Hort Innovation

Final Report

SITplus: Port Augusta QFLY SIT factory pilot operation

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Delivery partner:

PIRSA

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SITplus: Port Augusta QFLY SIT factory pilot operation (FF15000)

Disclaimer:

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Summary

In November 2016, a new state of the art Queensland pilot commercial fruit fly (Qfly) production facility was officially opened in Port Augusta, South Australia. The facility has been designed to enable the production and processing of Qfly by incorporating novel technologies developed by the research agencies contributing to the overall SITplus research project.

Since opening, production has increased to over 20 million flies per week. Depending on demand, the facility is capable of producing up to 50 million pupae per week when required, with only 15 days needed to be able to ramp up production within the facility.

Novel technologies implemented have included:

- The development of a larval gel diet which has enabled larger numbers of flies to be produced in a smaller physical space, while improving the consistency and fitness of flies produced
- Modified adult cages, incorporating horizontally located egg collection tubes to improve the efficiency of the egg collection method and to allow for quicker cleaning and turnaround of cages
- Changes to the yeast used to feed adult cages has resulted in a more than 10 fold increase in egg numbers produced, with more than 2.5 liters of eggs able to be collected each week
- Modified larval collection method to reduce the time and man power required to harvest multiple cages on any one day, and eliminate the need to physically move larval towers to drainage points in the floor when harvesting larvae
- Installation of a mechanical sieving machine to reduce labour and time required to separate vermiculite from mature pupae
- Installation of an automated packaging and sealing machine to speed up pupal packaging, irradiation and ensure safer transportation of sterile pupae
- Installation of larger capacity dye application equipment to reduce time taken to apply powdered dye to fly pupae prior to irradiation
- Installation of a 40 foot freezer container to be used to decontaminate used production cages/towers and waste products to allow for quicker cleaning and disposal of waste products such as uneaten larval diet. This system allows production cages to be cleaned outside the facility building once they have been decontaminated, and for more cages to be cleaned at any one time

The development of a single sex strain of Qfly is still under investigation, so the facility is currently producing both male and female flies. The factory and staff will be well placed to accommodate a single sex strain when it becomes available.

Site visits from fruit fly specialists from both overseas and within Australia have resulted in the further fine tuning of production methods, and have ensured that production practices meet with the International Atomic Energy Agency guidelines for both quality control and data collection.

Visits by the facility manager to Medfly production facilities in both Hawaii and Israel have served to develop a strong relationship between the Port August facility manager and the managers and technical staff of the facilities visited.

Keywords

Queensland Fruit Fly; Qfly; sterile; irradiation; SITplus; fruit fly eradication; fruit fly; PIRSA;

Introduction

Sterile Insect Control for Fruit Flies

Sterile Insect Technique (SIT) is a method of pest control using area-wide releases of sterile insects to reduce reproduction in a wild population of the same species. It is used with other techniques such as protein baiting and orchard hygiene to create fruit fly free areas, or areas of low pest prevalence. SIT is currently being used effectively against fruit flies in the USA, Mexico, Chile, Argentina, Peru, Israel, Jordan, Spain and South Africa.

SIT reduces the use of pesticides. It is very acceptable to export markets, which are increasingly focused on ecologically sustainable production techniques that reduce or eliminate pesticide residues.

The SITplus collaboration

SITplus is an R&D collaboration between six parties with interconnected interests in the development and uptake of science solutions for management of Queensland fruit fly, namely:

- Horticulture Innovation Australia Limited (Hort Innovation);
- Primary Industries and Regions South Australia (PIRSA) and the South Australia Research and Development Institute (SARDI);
- Plant and Food Research Australia Pty Ltd (PFRA);
- Commonwealth Scientific and Industrial Research Organization (CSIRO);
- Department Primary Industry New South Wales (DPINSW); and
- Macquarie University (MU)
- Biosecurity Tasmania

SITplus was announced on & December 2013 by the Premier of South Australia, Jay Wetherill. The announcement was supported by a Memorandum of Understanding signed by the parties. Under that Memorandum of Understanding, the parties agreed:

- To commit to the establishment of a SIT facility in Upper Spencer Gulf (the Facility) in South Australia and the development of a sterile male-only line of Qfly for commercial production and release in horticulture production areas for both eradication and maintenance of low pest prevalence status
- That the South Australian Government, through PIRSA would commit $3 million to establish a fit-for-purpose facility in 2013-14/2014-15. The South Australian Government will own this Facility.
- Hort Innovation, PIRSA/SARDI, PFRA, CSIRO, NSWDPI, and MU will collaborate and invest to develop a sterile male-only line of Qfly for commercial production and develop technology and protocols to underpin area-wide control approaches, under a collaborative research and development agreement that commenced in 2014-15
- In parallel with development of a sterile male-only line of Qfly, market analysis will be actively engaged and commercial partners will be sought out and ultimately licensed for the production and sale of the sterile male-only line of Qfly from the Facility to interested investors
- Intellectual Property (IP) and other matters pertaining to commercialisation of sterile Qfly technologies will be developed by the parties to the MOU under a separate collaborative research and development agreement.

