Know-how for Horticulture™

Developing the sterile insect technique for eradication of incursions of Mediterranean fruit fly in Australia

Dennis Hopkins
SA Research &
Development Institute

Project Number: AH01025

AH01025

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Developing the sterile insect technique for eradication of incursions of Mediterranean fruit fly in Australia.

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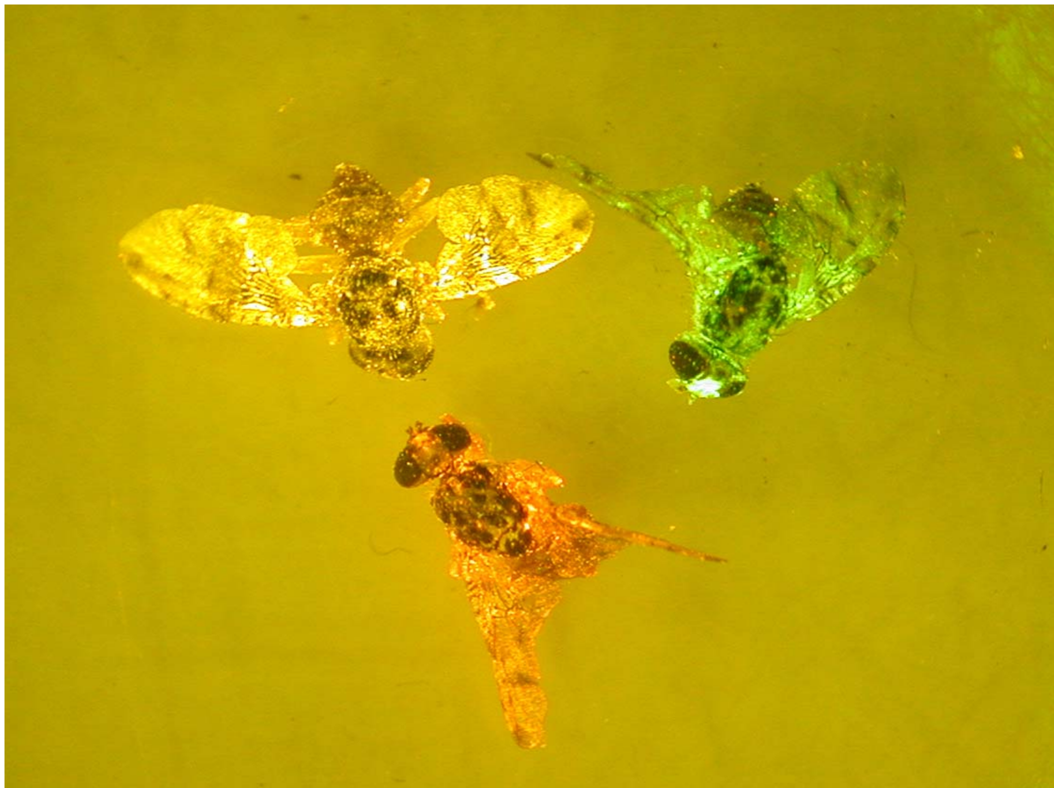

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FINAL REPORT

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Statement of purpose of report: This report details the research and development undertaken in Project AH 01025 on developing the sterile insect technique for eradication of incursions of Mediterranean fruit fly in Australia. The Mediterranean fruit fly sterile insect programs undertaken during the project period, and associated research, are described and recommendations are made for areas of future research.

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MEDIA SUMMARY

The Mediterranean fruit fly (medfly), an established pest in Western Australia, is a regular invader of South Australia. Early detection of fruitfly outbreaks is important, and strong community support through notification of any larvae found in fruit and an Adelaide-wide surveillance trapping grid in suburban back-yards has helped South Australia to remain fruitfly free. Fruitfly-free status enables South Australian fruit production industries to access lucrative overseas markets that have strict quarantine laws against fruitfly-infested regions.

In the past, medfly outbreaks were treated with chemical pesticides. As a result of this project, South Australia is now using the more environmentally friendly method of Sterile Insect Technique (SIT), combined with some baiting, to fight outbreaks. In a joint collaboration between the South Australian Government and the Western Australian Department of Agriculture (WADA) supported by Horticulture Australia Ltd, SIT has been evaluated for use against medfly incursions in South Australia. The three-year project has:

- ✓ Supervised two large SIT programs, assessing the quality of the released insects and monitoring their dispersal and density over the treatment area.

- ✓ Conducted research into improving the efficiency of the technique through possible modifications to the release method, and pre-release treatment of the flies to improve their success in the field.

- ✓ Assisted with the dissemination of acquired information through media reports, departmental publications and international presentations.

The results of the project support the continued use of SIT for medfly outbreak eradication with the following conditions:

- ✓ A reliable source of good quality sterile flies continues to be readily available. Excellent sterile flies are currently produced by WADA. This facility needs to be maintained, and the relationship with WADA fostered.

- ✓ SIT programs must be supported by a scientific assessment of the distribution and density of released insects. Unlike chemical control methods, the biological approach to eradication can be fraught with unexpected variation. Monitoring by qualified staff is needed to interpret the possible biological implications of any unforseen events.

The use of SIT for medfly control is widespread around the world. Intense scientific research, much of it supported by the International Atomic Energy Agency (IAEA), is ensuring that the efficiency of the method is improving rapidly. It is recommended that research collaboration with Western Australia continue, and that new innovations arising elsewhere are tested and incorporated as appropriate. In view of the advances in SIT in recent years, a process to evaluate the feasibility and economics of an a program to eradicate medfly from WA is warranted.

TECHNICAL SUMMARY

Until 2001, Mediterranean fruitfly (medfly, *Ceratitis capitata*) outbreaks in South Australia were eradicated through combined use of bait sprays, foliage cover sprays and ground sprays for a period of 10 – 12 weeks throughout a 1.5km radius of the outbreak centre. In 2000/2001, a series of outbreaks coalesced across an area of the Adelaide suburbs comprising more than 20 sq km and resulting in an extended eradication program. Public concern over the safety of chemical pesticides lead to a ban on cover sprays in May 2001 and a request by the Minister for Primary Industries and Resources for an independent review of the fruit fly program. This review recommended the introduction of the Sterile Insect Technique (SIT) for medfly incursions coupled with the appointment of a dedicated entomologist. An agreement was made with the Western Australian Department of Agriculture for the supply of sterile medfly (strain Vienna 7 / Mix 99).

The first SIT program was run in spring 2001 throughout the area affected by the 2000/2001 outbreaks (referred to subsequently as the Millswood SIT program) to address the potential for overwintering of immature stages in the area as a result of the ban on coversprays. A second SIT program was conducted following the declaration of two adjacent outbreaks in the Salisbury area in 2002. Both programs were considered to be successful as medfly were not detected in those areas thereafter. The method was also more favourably received by the public than previous chemical eradication campaigns.

- ✓ As a result of these programs, a Standard Operation Procedures manual for the conduct of future SIT programs was produced.

These SIT programs also afforded an opportunity to conduct research on a large scale, making use of the millions of flies released to assess density patterns and longevity. Trap calibration trials were conducted on a large scale to determine the efficiency of the traps during each program.

- ✓ Calibration trials formed the foundations for understanding the recapture data from SIT programs. Such trials should be conducted during each SIT program to provide standard data for comparison between programs, and to assist with the assessment of sterile fly density.

