lee, R. (2017). Raising Qfly SIT to world standard - Research activities at CSIRO. HIA project review (Dr Eric Chang visit) in Canberra (Australia).

Lynch, K. (2017). The evolutionary consequences of habitat selection. ECR Biology Research Showcase, Macquarie University, Sydney (Australia), September.

Lynch, K. (2017). Temperature preference in *B. tryoni*. Presentation for visiting HIA representatives, Macquarie University, Sydney (Australia), April.

Mainali, B. (2017). High-productivity gel-based diet for mass rearing of Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae): an overview. 5th Australian Biology of Tephritid Fruit Flies Meeting, Macquarie University, Sydney (Australia).

Majumder, R. (2017). The impact of domestication on the Queensland fruit fly gut microbiome. HDR Conference, Macquarie University, Sydney (Australia), 13th-15th June.

Nguyen, B. (2017). The effects of gut bacteria on immunity and reproduction of the Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), October.

Perez, J. (2017). Effects of domestication on sexual performance of two laboratory-reared colonies of Queensland fruit flies. 5th Australian Biology of Tephritid Fruit Flies Meeting, Macquarie University, Sydney (Australia).

Perez, J. (2017). Effects of domestication on pheromone production and release by male Queensland fruit fly. Lab Chat, Macquarie University, Sydney (Australia).

Popa, A. (2017). Genetic consequences of domestication in the Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), 13th-15th June.

Rathnayake, D. (2017). Identification of natural predators of Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), 13th-15th June.

Rathnayake, D. (2017). Efficacy of molecular techniques to identify Queensland fruit fly predators. Australian Entomological Society (AES) Conference, Terrigal (Australia), 17th-20th September.

Rempoulakis, P. (2017). Intrinsic and synthetic isotopes for reliable identification of wild and mass-reared Queensland fruit flies in SIT programs. Third FAO–IAEA International Conference on Area-wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques, Vienna (Austria), 22nd-26th May.

Rempoulakis, P. (2017). The Sterile Insect Technique as part of an AreaWide Integrated Pest Management system to control Q-fly. Persimon Conference, Melbourne (Australia).

Rempoulakis, P. (2017). The Sterile Insect Technique as part of an AreaWide Integrated Pest Management system to control Q-fly. Box Hill Institute, Master Class.

Rempoulakis, P. (2017). Lethal love: Using the knowledge of fruit fly biology for the development of environmentally benign control methods. ERC Showcase, Macquarie University, Sydney (Australia).

Rempoulakis, P. (2017). The Sterile Insect Technique as part of an Area-Wide Integrated Pest Management system to control Q-fly. Persimon Conference, Melbourne (Australia).

Shadmany, J. (2017). Prevalence and predictors of polyandry and paternity in the Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), 13th-15th June.

Shadmany, J. (2017). Postcopulatory sexual selection in the Queensland Fruit Fly. Lab Chat, Macquarie University, Sydney (Australia).

Siddiqui, M. (2017). The development of a monoclonal γH2AvB antibody to detect irradiated Queensland fruit fly. Third FAO–IAEA International Conference on Area-wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques, Vienna (Austria), 22nd-26th May.

Siddiqui, M. (2017). γH2AX : a dose-dependent marker to detect prior irradiation of Queensland fruit fly. SARDI seminar series, SARDI Plant Research Centre, Adelaide (Australia), 11th November.

Sultana, S. (2017). Impacts of climate change on habitat suitability for the Queensland fruit fly. Department presentation, Department of Biological Sciences, Macquarie University, Sydney (Australia), 14th June

2018

Adnan, S. (2018). Methoprene and dietary yeast as prerelease supplements for Queensland fruit fly SIT. HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Adnan, S. (2018). Dietary methoprene enhances sexual competitiveness of sterile male Queensland fruit flies in field cages. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Adnan, S. (2018). Dietary methoprene enhances sexual competitiveness of sterile male Queensland fruit flies in field cages. 10th International Symposium on Fruit Flies of Economic Importance, Tapachula (Mexico), 23rd-27th April.