The Key points regarding SITplus are as follows:

- SITplus is an inclusive, national R&D initiative which addresses clear industry need and will achieve impact.

- The SITplus Stakeholder Advisory Committee (SAC) comprises of representatives from each core research funding partner.
- The SITplus Technical Advisory Committee (TAC) is drawn from core partners, project leads (and other external advisors and stakeholders by invitation at specific times)
- Other research agencies/institutes are able to collaborate to achieve program aims
- Other commercial delivery partners are being sought to deliver sterile fruit fly solution packages to growers and regions
- Commercial partnering (public/private) strengthens the probability of success
- SITplus supports and delivers key elements of the National Fruit Fly Strategy and the National Plant Biosecurity RD&E Strategy

The SIT facility in South Australia

As part of the SITplus Initiative, the South Australian Government, with support from the Australian Federal Government through Hort Innovation (project HG14035), have constructed a SIT facility in Port August for commercial production of male-only sterile Qfly.

As initially proposed, the facility is being used as a pilot to:

- Develop the best method for producing fit sterile male-only Qfly pupae
- Develop cost-effective production processes for commercial production and release in horticulture production areas across Australia
- Prove the commercial case for Qfly SIT

The design of the facility allows for the requirements of a second phase when a commercial operator is expected to take over the enterprise. During the commercial phase of production, is it estimated that production will peak at 50 million sterile pupae per week.

This strategic co-investment under FF1500 has funded operation of the Sterile Insect Technique (SIT) facility for Queensland fruit fly (Qfly) production at Port Augusta in South Australia over the period 2016/17 – 2018/19. Funds from this project have purchased operating equipment for use in the factory and payed costs of operation of the facility for its first three years of operation. The Facility Manager has worked with SITplus researchers and the SITplus Program Director to assist in building the business case, which is currently being developed alongside production of the male-only Qfly strain.

Methodology

Construction of the SIT facility in Port Augusta was completed in 2016. As soon as construction was completed, testing of potential production equipment and processes began. Equipment used in Medfly rearing facilities was used as a starting point, and then modified prototypes developed to maximise production numbers and production quality within the new facility. Key areas of development were:

- Scaling up of production to commercially viable numbers, based on a calculation of the estimated minimum numbers of flies required to cover prophylactic releases over three release sites in non-pest free regions, and for curative releases of two potential outbreak sites at one time. The number agreed upon (after consultation between the Technical Advisory Committee, and Senior PIRSA outbreak management staff) was 10 million flies per week during the winter months, ramping back up to 20 million flies from September through to April/May
- Implementation and refinement of a gel larval diet to maximise production numbers from a small physical space
    - Implementing outputs of Macquarie Research project
    - Installation of a 300 liter jacketed stainless steel vat with internal heating elements and stirring mechanism to allow for larger more consistent diet preparation
- Fine tuning of diet ingredients to rationalise production costs
    - Bulk purchase pricing (for example - hydrolysed yeast cost reduced from $1300/25 lb bag to $830/25lb bag)
    - Use of food grade ingredients instead of more highly priced technical grades
    - Reduction in the amount of hydrolysed yeast used in adult diet as per recommendation from Carlos Di Caceres, IAEA Vienna and after in house trials
- Standardisation of larval towers
    - Adoption of a single design to standardize results
- Development of a cost effective and user friendly egg collection method
    - Use of long (1.2 meter) horizontal egg lay tubes as opposed to multiple smaller egg devices
    - Reduces time required to insert and remove devices
    - Reduces time taken to wash devices
- Design of a standard, easy to clean and maintain adult cage
    - Cage frames made of aluminum to reduce weight
    - All panels are able to be removed making cleaning easier
    - Removable panels enables panels to be repaired more easily and any changes to be made to the cages in future made more easily
    - Cages are able to be stacked on top of each other, enabling increased production out of the same physical floor space
- Determine the optimal egg density on larval trays
    - Multi replicate trials comparing the volume of eggs added per tray of diet to the number of larvae collected from each tray were undertaken. Current volume of eggs seeded per tray of diet is 4ml
- Determine the optimal diet volume for larval trays to minimize waste and maximise production
    - Multi replicate trials measuring the volume of uneaten diet left in diet trays after larvae have jumped (from trays containing varying volumes of diet) compared to the number of larvae collected from each tray. Due to slight irregularities in diet tray shapes, (not all tray bases are exactly flat), a volume of 2.5 liters of diet per tray was accepted as optimal. This volume produces the greatest number of larvae produced with the least amount of wasted diet