The literature provided indications that pre-release treatments, specifically the provision of protein in the diet or the exposure of the flies to ginger root oil (GRO), may have a beneficial effect on SIT programs through improvements in the mating competitiveness of the sterile male flies. Laboratory, orchard and field tent trials were conducted on these potential means of improving the potential success of the flies in the field. The effect on recapture rate of feeding the flies protein prior to release was tested on a large scale. Protein feeding effect on male calling ability was tested in the laboratory.

- ✓ The results of the large-scale release trial suggested that protein fed flies were recaptured at a lower rate, compared to sugar fed flies. Whether this result was due to differential survival, activity, or attraction to the traps was not determined.

- ✓ Protein feeding did not have a significant positive effect on the calling frequency of this strain of sterile male flies.

The effect on calling behaviour and attraction to traps of flies exposed to GRO prior to their release was also tested. Western Australia conducted complementary experimental cage trials to test the effect of GRO on the mating competitiveness of the sterile male medfly.

- ✓ There was a significant positive effect of GRO on calling behaviour.

- ✓ There was a significant effect of GRO on the recapture rate of Capilure-baited Lynfield traps, with GRO-exposed flies being less likely to be trapped in the first two days after release, relative to unexposed flies.

- ✓ The Western Australian trials demonstrated that exposure to GRO increased the mating competitiveness of sterile male medfly.

A simple aerial release method was also tested in one two-week trial. This trial returned lower recapture results for the aerial release method in comparison to ground releases, however, with compensatory increases in fly release numbers, the method could be cost-effective for very large outbreaks or those in country areas or inaccessible sites.

The SIT programs enabled Plant Health Operations PIRSA staff to gain experience in the running of a medfly SIT program. This is a complex process involving such diverse tasks as

- publicity and public relations tasks
- preparation of distribution routes and staff rosters,
- handling and distribution of pupae into rearing buckets with agar,
- implementation of standard quality control tests (IAEA/FAO/USDA, 2003),
- distribution methods
- facility hygiene and maintenance,
- trap monitoring techniques,
- sterile fly identification,
- data collation and reporting.

Overall, this project has demonstrated that SIT is a worthwhile method for medfly outbreak eradication in South Australia. Its continued use depends on a reliable source of sterile flies and ongoing scientific input to support Plant Health Operations.

INTRODUCTION

The use of the sterile insect technique (SIT) for Mediterranean fruit fly (medfly) control has been demonstrated to be effective in a number of countries. It is currently being used routinely in California, USA as a measure to prevent the establishment of medfly and reduce the need for expensive reactive eradication programs. In other countries with established medfly populations, the method is used to suppress the population to levels low enough for successful horticultural industries. In other areas, the method is even being used to attempt to strategically create fruit fly-free regions. In South Australia, the technique has been used for Queensland fruit fly eradication since 1993.

Mediterranean fruit fly outbreaks occur in South Australia nearly every year. They are generally detected via a suburban trapping grid or through notification to Primary Industries and Resources South Australia (PIRSA) Plant Health Operations division by members of the public finding larvae in fruit. In early 2001, a series of Mediterranean fruit fly outbreaks took place over an extensive area around Millswood in the Adelaide suburbs. The severity of the outbreak and the intense public concern over the chemical methods used to eradicate it served as the impetus to implement SIT for medfly in South Australia.

A supply of sterile male medfly for SIT in South Australia was secured from the Western Australian Department of Agriculture (WADA). Pre-emptive sterile medfly releases were planned for the period late September to the end of December, 2001 in the Millswood area (see following section on The Millswood Sterile Insect Program). In May 2002, another large SIT program was mounted against an outbreak in the Salisbury area, north of Adelaide (see section on The Salisbury Sterile Insect Program). These two large-scale releases produced data on recapture rates and provided opportunities for experimenting with release methods and rearing procedures. A large-scale release program was also conducted in Western Australia with the experimental aim of comparing the effectiveness of ginger-root treated sterile flies and non-treated flies. Outside of these large programs, small-scale experiments were conducted in the laboratory and in orchards and field tents with the aim of investigating exposure to ginger root oil, or modifications to the pre-release diet, on sterile fly behaviour. Experimental work was also conducted on the possible use of Spinosad as an alternative for malathion in the bait spray used prior to sterile fly release.

THE MILLSWOOD STERILE INSECT PROGRAM

In early 2001, a series of Mediterranean fruit fly outbreaks took place within a 27 sq km area of the Adelaide suburbs. The use of chemical cover and ground sprays caused public concern, resulting in ministerial intervention into the eradication campaign. Cover sprays of Fenthion were omitted for the last two months of the eradication program. The future use of such methods looked uncertain. There was concern that young medfly stages that may have developed on untreated trees, could survive the winter to establish a population in the following spring. Therefore, pre-emptive sterile medfly releases were planned for the period late September to the end of December, 2001 in a selected 20 sq km area of the outbreak zone.

GENERAL OPERATING PROCEDURES

Sterile fly production – Western Australia
The sterile medfly were provided by the Western Australia Department of Agriculture (WADA). The strain was Vienna 7/Mix99 with the genetic components: temperature sensitive lethal *tsl* and white pupae *wp*. This was a "male-only" strain which was reared in a system involving a hand-selected filter colony to keep recombination to a minimum. Unwanted females were killed at the embryo (egg) stage through exposure to high temperatures for 24 h. The remaining males were reared through the larval to the pupal stage. Females that were not killed by the temperature treatment could be detected at the pupal stage by their white pupae (otherwise dark brown in males). The proportion of females surviving heat treatment was kept to a minimum by the filter colony system, and was measured regularly in quality control tests. The proportion of females in brown pupae was also checked to make sure the quality control tests are reliable.

Pupae were irradiated for 10 minutes in nitrogen for a total exposure of 180Gray with a ^{60}Co source at WADA. They were then dyed by hand rotating them in a plastic cement mixer with fluorescent dye powder. Dyes used were Fiesta brand Magenta, Lunar Yellow, Flame Orange, and an unknown brand called Qfly orange (pale orange). A blue dye was also used in one trial, however their identification required the use of a UV flood-light, since the dye was completely invisible under the microscope UV lights. Long periods of work close to the UV flood light was deemed to be undesirable as UV monitors suggested the light was too strong in UVB, so this colour was not used again and is not recommended for further use. The pupae were then sealed in long slim plastic bags, packed in shredded paper in insulated foam boxes and air-freighted to South Australia at around 2pm Perth time.

Fly rearing - South Australia
Pupae were collected from the airport early in the mornings on the day following shipment from Perth and transported to the Netley rearing facility. Total weight was recorded, and the amount of pupae to be distributed into each bucket was calculated by dividing the total weight by the number of buckets required. There were six areas to be treated and the number of buckets varied slightly each day according to the size of the area that was scheduled to be treated that day (see Table 1).

Table 1. Details of release area sizes, release route distances and scheduled number of buckets to be released in each area.