Ahmed, A. (2018). A Proteomic Analysis to Investigate the Molecular Basis of Remating Inhibition in Queensland Fruit Fly, *Bactrocera tryoni*. MRes Final Pesentation, Macquarie University, Sydney (Australia), March.

Ahmed, A. (2018). What's in the Seminal Fluid of Male Qfly? A Molecular Approach to Fine Tune Sterile Insect Technique. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Baumgartner, J. (2018). Potential impacts of climate change on fruit flies in Australia. 2018 National Fruit Fly Symposium, Melbourne (Australia), 14th August.

Benelli, M. (2018). Use of cold storage to assist large-scale production of Queensland fruit fly. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Benelli, M. (2018). Effect of short-term suboptimal temperature storage to assist large-scale production of two dipterans: *Exorista larvarum* (L.) and *Bactrocera tryoni* (Froggatt). PhD Oral defense, University of Bologna, School of Agriculture and Veterinary Medicine, Bologna (Italy), 7th May.

Benelli, M. (2018). Effect of short-term suboptimal temperature storage to assist large-scale production of two dipterans: *Exorista larvarum* (L.) and *Bactrocera tryoni* (Froggatt). PhD Completion Seminar, Department of Biological Sciences, Macquarie University, Sydney (Australia), 23rd August.

Biswas, J. (2018). Integration of Queensland fruit fly, Bactrocera tryoni (Froggatt), Sterile Insect Technique (SIT) into an Integrated Pest Management (IPM) scheme. HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Biswas, J. (2018). Electrophysiological responses to Cue-lure odours: Raspberry Ketone treated vs control Q-flies. Lab Chat, Macquarie University, Sydney (Australia).

Biswas, J. (2018). The effect of Raspberry Ketone feeding on the electrophysiological responses of Queensland fruit flies to cue-lure stimuli. 49th AGM and Scientific Conference of the Australian Entomological Society (AES), Alice Springs (Australia), September.

Castro-Vargas, C. (2018). Physiology and genetics of reproductive fitness in Queensland fruit fly. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Castro-Vargas, C. (2018). Physiology and genetics of reproductive fitness in Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Colombo, V. (2018). Generating isofemales lines of *Bactrocera tryoni*. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Dinh, H. (2018). Effect of parental diet on offspring development and pathogen resistance in Queensland fruit flies. HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Dinh, H. (2018). Transgenerational effects of parental diet on offspring pathogen resistance. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Dinh, H. (2018). Nutritional effects on host defence induced by *Serratia marcescens* in *Batrocera tryoni*. ESA, ESC and ESBC Joint Annual Meeting, Crossing Borders: Entomology in a Changing World, Vancouver, British Colombia (Canada), November.

Gaire, S. (2018). Effect of domestication on quality parameters of the Queensland fruit fly from different locations. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Gaire, S. (2018). Evaluation of quality control parameters of *Bactrocera tryoni* (Froggatt). HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Gaire, S. (2018). Size distribution of pupae and quality control parameters of the Queensland fruit fly *Bactrocera*

tryoni. 49th AGM and Scientific Conference of the Australian Entomological Society (AES), Alice Springs (Australia), September.

Inskeep, J. (2018). Differences in the microhabitat distribution and behavior of sterile mass-reared and wild Queensland fruit flies, *Bactrocera tryoni* (Froggatt). 10th International Symposium on Fruit Flies of Economic Importance, Tapachula (Mexico), 23rd-27th April.

Inskeep, J. (2018). The behaviour of sterile Queensland fruit flies, *Bactrocera tryoni* (Froggatt). HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Inskeep, J. (2018). Differences in the microhabitat distribution and behavior of sterile mass-reared and wild Queensland fruit flies, *Bactrocera tryoni* (Froggatt). Visiting talk at the Institute of Biotechnology and Applied Ecology Xalapa (Mexico), 19th April.

Kemparaju, V. (2018). Queensland fruit fly – predator interaction: a Chemical Ecology perspective. HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Kemparaju, V. (2018). Queensland fruit fly – predator interaction: a Chemical Ecology perspective. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Lynch, K. (2018). Nature via Nurture: estimating habitat selection and its impact on behavioural evolution. Workshop on Culture and Cognition, CAVE, Macquarie University, Sydney (Australia), October.