- Determine the optimal volume of pupation media (vermiculite) to be added to larvae prior to pupation to provide the heaviest pupal weights possible (a key factor affecting adult competitive advantage in the field)
    - Two different pupation media (vermiculite and corn grits) were trialed alongside naked pupation (no media was added)
    - Varying volumes of each type of media were added to a constant volume of harvested larvae. Pupal weights and numbers, pupation speeds and flightability results were used to determine the optimal volume of pupation media required to produce the most and highest quality flies
    - Both vermiculite and corn grits resulted in similar production outcomes (both in quantity and quality), however the cost of using corn grits is twice the cost of vermiculite but without any appreciable benefit
- The development of a rigid form of pupal packaging to speed up the handling of pupae prior to and during irradiation, and to minimize potential damage to pupae during transportation
    - Various forms of rigid packaging was trialed to firstly determine if the packaging could be used safely in the irradiator, and still result in even levels of irradiation though the entire container
    - Samples of flies irradiated and packaged in each type of packaging was dispatched to SARDI and the quality of resulting flies compared with flies sent in plastic bags (standard used in many overseas facilities for transportation)
    - The ability of each type of potential canister to maintain hypoxic conditions was determined
        - Included trialing various sealing devices
    - The cost of each type of canister was compared
    - The ease of recycling/disposal of each canister type was compared
    - The ability of the canister to be part of a mechanized filling and sealing process was considered
    - Final recommendation is to use a cardboard canister (100mm diameter x 220mm height) with a pressed aluminum base, and heat sealed with a polyethylene membrane
- Investigation of the optimal irradiation dose required by Q flies to maintain peak field fitness while imparting the required level of sterilization
    - Supporting Macquarie research project
- The development of a larger capacity dye application machine to speed up the process allowing for greater numbers of pupae to be processed in a shorter period of time
    - A variable speed modified gem tumbler was trialed against the existing dye application machine
    - New machine does not require the machine to be stopped to remove/change dye canisters, reducing time taken to apply dye
    - Larger volume of pupae is able to be dyed at any one time
    - Multiple canisters (four per applicator) are able to be used at one time allowing for different colour dyes to be applied to different canisters simultaneously
- The development of a mechanised packaging machine to pack pupae into rigid canisters, to enable greater numbers of pupae to be packaged and sealed over a shorter period.
    - Reduces the amount of physical labour required to carry out the procedure,
    - Reduces the likelihood of repetitive strain injuries to staff, and
    - Minimizes staff exposure to powdered dyes (potentially hazardous substance)
- Investigate the possibly deleterious effects of using different amounts of powdered (and dyes from different dye manufacturers) on the flightability and health of resulting adult flies
    - Dyes from two available suppliers were compared to determine if the resulting adult flies showed differences in quality control parameters

        - Results showed no significant difference in quality control results from flies dyed with material from either supplier
    - Varying amounts of dyes were applied to set volumes of pupae and the resulting quality control parameters were compared
        - Found that 2g of dye per one liter of pupae gave the best results in respect to quality control results and ease of identification of sterile vs wild flies in recapture situations