Area number	Area size (sq km)	Release route distance (km)	Number of buckets
1	3.45	33.5	209
2	3.02	34.8	218
3	3.42	34.4	215
4	3.41	34.8	218
5	3.34	31.5	197
6	3.33	32.6	204

Pupal samples were taken at random from different bags for quality control tests. The pupae were then gently mixed together in a large basin, and weighed out into the appropriate sized batches (usually around 14 g per bucket, about 1600 pupae). The pupae were placed into paper bags with additional paper inserts (surface area for emerging flies to rest on whilst drying and hardening off). One paper bag was then placed in each 5L cardboard bucket along with a small plastic cup holding 40 ml of a sugar/agar food source. The agar source was made up in large volumes according to the following proportions of ingredients: 1L water, 4.84g agar, 0.1g methylparaben (preservative) and 178.6g sugar. The cardboard lid of the bucket was positioned and the buckets were taken in trays of 18 to be stacked in the rearing room and held at 26°C, 65- 80%RH until release. Stacks were labelled with batch number and release date.

Quality control

Quality control tests were carried out according to the protocol outlined in the USDA publication: (1998) Product Quality Control, Irradiation and Shipping Procedures for Mass-Reared Tephritid Fruit Flies for Sterile Insect Release Programs. Other tests were carried out to provide additional information of the program managers. Despite initial problems with low flight ability, the quality of the flies overall was exceptionally good with the quality results generally well above the USDA acceptable minimum.

The average results were:

- Pupal weight 8.3mg, USDA acceptable mean is 7.2mg
- Flight ability 72%, USDA acceptable mean is 55%
- Emergence 75%, USDA acceptable mean is 65%
- Longevity 21 days to 50% mortality.

Pupal weights

The weights of at least three samples of 100 pupae were measured on a 4 digit Mettler balance and the average calculated. This test was carried out for most of the batches received.

Flight ability and emergence

Flight ability and emergence was taken by placing three samples of 100 pupae inside a smooth black plexiglass tube (8.9cm outside diam, x 10 cm in height) lined with talcum powder. Each of these tubes was placed in a separate cage, along with one empty tube as a control. The control tube provided an estimate of the number of flies that could fly out, but might then have got back into a tube and were unable to get out a second time. On the day of release, the number of flies outside the tubes was used in conjunction with the number of flies that were found inside the control tube to estimate the flight ability of the batch. Emergence was also calculated by counting the total number of emerged flies.

Daily emergence
This was initiated before the flight ability tests were implemented to give emergence results. They were continued because they allowed the visualisation of when the majority of flies emerged, so that if flies emerged early or late this would be known. Three samples of 100 pupae were placed in a petri-dish and counted daily for emerged flies. In this method, the flies were temporarily placed in the refrigerator to immobilise them for counting. In most batches, the majority of flies emerged on the second day after arrival, indicating that the time of irradiation was appropriate to ensure their sterility. As total emergence was also recorded from the flight ability tests, the details of the daily emergence results are not given here.

Longevity
In this test, one hundred flies were placed in a cage with agar (replaced twice weekly), and the number dead counted and removed daily. The cages were maintained outside in shady ventilated cabinets, protected from ants and rain. Flies from two batches (received Mon and Thurs) each week were tested for longevity.

Fly release method
Releases were initially planned to take place on the fourth day after the pupae arrived in Adelaide, however, emergence was found to be complete by the third day so after the first few releases, the schedule was changed to release the flies on the third day. The release vehicles were standard utility type vehicles onto the back of which was fixed a custom-built release unit. The release vehicle unit consisted of a temperature-controlled room with a large rear-facing sliding door. Behind the room was a chair (with seatbelt) for the release person and bins for used buckets and waste materials. The vehicle room was pre-warmed to 25 °C, then stacked with the buckets of flies and driven to the start of the release site route. One person then took the seat at the back, and in radio contact with the navigator who was also measuring the distance travelled, opened a bucket and gradually released the flies over a distance of about 160 m before opening the next bucket and so on.

Release schedule
Each of the six areas was treated with flies every third day. At the start of the program, while WA were increasing their supply capacity, the number and quality of flies was lower than anticipated, so these flies were released into areas selected on the basis of the severity of the infestations found there earlier in the year. When the supply became consistent, the three-day schedule was adhered to without variation.

Fly recapture through trapping
The fruit fly detection trapping grid, which uses Capilure-baited Lynfield traps on about a 400m offset grid, was used to analyse the distribution and density of the released sterile flies. The fluorescent dye on the pupae was transferred to the fly body as they emerged, in particular the balloon-like structure (ptilinum) on the head which retracts after emergence trapping dye particles permanently in the head. The dye was visible under UV-illuminated microscopes. Supplementary traps, previously placed during the outbreaks, were removed part way through the program since their placement did not assist the distribution analysis, and the number of flies being caught was becoming more than the fly identifiers could handle.

Recapture data were entered into a custom designed Microsoft Access database and every entry was double checked for errors. The database was designed with the assistance of Paul Hughes from Morton Blacketer Pty Ltd (153 Greenhill Rd, Parkside SA 5063). His expertise provided for a database that stored historical information, but allowed changes to be made to present data characters. For example, the traps are identified by area, round and trap number, however the physical location of that trap

may be different in different years since traps sometimes have to be moved. The database was set up so that the address of the trap number could be kept updated in one table, which was then used by the software to allocate the address to the recapture at the present time. The combined data were saved in separate archive type database. Thus, if round 31, trap 4 was in one street in 2001, and in a different street in 2002, the archived data showing the number of flies caught in each year will also show different addresses. The GPS locations of all traps in the trapping grid were also recorded and entered into the database address table. This facilitated the charting of fly captures using scatter plots and bubble charts to visualise fly recapture numbers spatially.

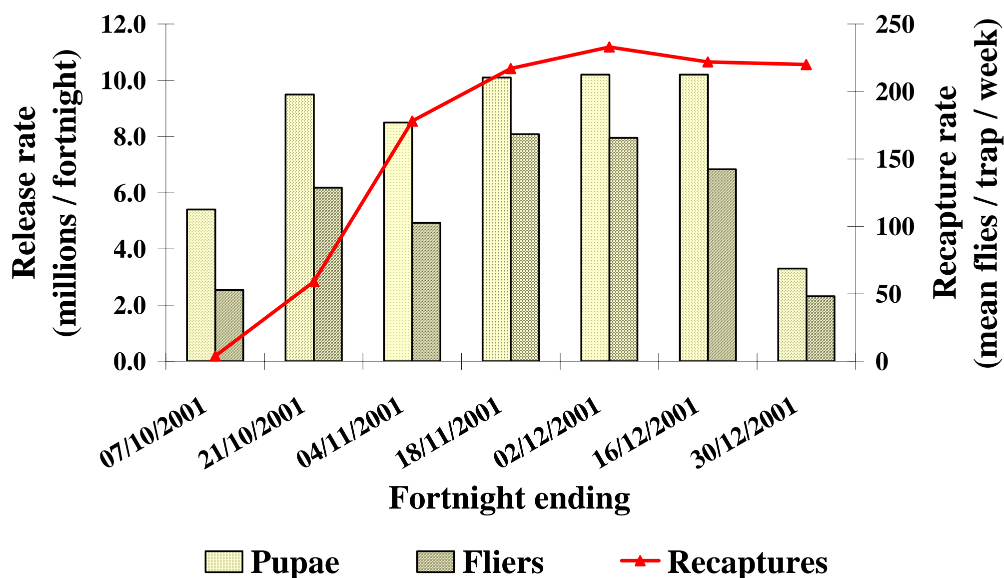
Experimental releases

Experimental releases were made with sterile flies marked with different fluorescent dye colours. Some were conducted within the main release area using the roving release vehicle. Others were conducted outside the release area by hand releasing small numbers of buckets. Details are given in the results section.