Mainali, B. (2018). Pre-release diet supplement affect survival and dispersal of Queensland fruit fly, *Bactrocera tryoni* in the field. 10th International Symposium on Fruit Flies of Economic Importance, Tapachula (Mexico), 23rd-27th April.

Mainali, B. (2018). Pre-release diet supplement affect survival and dispersal of Queensland fruit fly, *Bactrocera tryoni* in the field. 10th International Symposium on Fruit Flies of Economic Importance, Tapachula (Mexico), 23rd-27th April (Poster).

Majumder, R. (2018). The impact of domestication on the Queensland fruit fly gut microbiome. HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Majumder, R. (2018). Dynamics of the Queensland Fruit Fly Gut Microbiome under Changes in Host Environment. Lab Chat, Macquarie University, Sydney (Australia).

Morimoto, J. (2018). Commensal microbial communities modulate larval foraging decisions and developmental traits in *Bactrocera tryoni*. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Nguyen, B. (2018). Effects of gut bacteria on development and behavior of the Queensland fruit fly (*Bactrocera tryoni*). HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Perez, J. (2018). Domestication-related changes in sex pheromone released by laboratory-reared *Bactrocera tryoni* males. 10th International Symposium on Fruit Flies of Economic Importance, Tapachula (Mexico), 23rd-27th April.

Popa, A. (2018). Geographical variation in the Queensland Fruit Fly (Qfly): a genome-wide approach. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Prasad, S. (2018). Genetics of stress and domestication related traits in *Bactrocera tryoni*. HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Prasad, S. (2018). Genetics of stress and domestication related traits in *Bactrocera tryoni*. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Rathnayake, D. (2018). Interaction between natural predators and Sterile Insect Technique (SIT) for the Queensland fruit fly control. ESA, ESC and ESBC Joint Annual Meeting, Crossing Borders: Entomology in a Changing

World, Vancouver, British Colombia (Canada), November.

Rathnayake, D. (2018). Predator-prey interactions in Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), October.

Rathnayake, D. (2018). Significance of natural predators and Sterile Insect Technique (SIT) on Queensland Fruit Fly control. 49th AGM and Scientific Conference of the Australian Entomological Society (AES), Alice Springs (Australia), September.

Rathnayake, D. (2018). Effect of natural predators on Q-fly reproduction and SIT. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

Rempoulakis, P. (2018). The use of stable isotopes for identification of fruit flies in SIT releases. HIE Stable isotopes workshop, Western Sydney University, Sydney (Australia).

Rempoulakis, P. (2018). Fruit flies in their habitats: A journey in space and time. Seminar upon invitation, Western Sydney University, Sydney (Australia).

Rempoulakis, P. (2018). A glimpse of the portfolio supporting fruit fly research at Macquarie University. 2018 National Fruit Fly Symposium, Melbourne (Australia), 14th August.

Shadmany, J. (2018). Prevalence and predictors of polyandry and paternity in the Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), 7th-8th June.

Siddiqui, M. (2018). The Development of a Monoclonal γH2AvB antibody to detect irradiated Queensland Fruit Fly. 10th International Symposium on Fruit Flies of Economic Importance, Tapachula (Mexico), 23rd-27th April.

Siddiqui, M. (2018). The Development of a Monoclonal γH2AvB antibody to detect irradiated Queensland Fruit Fly. Opening of new SITplus Fruit Fly Facility in Port Augusta (Australia).

Siddiqui, M. (2018). Adapt novel molecular methods for comprehensive diagnosis of pathogenic microorganisms in insect mass rearing. SARDI Entomology Annual Update Seminar, SARDI Entomology, Adelaide (Australia), 6th November.

Siddiqui, M. (2018). DNA damage and hatching rate of Queensland fruit fly after exposure to Ionizing radiation. SITplus meeting, Auckland (New Zealand), 11th November.

Sultana, S. (2018). What the future may bring: fruit fly pests of the horticultural industry. Ecological Society of Australia (ESA) Conference, Brisbane (Australia), 26th November.