- Installation of a minus 20 degree freezer unit to allow for quicker decontamination of used equipment and uneaten diet
    - Enabled larger volumes of waste to be frozen and in a shorter period of time than previously
        - Waste diet material able to be spaced out to allow for quicker freezing time
        - General decontamination of rubbish materials, materials to be removed from the facility, used production equipment achieved more quickly (24 hours vs up to 6 weeks previously)
        - Ability to quickly and safely decontaminate production cages, to allow them to be cleaned outside the facility (without jeopardizing biosecurity requirements)
- Development of a comprehensive database, with “dashboard” capabilities allowing for the generation of trend reports and graphs
    - Redesign of production data collection tables to allow for real time data input
    - Inclusion of “data input error” detection capability to reduce chances of data input errors
    - Linking of all data to eliminate the need for data entry duplication
- Development of a suitable pupal bag to prevent larval from jumping away during pupation, and to improve pupal weights and health
    - A range of fabrics trialed to determine which resulted in the best quality control results (highest pupal weight, best flightability results, lowest pupal mortality) and lowest rate of larval escape.
    - Curtain “voile” was found to produce the best quality control results, is easy to clean and has a thread spacing small enough to prevent larvae from escaping, but large enough to have larvae dry quickly and therefore prevent the potential buildup of fungi and molds inside the bags
- Investigation of a mechanised sieving machine to separate vermiculite from pupae more quickly and efficiently, and reduce the amount of physical labour required
    - A bulk sieving machine capable of sieving up to 30kg of pupae at any one time has been identified
    - Machine sieves using lateral movement rather than vertical movement therefore reducing potential damage to pupae during the sieving process
    - Machine reduces potential for repetitive strain injuries to staff
    - Mechanized sieving provides more “consistent” sieving results and removes the potential for variation between staff sieving techniques
- Investigate the effects of “bubbling” harvested eggs to synchronize and improve egg hatch to maximise the number of eggs able to be seeded on any given day
    - After visiting 2 facilities overseas, and observing the results obtained by “bubbling” eggs, trials were set up on site to determine the effect of bubbling Qfly eggs on hatching speed, hatchability of eggs, and the maximum time Q-flies are able to be bubbled, before detrimental effects are observed
    - Bubbling was found to increase the number of eggs that hatched into viable larvae
    - Bubbling eggs resulted in the ability to maintain egg viability from previously collected eggs (up to 48 hours previous) and to be able to seed eggs collected from multiple days at one time, increasing the volume of larvae able to be collected from 1 days seeding (thereby better

synchronizing pupal collections)

- Development of a more efficient larval collection method to reduce time spent harvesting larvae and subsequent clean up afterwards
    - A removable stainless steel trough has been installed between two rows of larval towers. Collection sieves fit into the trough eliminating water spills onto the floor and reducing the incidence of escaped larvae onto the floor from the sieves
    - Allows for the collection of larvae without having to move towers to drainage points within the room
        - Reduces manual labour required
        - Reduces time needed for cleaning
- Implementation of effective cleaning equipment utilising steam for cleaning for facility floors, ceilings and walls to reduce chemical use, physical labour required, and potential fungal and bacterial pathogen loadings on facility walls, ceilings and floors
- Development and implementation of Standard Operating Procedures to ensure optimized consistent Qfly production (quality and quantity).

Outputs

The primary outputs of the project are development and refinement of factory SOPs and production of sterile flies for a range of applications, including outbreak response, area wide management and buffering around the Riverland Pest Free Area. A full list of SOPs is provided in Appendix 1.

Media

A large number of media releases have been developed as part of the project. Some highlights include:

- '*Irresistible' sterile flies released to mate with female fruit flies in SA*. ABC News, 3 April 2018.
- Elite sterile fruit flies released across Adelaide. Stock Journal, 4 April 2018.
- Sterile fly release in Adelaide. Asia Fruit, 4 April 2018.
- Sterile fruit flies release in SA to prevent species from reproducing. Asia & Pacific Edition, 4 April 2018.
- Measures taken to keep Sa fruit fly free. Wes cost Sentinel, 20 September 2018
- Super sexy sterile fly enlisted in fruit fly fight. Australian Interstate Quarantine. 3 April 2019.

Outcomes

Overview

The factory has been 'recommissioned', and is now operating to specifications. The factory continues to use an established bi-sex strain and has a consistent production run of between 15-20 million pupae per week. Plans for full commercialization have been delayed due to unforeseen delays in development of the male-only strain. It is anticipated that this work will be completed and the male-only strain introduced under project FF18003.

The SITPlus program director is continuing to actively seek out and engage commercial partners. This aspect of the project has been pushed back due to delays in developing the male-only strain.

Both static and ground releases of sterile flies have been used through 2018-2019 to assist with the eradication of Qfly outbreaks across regional South Australia (Loxton) and regional Adelaide. Field releases of a bi-sex strain were included as part of the Queensland fruit fly eradication program in Loxton (beginning in Feb 2019), regional SA while prophylactic releases are being employed in both Lindsay Point (static releases from Sept 2018), and Shepparton regions, Victoria (starting March 2019).

SOPs have been developed to govern factory procedures and protocols and staff have been trained in there implementation, to ensure consistently optimised Qfly production, quality and fitness. These have been reviewed and approved by the Manager, National SITplus Facility and General Manager, Plant and Food Standards (PIRSA). Further input of research and technical experts is occurring to enhance and improve production. The factory and

staff have the capacity to accommodate a male only strain once it has been developed. Staff also have the technical expertise to be able to develop new procedures adapt current SOPs to suit male only production.