STERILE FLY DISTRIBUTIONAL RESULTS

Overall, there were 38.8 million flying males (pupal number adjusted according to flight ability QC results) released into the area over the 12-week release period. The average recapture number per trap per week is indicated in fortnightly periods in Figure 1. The average number of flies caught per trap per week was greater than 100 after four weeks, and stabilised at more than 200 flies per trap per week after six weeks. The catch per trap ranged from 0 to 2094, but after the first fortnight, 75 – 88% of the traps caught between 100 – 600 flies per week. This indicates that the roving release method was effective in creating a relatively even spread of flies across the area.

Figure 1. Release and recapture rates during the Millswood sterile insect program in 2001.



There was variability in the recapture number at any one trap in different weeks, but three traps were identified as catching low numbers of flies (<10) in three consecutive weeks. When investigated, one of these was found to be just outside the release area, another was in a nursing home grounds (lawns and no fruiting hosts present) and the third was located on a street that had no street trees. None were directly passed by the release vehicle. The low numbers in these areas were likely a combination of poor habitat and lack of direct treatment. These areas, once identified, could have been treated individually, but in the case of this preventative release it was not deemed necessary.

The vast majority of flies were recaptured within the release area, however, 7639 flies (0.02%) were trapped 1 – 10 km from the release area, and 224 flies (0.0006%) were trapped more than 10 km from the release area. Medfly are generally thought to be poor dispersers, and the trapping data support this idea. However, in an urban situation, dispersal may be facilitated by vehicles.

EXPERIMENTAL RELEASES

Grid calibration

Experimental releases within the release area were designed to estimate the trapping efficiency of the surveillance grid in the area. The effectiveness of the traps is expected to vary according to how fresh the lure is, as well as other factors such as potential contamination, trap location and weather conditions (e.g. wind strength, temperature). The responsiveness of the flies will also vary according to the weather (which affects their activity levels). The results of grid calibration trials can therefore be expected to vary considerably, but an estimate of the range and average efficiency is very valuable to the interpretation of recapture results. Thus, seven trap calibration trials were conducted in three areas selected because all of the traps in those areas were serviced on one day each week. To obtain more data, three additional trials were carried out after the release period, in January 2002.

The trials were conducted by distributing the flies in the manner normally used for release. The flies were marked a distinct colour and were released into an area on the day before the traps in that area were due to be serviced. Thus the traps were catching flies for about 24 h between release and recapture. This method enables the calculation of the percent released flies recaptured over a one-day period, during which losses due to emigration or mortality may be relatively small.

The results of the ten trials are given in Table 2, and the total average daily recapture rate from those trials was 0.15%. This means that in a weekly trapping period, each trap can be considered to be catching approximately 1% (0.15 x 7) of the flies in the immediate vicinity of the trap, and the grid will catch about 1% of the population as a whole.

Table 2. Recapture rates from ten grid calibration trials.

Day released	# fliers released	# recaptured	% recaptured
31/10/2001	194400	310	0.16
5/11/2001	265320	223	0.08
19/11/2001	247500	178	0.07
21/11/2001	272580	509	0.19
26/11/2001	277200	301	0.11
10/12/2001	298800	544	0.18
12/12/2001	159720	515	0.32
21/01/2002	298200	665	0.22
28/01/2002	361872	487	0.13
4/02/2002	69300	34	0.05

In one trial, the traps were serviced daily for four days. This trial was designed to see how quickly the fly recapture rate dropped off. On the first day, the recapture rate was 0.13%, close to the overall average. On the second day, it was 0.09%; on the third day 0.03%, and on the fourth day it was 0.02%. In other words, the recapture rate on the fourth day was only 16% of that seen on the first day.

Discussions with sterile medfly workers from California indicate that a similar pattern is seen in their program of releases (K. Hoffman, pers. com.). The hypothesis is that most released flies are subject to early mortality, probably as a result of their inability to find a suitable habitat before they succumb to predation or climatic factors. It is possible that with females rare or absent, males may spend more time in potentially more risky behaviour related to mate location. It appears that a small proportion of the released flies do find suitable conditions and may have relatively long life-spans. The single release of a blue cohort of flies during this release period found that the average daily recapture rate halved each week for three weeks. By the third week, some flies were found about 2 km from the release area. The last blue flies were captured six weeks after their release. Releases of other colours were less definitive due to the more prolific use of the other colours, but generally showed that the number recaptured in the third week was only about 10% of the number recaptured in the first week.

Fly age of response

Fly age is not thought to be important to recapture results for sterile Mediterranean fruit fly since they mature early, however experimental releases outside the release area were conducted to confirm this. For this experiment, traps were identified from the trap information as being located in front yards. Letters were distributed in advance to the trap property resident, and adjacent properties, informing them of planned experiment and inviting comment or dissent. The selected traps were more than 1 km from each other. Ten grams of pupae were placed into buckets and kept in the rearing room for either 3, 4, 5, or 6 days prior to release. The buckets had specially ventilated lids (mesh inserts) and food agar was provided through the mesh prior to emergence and replaced on Day 3. All buckets were released on the same day, at the same set of traps. The flies were therefore raised from egg batches collected from the colony on four consecutive days. The flight ability QC result of each batch was used to estimate the number of flies in each 10g pupal sample in each bucket. Dead flies in the bottom of each bucket were counted after release. Very few flies of any age died prior to the day of release.

This experiment was conducted with six replicate traps on two occasions (21/11/01 and 12/12/01). The buckets were taken to the trap location in the morning (9.30 - 11am) and opened on the ground within five metres of the tree in which the trap was located. The flies were allowed to fly out on their own for five minutes, and the stragglers were then emptied out into the air. The number of flies in the traps was counted the next day. In Trial 1, no Day 4 flies were released since when the buckets were opened at the first trap, they were found to be dyed the same colour as the Day 3 flies. The results of that first trap were omitted for that trial. In Trial 2, one trap caught no flies at all. This was probably due to the trap or lure, rather than the flies, since all five other replicates caught flies. The results, summarised in Table 3, show that flies on the day of release (Day 3) respond to the trap lure as well as older flies. The higher recapture rate in Trial 2 relative to Trial 1 could be due to differences in the attractiveness of the lure – Trial 2 was conducted one week after lure wicks were replenished.

Table 3. Summary of age of response experimental trial.

Fly Age	Average % released flies recaptured	
	Trial 1 (n=5)	Trial 2 (n=6)
Day 3	0.15	0.23
Day 4		0.26
Day 5	0.12	0.19
Day 6	0.14	0.22

CONCLUSIONS

- No wild flies were recorded in the area throughout the release period, or for the remaining summer of 2001/2002. It was not possible to ascertain if this was the result of successful reproductive suppression of an over-wintering population of flies, or whether no over-wintering took place.

- The Access Database of fly recaptures serves as a useful tool for distribution analysis, and could be further utilised in managing the fly releases. Identification of fly distribution "holes", areas of low fly recapture, could be routinely carried out by the Operations Leader, and these could be addressed by applying extra buckets of sterile flies when and where needed.

- The trap calibration trials provided an estimate of how effectively the trapping grid was operating in that area, at that time. This process should be carried out routinely in future programs to provide information on the spatial and temporal variability of the grid. It does not require very much special preparation since the flies can be distributed in the normal schedule. The only planning needed is to release a unique colour on a day before the traps are due to be serviced. This requires early notification to WA so that they can dye the appropriate pupal batch with the requested colour.