Sultana, S. (2018). Modelling habitat suitability of 10 fruit fly species in Australia under climate change. 6th Australian Biology of Tephritid Fruit Flies Meeting, Canberra (Australia), 6th March.

2019

Adnan, S. (2019). Dietary caffeine supplements as a novel promotor of sexual development for fruit fly sterile insect technique. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Ahmed, A. (2019). Sexual inhibition in Qfly, a search for selfish male molecules that prevent females from remating. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Ahmed, A. (2019). Heavy vs Light Fly, an isotopic label-based proteomics approach to identify remating inhibitory molecules in Qfly. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Benelli, M. (2019). Response of Queensland fruit fly to post-production stressors: effects of hypoxia, irradiation and vibration on adult quality. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Biswas, J. (2019). Extended holding period and yeast hydrolysate supplementation prior to release enhances prevalence and dispersal of sterile male Queensland fruit fly in the field. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Biswas, J. (2019). The effect of Raspberry Ketone feeding on the electrophysiological responses of Queensland fruit flies to cue-lure stimuli. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Biswas, J. (2019). Electrophysiological responses of Raspberry Ketone fed Queensland fruit flies to cue-lure stimuli. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Biswas, J. (2019). Extended holding period and yeast hydrolysate supplementation prior to release enhances prevalence and dispersal of sterile male Queensland fruit fly in the field. Entomological Society of America (ESA) Annual meeting and Conference, St. Louis, Missouri (USA), 17th-20th November.

Castro-Vargas, C. (2019). Physiology and genetics of reproductive fitness in Queensland fruit fly. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Castro-Vargas, C. (2019). Physiology and genetics of reproductive fitness in Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Dinh, H. (2019). Effects of larval diet on adult fitness and pathogen resistance. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Dinh, H. (2019). An integrated approach of nutritional immunology in fruit flies. 2019 National Fly meeting.

Gaire, S. (2019). The effect of egg seeding density on pupal size distribution and quality control parameters of the Queensland fruit fly *Bactrocera tryoni*. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Gaire, S. (2019). Size distribution of pupae and quality control parameters of the Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Gaire, S. (2019). The effect of domestication on quality control parameters of the Queensland fruit fly *Bactrocera tryoni*. Entomological Society of America (ESA) Annual meeting and Conference, St. Louis, Missouri (USA), 17th-20th November.

Inskeep, J. (2019). Arid conditions affect the survival and activity of Queensland fruit flies, *Bactrocera tryoni* (Froggatt). 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Inskeep, J. (2019). Arid conditions affect the survival and activity of Queensland fruit flies, *Bactrocera tryoni* (Froggatt). HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Kemparaju, V. (2019). Olfactory cues of predators influence vital life processes of *Bactrocera tryoni* (Qfly). 10th Conference of Asia-Pacific Association of Chemical Ecologists, Hangzhou (China), 9th-13th October.

Kemparaju, V. (2019). Queensland fruit fly–predator interaction: a Chemical Ecology perspective. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Kemparaju, V. (2019). Chemical Ecology of Qfly and Green Tree Ant interaction. Lab Chat, Macquarie University, Sydney (Australia).

Lee, R. (2019). A second-generation genome assembly of the Queensland fruit fly: How good is it?. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Lee, R. (2019). Assessment of environmental stress resistance traits relevant to ecological competence in the Queensland fruit fly. IAEA Research Corporate Meeting in Adelaide (Australia).

Lee, R. (2019). Tephritidbase: a bioinformatics resource for tephritid comparative genomics. FF17000 project meeting in Hahndorf (Australia).

Mainali, B. (2019). Pre-release raspberry ketone (RK) feeding and non-responder selection as approaches for developing cuelure non-responsive Queensland fruit flies for combined SIT and MAT. First Research Coordination Meeting on Simultaneous application of SIT and MAT to Enhance Pest Bactrocera Management, Vienna (Austria).