Monitoring and evaluation

The following immediate project outcomes were identified in the Monitoring and Evaluation Plan developed in MS103.

Industry level

Support for SIT and willingness to invest – Industry awareness and support for SIT has been substantially raised through the project. An indicator for this was strong industry support for SIT releases during the Loxton Q-fly outbreak. Industry has also expressed strong support for area wide management and prophylactic use of SIT, once any market access concerns have been addressed through development of the relevant sections of the National Fruit Fly Management Protocols. There has also been significant interest in investment in the facility, which is likely to bear fruit once the aforementioned issues have been resolved and industry is further down the pathway to widespread adoption.

Capacity and practice change

Full staff complement hired and trained – The factory is operating at required capacity and a full complement of technical, operational, management and administrative staff are in place.

Equipment installed in factory – All required equipment is now installed in the factory and operational. This includes larval towers, breeding cages, sifting and dyeing equipment, washing facilities and an irradiator.

SOPs in use – SOPs have been develop and are in use. An ongoing training and compliance program is in place to ensure application of and adherence to SOPs by all staff.

Fly production at level of proof of concept – Sterile flies from the facility have been successfully deployed in a range of real world situations including population suppression, buffering pest free areas from pest pressure, and eradication of outbreaks. This demonstrates the factory can produce sterile flies of sufficient number an quality for practical application.

Benefits

Flies produced at a cost that allows an attractive profit margin for a factory operator. Currently the factory is able to produce sterile flies at a cost of approximately $1,200 per million. By contrast, Western Australia has been charging $7,500 per million for sterile Medflies. It is anticipated that factory costs can be further reduced with economies of scale and ongoing refinement of procedures.

Recommendations

1. Continuing support and development of the male –only Qfly strain
2. Further refinement of factory SOPs to optimize Qfly production and fitness under F18003.
3. Development, adaptation and update of appropriate SOPs once male-only strain is available
4. Continued supply of SIT flies for outbreak response, area wide management and buffering around Pest Free Areas
5. SITPlus Director to continue pursuing commercialisation opportunities
6. Ongoing work with Department of Agriculture and Water Resources to facilitate prophylactic use of SIT in Pest Free Areas.

Intellectual property, commercialisation and confidentiality

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

Appendix 1: List of Factory SOPs

1. Adult cage set up SOP
2. Bush fire drill template
3. Bush fire evacuation SOP
4. Cleaning adult cages SOP
5. Cleaning larval trays SOP
6. Egg collection and seeding SOP
7. Facility system adjustment SOP
8. First aid checklist, first aid defibrillator SOP and first aid register template
9. Forklift safety checklist
10. Forklift use SOP
11. Gel diet preparation SOP
12. General cleaning duties SOP (covers vacuum and drain cleaning and pressure cleaner use)
13. General entry and exit SOP
14. General fly maintenance SOP
15. Generator use SOP
16. Irradiator operation procedure SOP
17. Larval tower cleaning SOP
18. On-call duty information SOP
19. Pest control SOP
20. PIRSA Check of irradiator power supply
21. PIRSA Chemical use
22. PIRSA Diet preparation
23. PIRSA electrical testing SOP
24. PIRSA emergency response checklist
25. PIRSA emergency response insect bite or sting
26. PIRSA emergency response medical emergency
27. PIRSA emergency response plan for chemical exposure
28. PIRSA emergency response plan for chemical spills
29. PIRSA emergency response plan for snakes and spider bites
30. PIRSA emergency response plan site evacuation
31. PIRSA Ergonomic SOP
32. PIRSA eye wash station SOP and testing register
33. PIRSA Forklift use SOP
34. PIRSA Irradiator interlock tests
35. PIRSA Irradiator operation hazard identification
36. PIRSA Manual handling risk register
37. PIRSA Manual handling SOP
38. PIRSA PPE selection and use SOP
39. PIRSA Vehicle checklist template and instructions for logbook use
40. PIRSA Working from heights SOP
41. Quality assurance testing SOP (pupal weights, sex ratios, flight ability and fertility testing)
42. Quarantine procedures SOP
43. Security processes SOP
44. SOP for dosing drains
45. SOP for dyeing pupae
46. SOP for larval and pupal collection
47. SOP for measuring balances (including calibrations)
48. SOP training template
49. Staff induction SOP

50. Steam cleaner use SOP
51. Waste removal SOP
52. Yearly workplace safety checklist - laboratory
53. Yearly workplace safety checklist - office and outside