- Although the flies tend to die off quickly after release, any trials using the same colour in the same area should be at least three weeks apart to limit the likelihood of excessive captures from the previous trial confounding the results of the later trial. In cold weather, this time period may need to be greater since adult survival may be lengthened in cooler conditions.

THE SALISBURY STERILE INSECT PROGRAM

In late 2001, a male-only strain of sterile medfly was used as a preventative measure against a potential over-wintering population in the Millswood area of Adelaide. That project provided valuable experience in the use of the roving release method of fly distribution. In April 2002, wild medfly were detected in traps in the Salisbury district, north of Adelaide. An integrated chemical and sterile fly eradication program was conducted in the area from May to October 2002.

WILD FLY DETECTIONS AND CHEMICAL PROCEDURES

Plant Health Operations recorded the finding of a number of wild flies and infestations in the Salisbury area, north of Adelaide. The first flies were found at Universal Road and Gainsborough St, Salisbury Downs between the 26th to the 28th of April, 2002. Larvae were detected in Martins Road and Universal Road on the 28th of April. The trees were stripped and plastic was spread below the infested tree at Universal Rd. The Martins Rd. property had long grass below trees. With the owner's permission, the ground beneath the infested trees was treated with Lorsban® at a rate of 500 g/l (0·176% Chlorpyrifos) with 2 to 3 litres of solution applied per square metre of ground depending on soil conditions.

On the 29th of April, 2002, one female fly was trapped in Aquamarine Drive, Salisbury East. This fly formed the basis of the declaration of a second outbreak, however no more flies were detected in the Salisbury East area.

More flies and larvae were detected in the Salisbury Downs area until the 20th of May 2002. Some of the later trappings necessitated extensions to the outbreak zone and the 1.5k treatment areas. Bait spotting and hygiene were commenced on May 1st. Bait spotting involved the application of a solution of 2% protein (low salt yeast autolysate, 420 gms/L protein) and 1% Hymal® (1150 gms/L maldison) to tree foliage at a rate of 100 spots (100 ml each) per hectare. Hygiene involved the removal of all fallen fruit around fruit trees.

33 flies and a number of infestations were detected on a property on Kings Rd. Salisbury Downs. Fruit stripping and ground treatment with Lorsban were completed on the property on the 7th of May, 2002.

Bait spotting and hygiene continued until the 22nd of May. The original outbreak zone, a 200m radius circle around the first three fly finds, was treated five times. Three extension zones and the Salisbury East outbreak zone were treated with four applications of bait. The outbreak area, that is, the area within a 1.5 km radius of larval and fly finds, was treated two to three times during the three week baiting period. Lynfield supplementary traps were removed from Salisbury East on the 23rd of May and from Salisbury Downs on 24th of May, 2002.

The first sterile release began on Sunday the 26th of May, 2002. The structure of the release program consisted of two different phases; a 10-week eradication phase and a 12-week preventative phase. The eradication phase consisted of four weeks of high intensity releases (300,000 pupae per sq km per week) to ensure a rapid rate of coverage of flies within the area following the end of baiting. Then the release rate was reduced to a moderate rate (200,000 pupae per sq km per week) for the remainder of the 10-week eradication phase of the release. The preventative phase involved low level releases (100,000 pupae per sq km per week).

STERILE INSECT TECHNIQUE: GENERAL OPERATIONS

Sterile fly production – Western Australia

The same sterile medfly strain used in the Millswood SIT program in 2001 (Vienna 7/Mix99, *tsl wp*) was again provided by the Western Australia Department of Agriculture (WADA). The same production and shipping methods were used. Pupae were dyed with fluorescent powders before shipping.

Fly rearing - South Australia

Pupae were collected from the airport early in the mornings and transported to the Netley rearing facility. Total pupal weight was recorded, and the amount of pupae to be distributed into each bucket was calculated by dividing the total weight by the number of buckets required. Pupae were handled, distributed and maintained until emergence as described in the section on the Millswood Sterile Insect Program. Unlike the Millswood program in which the number of buckets used per day varied according to the distance to be travelled in the scheduled treatment area, in this program, the number of buckets for each area was set at 220. The number of pupae per bucket therefore varied according to the total weight of pupae sent from Western Australia, ranging from approximately 3000 pupae per bucket for the first four weeks of the program, to around 1650 pupae per bucket for the main eradication phase of the program, to about 1160 pupae per bucket for the preventative phase.

Pupal samples were taken at random from different bags for quality control tests. The pupae were then gently mixed together in a large basin, and weighed out into the appropriate sized batches. The pupae were placed into paper bags with additional paper inserts (surface area for emerging flies to rest on whilst drying and hardening off). One paper bag was then placed in each 5L cardboard bucket along with a small plastic cup holding 40-80 ml of a sugar/agar food source. The agar source was made up in large volumes according to the following proportions of ingredients: 1L water, 4.84g agar, 0.1g methylparaben (preservative) and 178.6g sugar.

Quality control

Quality control tests were carried out according to the protocol outlined in the USDA publication: Product Quality Control, Irradiation and Shipping Procedures for Mass-Reared Tephritid Fruit Flies for Sterile Insect Release Programs (1998). These are described in the Millswood SIT Program section of this report. Other tests were carried out to provide additional information for the program managers. Despite initial problems with low flight ability, the quality of the flies overall was good with the overall average quality results being just above the USDA acceptable mean.

The average results were:

- pupal weight 0.75 mg, USDA acceptable mean is 7.2mg
- flight ability 63%, USDA acceptable mean is 55%
- emergence 67%, USDA acceptable mean is 65%
- and longevity 26 days to 50% mortality.

Fungus in longevity cages

A species of *Entomophthora* was discovered in the outdoor longevity cages. It killed a large proportion of the caged flies. It seems likely that the infection originated through an infected wild fly (of a different species) getting into the cabinet, and dispersing conidia into the cages. The literature suggests it thrives in cool moist conditions, therefore it is not expected to cause a problem in the relatively warm rearing room. Samples of three batches of flies set up in longevity cages in the rearing room did not demonstrate any evidence of fungal infection. It may however be a source of mortality for released (and wild) flies in the field during the cooler months. The fungus was

eliminated when the cages and cabinets were thoroughly cleaned with bleach solution and left without flies for one week.

Fly release method

Releases took place on the third day after the pupae arrived in Adelaide (72 h in the rearing room). In some instances, due to poor weather conditions, emerged flies were held over for a day or two in the rearing room at Netley until releases were possible. The release vehicles were the same as those used in the Millswood Sterile Insect Program. To limit fly wastage, the paper bags containing the pupae were placed into an open bin to allow any flies that were still emerging to fly away throughout the day. This bin was closed when the vehicle parked over the lunch period and for the return trip to the rearing facility.

Release schedule

There were six areas to be treated (see Table 4) and flies were distributed into each area twice per week. For the first three days, only one release vehicle was available. When two release vehicles were available, two areas could be treated each release day. A third backup vehicle became available in June to enable additional releases to take place in the event of a back-log occurring due to rain delays. Areas A and B were treated on Mondays and Thursdays, areas C and D were treated on Tuesdays and Fridays, and areas E and F were treated on Wednesdays and Saturdays. Sundays were left free so that if releases were delayed due to rain during the week, the spare day would reduce fly wastage by providing a second opportunity for release.

Table 4. Details of release area sizes and release route distances.