Majumder, R. (2019). Fungal microbiome analysis of the wild polyphagous fruit fly *Bactrocera tryoni* larvae and various host fruit using Next-Generation Sequencing. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Majumder, R. (2019). Next-Generation Sequencing reveals the relationship between the gut microbiota and the food substrate in the polyphagous fruit fly *Bactrocera tryoni*. 8th Congress of European Microbiologists, SEC center, Glasgow, Scotland (UK), 7th-11th July.

Majumder, R. (2019). Fungal microbiome analysis of the wild polyphagous fruit fly *Bactrocera tryoni* larvae and various host fruit using Next-Generation Sequencing. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Morimoto, J. (2019). Larval density modulates larval foraging behaviour, developmental traits, and adult fitness in Qflies. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Nguyen, B. (2019). Trans-generational effects of commensal microbiota on pupal and adult traits in a polyphagous fly. Congress of the European Society for Evolutionary Biology (ESEB), Turku (Finland), August.

Nguyen, B. (2019). Trans-generational effects of commensal microbiota on pupal and adult traits in a polyphagous fly. Australian Society of Microbiology Meeting (ASM), Adelaide (Australia), July.

Nguyen, B. (2019). Effects of commensal microbiota and larval density on the development and behaviour of the Queensland fruit fly (*Bactrocera tryoni*). HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Nguyen, B. (2019). Effects of commensal microbiota on development of the Queensland fruit fly (*Bactrocera tryoni*) in parental and offspring generation. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Perez, J. (2019). Behavioural and electrophysiological response of Queensland Fruit Fly ('Q-fly') to emissions from male rectal glands. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Popa, A. (2019). Variation in stress tolerance in Queensland fruit fly. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Prasad, S. (2019). Genetics of stress and domestication related traits in *Bactrocera tryoni*. 7th Australian Biology of Tephritid Fruit Flies Meeting, Shepparton (Australia), 28th-29th May.

Prasad, S. (2019). Genetics of stress and domestication related traits in *Bactrocera tryoni*. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Shadmany, J. (2019). Prevalence and predictors of polyandry and paternity in the Queensland fruit fly. HDR Conference, Macquarie University, Sydney (Australia), 11th June.

Shadmany, J. (2019). Novel methods of sperm quantification in the Qfly. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Shadmany, J. (2019). Prevalence and predictors of polyandry and paternity in the Queensland fruit fly. Lab Chat, Macquarie University, Sydney (Australia).

Siddiqui, M. (2019). The Development of a Monoclonal γH2AvB antibody to detect irradiated Queensland Fruit Fly.

SARDI seminar series, SARDI Plant Research Centre, Adelaide (Australia), 6th June.

Sultana, S. (2019). Habitat suitability for economically-relevant fruit fly species in Australia under climate change. HDR Supplementary Conference, Macquarie University, Sydney (Australia), 18th Feb.

2020

Adnan, S. (2020). Domestication modifies metabolic rate and activity level in Queensland fruit fly. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Adnan, S. (2020). Methoprene treatment increases activity, and dietary intake of Queensland fruit fly. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Ahmed, A. (2020). Biochemistry of mating-induced sexual inhibition in Queensland fruit fly. ESA 2020: Entomology for All Virtual Meeting, Online Conference, 11th-25th November.

Biswas, J. (2020). Effect of raspberry ketone and methoprene as the pre-release dietary supplement on sexual maturation, prevalence, and dispersal of sterile Queensland fruit fly. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Biswas, J. (2020). Pre-release dietary supplements of methoprene and raspberry ketone increase field abundance of sterile Queensland fruit fly. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Gaire, S. (2020). Effect of prolonged hypoxia on quality control parameters of the Queensland fruit fly (*Bactrocera tryoni*). Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Gaire, S. (2020). Plant based proteins do not compensate yeast hydrolisate for mating success in mass reared Queensland fruit fly. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Kemparaju, V. (2020). Qfly subtly uses raspberry ketone to mitigate spider attacks. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Kemparaju, V. (2020). Computational Reverse Chemical Ecology. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Majumder, R. (2020). Microbiome of the Queensland fruit fly through metamorphosis. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Shadmany, J. (2020). Polyandry in wild populations of the Queensland fruit fly. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Shadmany, J. (2020). Enhanced sperm quantification in the Queensland fruit fly using a Y-specific assay. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

2021

Adnan, S. (2021). Domestication modifies metabolism in Queensland fruit fly. Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Mainali, B. (2021). Stable isotopes for reliable identification of wild and mass-reared Queensland fruit flies (Q-fly). Applied BioSciences Lab Chat, Macquarie University, Sydney (Australia).