Area	Area size (sq km)	Release route distance (km)
A	3.48	26.4
B	3.51	27.7
C	3.44	30.9
D	3.86	28.7
E	3.48	30.3
F	3.86	24.8
Total	**21.63**	**168.8**

Fly recapture through trapping

The fruit fly detection trapping grid, which uses Capilure-baited Lynfield traps on about a 400 m offset grid, was used to analyse the distribution and density of the released sterile flies. Traps were checked and flies collected into vials on a weekly basis. The flies were brought back to the Plant Health Operations building at Prospect and sorted and examined for the presence of fluorescent dye under the UV microscope.

Quality control of fly recaptures

Quality control of the fly examination process was achieved by the arbitrary placement of dead, unmarked flies in Salisbury traps without the knowledge of the fly identification staff. The first quality control test of this kind resulted in one of three flies not being detected. Staff were then assisted in how to discriminate sterile, dyed flies from wild flies that may have been contaminated with dye in the traps. In an afternoon training session, unmarked flies were shaken around in vials containing ~ 20 dyed flies, then sorted under the UV microscope. There was considerable dye transfer including specks of dye on the head, however, the pattern of dye particles was different and the glow from within the head (the ptlinum) was absent. Following treatment, the unmarked flies had dye particles spread evenly around the body, whereas in marked flies, there

tended to be concentrations of dye particularly on the underside of the body and the joints to appendages, as well as the ptlinum. Unmarked wild flies were more difficult to detect among pink dyed flies due to the low fluorescence of all pink flies compared to other dye colours. Subsequently, 10 flies were planted in Salisbury traps at random times throughout the program without the knowledge of the identification staff, and every one of these flies were correctly identified as a non-marked fly.

Data records

Recapture data were entered into same Microsoft Access database used for the Millswood SIT program. The GPS locations in Australian Map Grid coordinates (Eastings and Northings) of all traps in the trapping grid were also recorded and entered into the database address table. This facilitated the spatial representation of changes in fly recapture numbers.

Experimental work

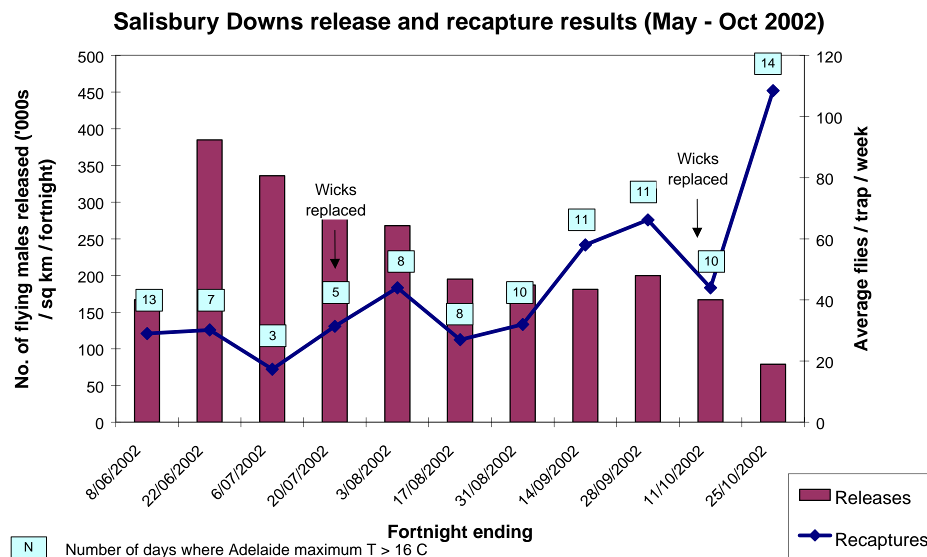
Experimental field releases were conducted from the roving release vehicle within the main release area using sterile flies marked with different fluorescent dye colours. Field experiments included a longevity trial, grid calibration trials and a trial to compare the recapture rates of flies fed the usual sugar agar with some fed on sugar agar with extra protein. Four aerial releases were conducted in the last two weeks of the program. Laboratory experiments comparing the calling activity of males fed on sugar agar or on agar with protein were also conducted.

RESULTS OVERVIEW

General distribution

Overall, 32.3 million flying males (pupal number adjusted according to flight ability QC results) were released into the entire area during the 10 week eradication phase of release period (June – August), and 16.5 million flying males were released into areas A – D in the preventative releases (August – October). The release number and average recapture number of flies per trap per week are graphed in fortnightly periods in Figure 2. The graph also shows the number of days per fortnight that Adelaide maximum temperatures exceeded the activity threshold for medfly (16°C).

Figure 2. Release and recapture rates during the sterile insect program in Salisbury Downs, SA 2002.



Although lure age no doubt also plays a part in the recapture rate in any one week, whether or not the weather is warm enough for medfly flight is likely to be an important factor in estimating the target recapture rate. This is discussed in more detail in the section below on grid calibration.

The distribution data were sent to PIRSA's Spatial Information Services, GIS Mapping Unit where they were digitally superimposed over a map of the area each week using Arcview software. The trap catches were represented by coloured spots: red = no flies caught, orange = 1- 50 flies caught, yellow = 51 – 100 flies caught, and green = >100 flies caught. The actual number of flies caught was printed inside the coloured spot. These maps enabled a rapid visual inspection for locations where no flies were caught. One trap (the most northerly trap), which only caught flies once, was actually found to be outside the release area. The maps also facilitated checks that properties where the most wild flies were caught originally were being treated sufficiently. However, the maps could be made simpler and more useful in future SIT programs. It is recommended that trap catches from future programs are plotted on the basis of whether or not they achieved the weekly target recapture rate (ie two categories). This target rate would be set making use of information from calibration trials (see Grid calibration section below) and the estimated ambient temperatures for the period of the program.

EXPERIMENTAL RELEASES

Grid calibration

Experimental releases within the release area were designed to estimate the trapping efficiency of the surveillance grid in the area. The effectiveness of the traps is expected to vary according to how fresh the lure is, as well as other factors such as potential contamination, trap location and weather conditions (wind strength, temperature). The responsiveness of the flies will also vary according to the weather (which affects their activity levels). The results of grid calibration trials can therefore be expected to vary considerably, but an estimate of the range and average efficiency is very valuable to the interpretation of recapture results. Thus, seven trap calibration trials were conducted in two areas (A and B) selected because all of the traps in each area were serviced on the one day each week.

The trials were conducted by distributing the flies in the manner normally used for release. The flies were marked a distinct colour (either pale orange or pink) and were released into an area on the day before the traps in that area were due to be serviced. Thus the traps were catching flies for about 24 h between release and recapture. This method enables the calculation of the percent released flies recaptured over a one day period, and limits losses due to emigration or mortality (Fletcher 1974).

The results of the trials are given in Table 5, and the total average daily recapture rate from those trials was 0.093%. This compares with 0.15% found during releases in Spring 2001 at Millswood. The density of traps in the calibration trials of each program was slightly different, with about five traps per sq km in Salisbury compared with about six traps per sq km in Millswood. Accordingly, this lower trap density in Salisbury may reduce the expected recapture by 17% to 0.125%.

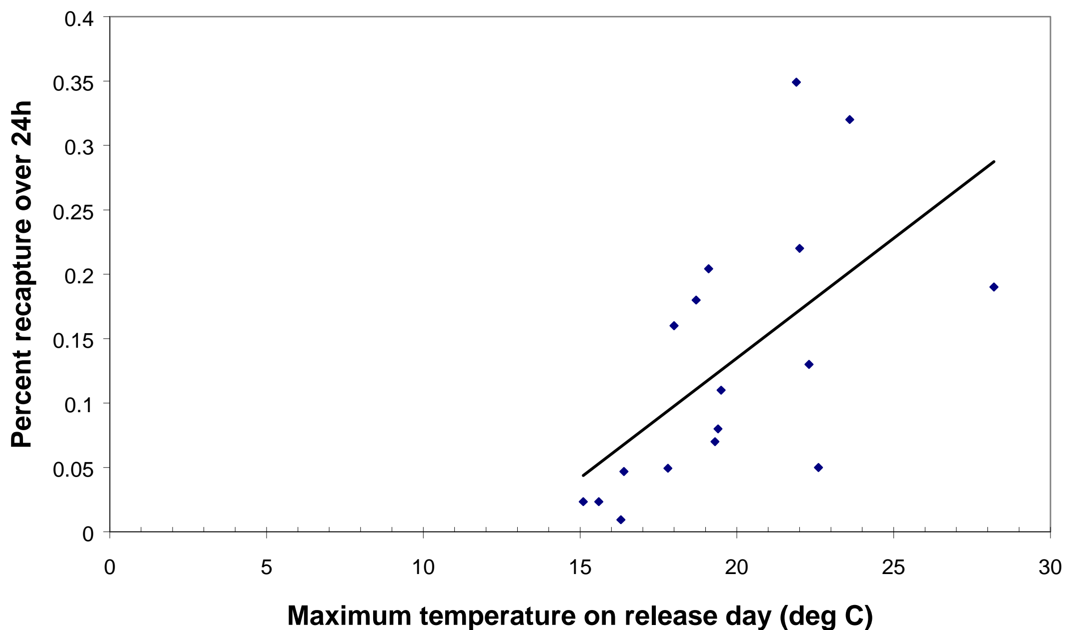
Table 5. Details of the grid calibration trial conditions.

Day released	Dye colour	Temp (°C) release day	# fliers released	# flies recaptured	% flies recaptured
4/7/02	Pink	15.1	239000	46	0.02
1/8/02	Pale orange	15.6	243540	56	0.02
15/8/02	Pink	16.4	400000	164	0.04
29/8/02	Pale orange	16.3	314670	25	0.008
12/9/02	Pink	21.9	244800	913	0.37
27/9/02	Pale orange	17.8	184800	78	0.04
10/10/02	Pink	19.1	348330	608	0.17
				Average	**0.093**

When the results of the Millswood and Salisbury (adjusted for trap density differences) grid calibration trials were combined and analysed in relation to the maximum ambient temperature on the day of release, a significant positive correlation was demonstrated (Figure 3, $r^2 = 0.37$, $F = 8.898$, $p = 0.009$, $N = 17$).

At temperatures above 20°C, there is more spread around the regression line suggesting that other factors (for example wind strength or variations in lure attractancy) may start to play a greater role in determining the recapture rate on any one day.

Figure 3. Regression of recapture rate with temperature using grid calibration data from the Millswood and the Salisbury Mediterranean fruit fly SIT programs.



These results suggest that the lower temperatures during the Salisbury trials which were held in autumn and winter (average = 17.4°C), as compared with the Millswood trials which were conducted during spring and summer (average = 21.3°C), may account for much of the difference between the recapture rates of the two programs. They also suggest that the target recapture rate for any eradication program needs to be related to the ambient temperatures of the program. Programs conducted under warm weather conditions (20 – 28°C) could be guided by the current rule of thumb of aiming for an average of 100 flies per trap per week. By further separating the grid calibration trials into temperature groups of 17 – 20°C and < 17°C and averaging the results in each group, it is estimated that on days where temperatures are between 17 – 20°C, the recapture rate may be expected to be only around 55 – 60% of that, and when temperatures are below 17°C, the recapture rate could be only 10 – 15% of the warm weather target. It was not possible to collect data on very hot days (>28°C), however future SIT programs are likely to provide such conditions at some point. This will make it possible to determine whether the observed relationship between temperature and recapture rate is maintained, or whether high temperatures affect recapture rates differently.

Longevity

An estimated total of 3.4 million uniquely coloured (yellow) flying males were released for a period of one week (24 – 29/6/02). Yellow flies were recaptured up to and including the week of 3-9/8/02 – that is, for five weeks after the yellow releases ceased. They were not subsequently captured until after this colour was released again on 26/9/02. It was expected that if adult winter survival was good, these yellow flies may have been recaptured early in the spring. This was not found to be the case, providing no evidence for the hypothesis that released medfly adults could over-winter in Salisbury.

Field protein trial

Recent publications in the scientific literature point towards the improved sexual competitiveness of medfly males fed protein compared to those given only a sugar diet (Kaspi and Yuval 2000, Papadopoulos *et al* 1998, Shelly *et al* 2002, Taylor and Yuval 1999, Yuval *et al* 1998). A possible drawback to the use of protein meals for sterile flies appeared to be higher mortality if the flies experienced 24 h starvation following release. The authors of one of these publications is following up on this work by examining the foraging behaviour of released medfly in comparison to wild flies. We attempted to compare the survival of released flies previously fed protein or sugar by examining the recapture rate of flies dyed different colours according to the diet treatment. The first field trial of this type was conducted on 26/9/02, in which half of one day's release (264,000 yellow pupae) were reared in buckets with protein (2% protein in sugar agar) and the other half (264,000 pale orange pupae) were reared with the usual sugar agar. A bucket of each type was released alternately throughout the release areas A/B by both release vehicles. It was noted that a small proportion of the flies in the protein-fed buckets got stuck in the diet due to the protein agar being softer and more liquid at the surface. The recapture rate of each group is given in Table 6.

Table 6. Results of field release trial comparing sugar-fed with protein/sugar fed fly recapture data.

Recapture date (period)	Sugar agar fed flies (pale orange)			Protein/sugar agar fed flies (yellow)		
	# traps	# flies recaptured	% flies recaptured	# traps	# flies recaptured	% flies recaptured
27/9/02 (~ 24 h)	18	78	0.042	10	47	0.025
28/9 – 4/10/02 (7 d)	28	309	0.167	30	176	0.095
5 – 11/10/02 (7 d)	13	25	0.014	10	21	0.011
12 – 18/10/02 (7d)	16	41	0.022	16	32	0.017
Total	**75**	**453**	**0.245**	**66**	**276**	**0.148**

This first trial suggests that there may be a decrease in the recapture rate of protein-fed flies. The reason for this is unknown, and this trial triggered a series of laboratory cage experiments that will be described in detail in a later section.

Aerial release trials

Four aerial release trials were conducted on October 19, 21, 26 and 28, 2002. Flies were distributed into release areas A, B and C via roving release and via 4 seater Piper Lance aircraft operated at an altitude of 305 m (1000 ft) by Australian Maritime Resources (AMR). Average air speed was about 120 knots, and the seven aerial release transects were 400 m apart and varied from 2.4 – 3.6 km in length. Pupae for roving release were marked with the pale orange dye and reared in cardboard buckets in the same way as for the previous releases. Pupae for aerial releases were marked with yellow dye and distributed into short paper bags (33g per bag) stapled once in the middle. Three bags were placed with one agar cup into each of 40 large rubbish bins to make a total of approximately 12,500 pupae per bin (depending on emergence). Two additional cups of agar were placed on a mesh insert in the wooden lid of the bins. The bins were placed with the buckets into the rearing room at Netley until the day of release.