Popular Articles

Australian Fruit Grower Autumn Edition 2020 - *Sterile flies, sexual development and the surprising role of caffeine in Qfly control*

Australian Tree Crop Magazine Aug/Sep 2020 – *Rough handling causes 25% fruit fly deaths*

Australian Tree Crop Magazine Feb/Mar 2020 – *Caffeine, sex and fruit flies*

Mango Matters Winter 2020 - *Developing a solution for Queensland Fruit Fly*

Hort Journal March 2020 – *The Sterile Insect Technique making an impact on Queensland Fruit Fly*

Australian Fruit Grower Autumn edition 2021 - *SITPlus – Progressing a solution to Queensland Fruit Fly*

Australian Tree Crop Magazine Feb/Mar 2021 – *Tracing the origins of fruit fly incursions*

Media Releases

Media Release Hort Innovation 08 Sep 2020 *Sterile fruit flies contribute to reduction in Queensland fruit fly population*
https://www.horticulture.com.au/hort-innovation/news-events/sterile-fruit-flies-contribute-to-reduction-in-queensland-fruit-fly-population/

Media Release Hort Innovation 29 Jan 2020 *Caffeine linked to rapid testicle growth, stronger sex drive in male fruit flies*
https://www.horticulture.com.au/hort-innovation/news-events/caffeine-linked-to-rapid-testicle-growth-stronger-sex-drive-in-male-fruit-flies/

Media Release Macquarie University 01 May 2020 *A Nose for Trouble*
https://www.mq.edu.au/newsroom/2020/05/01/a-nose-for-trouble/

National Rural News Broadcast Wed 9 Sep 2020 11:00am Eddie Summerfield interview with Dr Bishwo Mainali
https://omny.fm/shows/the-rural-news/national-rural-news-september-9-1?t=3m40s

Tasmanian Country Hour Broadcast: Wed 9 Sep 2020, 12:00pm Tony Briscoe interview with Dan Ryan
https://www.abc.net.au/radio/programs/tas-country-hour/sterile-flies/12656868

Victorian Country Hour Broadcast Wed 9 Sep 2020, 12:00pm Jane McNaughton interview with Prof Phil Taylor
https://www.abc.net.au/radio/programs/vic-country-hour/victorian-country-hour/12625218

Impact of Media Releases

Industry Publications
AUSVEG 29 Sep 2020
https://ausveg.com.au/articles/sterile-fruit-flies-contribute-to-reduction-in-qld-fruit-fly-population/

APAL 09 Sep 2020
Sterile fruit flies helping to bring down QFly numbers
https://apal.org.au/sterile-fruit-flies-helping-to-bring-down-qfly-numbers/

Good Fruit and Vegetables 16 Oct 2020 (syndicated publication)
Aerial drop of sterile Qld fruit flies proves a winner
https://www.goodfruitandvegetables.com.au/story/6919179/aerial-drop-of-sterile-fruit-flies-proves-a-winner/
https://www.stockjournal.com.au/story/6919179/aerial-drop-of-sterile-fruit-flies-proves-a-winner/?src=rss

Australian Tree Crop Magazine 10 Oct 2020
Sterile fruit flies contribute to reduction in QFF
https://www.treecrop.com.au/news/sterile-fruit-flies-contribute-reduaction-qff/

Fresh Plaza 08 Sep 2020
https://www.freshplaza.com/article/9247904/sterile-fruit-flies-contribute-to-reduction-in-queensland-fruit-fly-population/

Other news channels

ABC Rural 14 Sep2020
Queensland fruit fly population aerial eradication trial yields better than expected results
https://www.abc.net.au/news/rural/2020-09-14/queensland-fruit-fly-population-aerial-eradication-trial-yields/12646902

Food and Beverage Industry News 08 Sep 2020
Sterile fruit flies reduce pests' numbers
https://foodmag.com.au/sterile-fruit-flies-reduce-pests-numbers/

Mirage News 08 Sep 2020
https://www.miragenews.com/sterile-fruit-flies-contribute-to-reduction-in-queensland-fruit-fly-population/

North Queensland Register 16 Oct 2020
Aerial drop of sterile Qld fruit flies proves a winner
https://www.northqueenslandregister.com.au/story/6919179/aerial-drop-of-sterile-fruit-flies-proves-a-winner/?cs=4752

Mango Matters July 2020 pp 22-23
https://static1.squarespace.com/static/53b0ef57e4b04ed3debabc4f/t/5ef5476bf22174273c597569/1593132949140/MM+Winter+2020+FINAL+%28for+web%29.pdf

Research for Ag 6 May - Queensland fruit flies have a nose for trouble
https://researchforagriculture.com.au/2020/05/06/queensland-fruit-flies-have-a-nose-for-trouble/

Hort Daily A nose for Trouble
https://www.hortidaily.com/article/9213683/a-nose-for-trouble/

Lab Down Under17 June 2020 - Sniffing out danger: study suggests fruit flies use smell to adjust their reaction to predators
https://labdownunder.com/sniffing-out-danger-study-suggests-fruit-flies-use-smell-to-adjust-their-reaction-to-predators/

Hillston-Ivanhoe Spectator Newspaper 29 April 2020
https://www.facebook.com/hillstonivanhoespectator/photos/a.325365291741249/547504326194010/?type=3&theater

Hort Journal March 2020 pp26
https://hortjournal.com.au/backissuedisplay.php?issue=March2020&pages=44

Australian Fruit Grower Magazine Autumn 2020 pp28
https://apal.org.au/wp-content/uploads/2020/03/AFG-2020-Autumn-final-LR.pdf

Intellectual property, commercialisation and confidentiality

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

Acknowledgements

Acknowledgements relating to each specific Theme and Project outcome are presented in the associated peer-reviewed publications, theses and reports. HG14033 has been a large and diverse program of research, requiring substantial contributions of cash and in kind from numerous organisations, including Macquarie University, New South Wales Department of Primary Industries (NSW DPI), Department of Primary Industries and Regions (PIRSA)/ South Australia Research and Development Institute (SARDI), Plant and Food Research New Zealand (PFR), and Commonwealth Scientific and Industrial Research Organisation (CSIRO).

The SITplus program arose initially from the vision and commitment of Will Zacharin (Executive Director Biosecurity South Australia) and David Moore (General Manager of Research Marketing and Investment, Hort innovation), who both recognised the need for transformational change in the approaches taken to management of fruit flies in Australia, and the potential for SIT to play an increased role as a sustainable tool for use in area-wide management programs. Their efforts in establishing the SITplus program, and advocating for broad and deep foundations of Strategic Basic Science and Applied Science to sustain the establishment and future development of SIT was of critical importance in the development of HG14033.

Working closely with Will and David, Dan Ryan (SITplus Director) deserves special mention as a guiding force in the strategy and operations of SITplus, and as a principal mediator of dialogue amongst the stakeholder organisations as well as representing the SITplus program to the Hort Innovation Investment Committee and Board.

The SITplus Technical Advisory Committee (TAC) provided routine oversight of HG14033 operations, with reports supplied and discussed at each quarterly meeting. The SITplus Stakeholder Advisory Committee (SAC) provided strategic oversight, reviewing and approving reports on HG14033 as required by TAC. The active engagement of members of each of these committees, and the organisations they represent, is gratefully acknowledged.

Hort Innovation project managers Dr Brenda Kranz, Dr Penny Measham and Dr Greg Chandler have provided a consistently high level of support and guidance through the course of HG14033, assisting in developing the initial scope (principally Dr Brenda Kranz), then activities toward the specific outcomes (principally Dr Penny Measham), and finally the outputs produced (principally Dr Greg Chandler).