On the release day, the buckets for roving releases in two of the areas were loaded into the release vehicles and taken for release as usual. The buckets for the third area were delivered to the release vehicles in a covered trailer at around midday. A max-min thermometer was placed in the trailer with the buckets, and indicated that temperatures in transit (~ 1 h) ranged from 14 – 28°C.

The bins for the aerial releases were transferred into a portable refrigeration unit (2.4 x 3.3 m) that had the thermostat set to 0°C. The bins were cooled for 15 - 30 mins, then three staff collected the flies into a single bin by removing the lids and knocking the flies into the bin, then removing the pupal bags and agar cup and bumping the bin on the floor of the cooler to knock them to the bottom of the bin. The flies were poured into the single collection bin, then transferred to a smaller bucket and finally distributed among seven 1.75L Décor™ plastic containers (11.5 x 7.5 x 20.5 cm). These were stacked in an esky with ice packs and transported to Parafield airport. The esky was transferred to the aircraft and, during flight, the contents of each container was distributed via suction from a flexible hose (1m long, 2cm diam.) attached to a PVC pipe protruding through the floor of the aircraft to the outside by 33cm. The outside end of the pipe was cut on a sharp angle, and the amount of suction could be regulated by turning the pipe relative to the direction of travel of the aircraft and by changing how far into the container of flies the hose was held. The process was co-ordinated between the person

operating the release mechanism and the pilot through the pilot calling out along each transect to indicate the start, quarter, half, three quarters and finishing points. The flight transects were mapped out manually, and transferred by AMR into GPS grid co-ordinates on a digital map in Ozi-Explorer to guide the pilots. Observations from ground points indicated that the flight transects were repeated fairly accurately.

Operationally, the releases ran smoothly. An operations plan developed prior to the project outlined tasks required in preparation for, and during the releases. It also included contact numbers of key personnel and formed the basis of communications among the many participants (SARDI, WADA, PIRSA and AMR).

Flight ability quality control tests were used to estimate the number of flying males released on each day by each method (Table 7). In total about 52,000 flying males/sq km/week were released of each colour in each week. In addition, the total volume of flies used in the aerial releases was recorded when they were collected into the small release containers.

Table 7. Estimated number and volume of flying males released during the aerial trials.

DATE	**Number of pupae**	**Flight ability**	**Estimated number of flying males released**	**Volume (L) of flies (aerial)**
19/10/02	510,000	0.61	311,100	6.9
21/10/02	510,000	0.45	229,500	4.9
Weekly total	1020,000		540,600	11.8
26/10/02	510,000	0.41	210,630	5.9
28/10/02	510,000	0.63	322,830	6.3
Weekly total	1020,000		533,460	12.2

These numbers translate to between 45,000 – 51,000 flies per litre, except for the batch released on 26/10/02. For that batch, given the volume of flies obtained, it seems likely that the flight ability result was an underestimate. There were 55 traps in the target area. Recapture data for each week are summarised in Table 8.

Table 8. Summary of aerial and roving recapture data for each fly colour and week.

	From aerial releases (yellow)		**From roving releases (pale orange)**	
	Week 1	**Week 2**	**Week 1**	**Week 2**
Flies recaptured in target area	1381	2616	2282	4051
Number of traps in target area found with flies (% of total)	41 (75%)	47 (85%)	51 (93%)	50 (91%)
Total flies recaptured anywhere	1665	2888	2312	4132
Total number of traps found with flies	63	103	66	86
Number of traps containing >100 flies	3	5	5	14

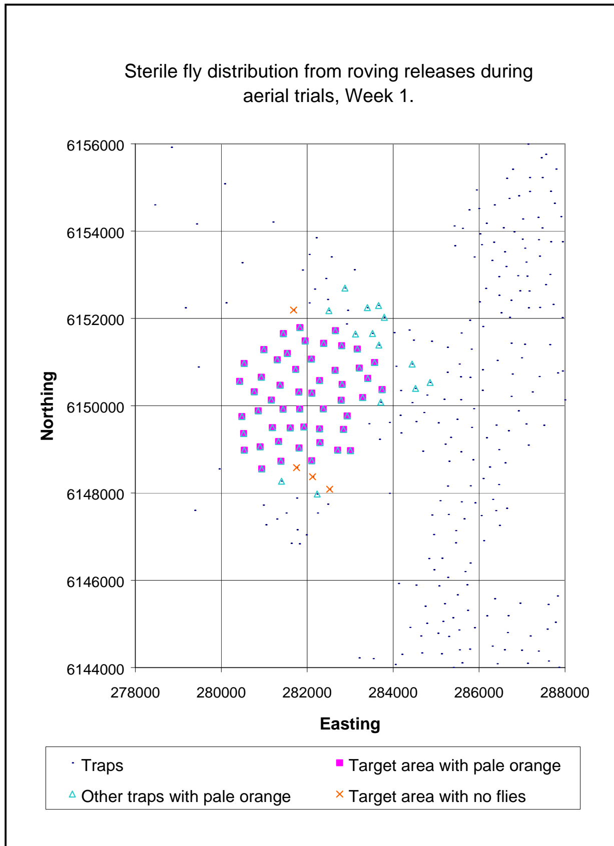
The distributions of the flies released with each method are illustrated in Figure 4. In the first week, 40% fewer flies were recaptured in the target area from the aerial method

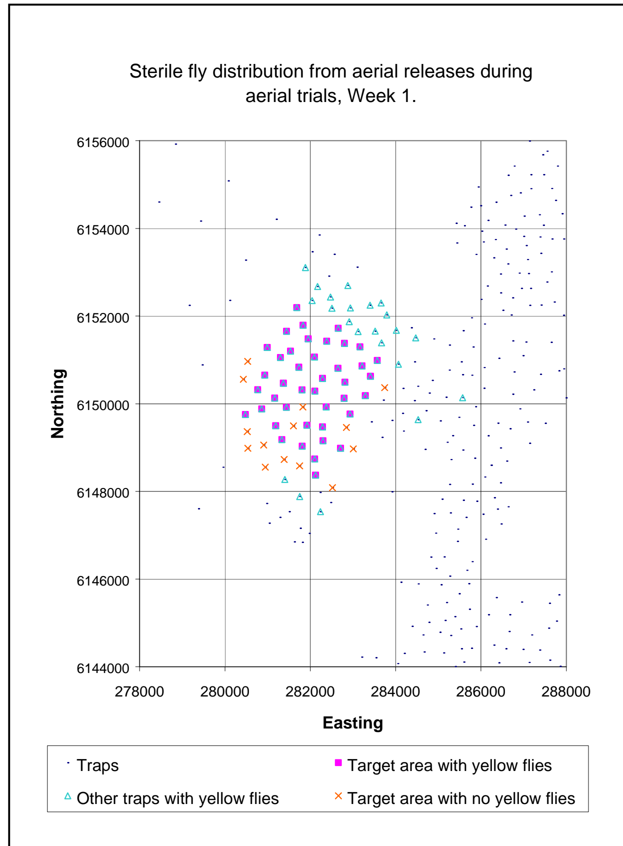
(1381 flies) compared to the roving release method (2282 flies). It was apparent in the first week that, in the target area, most of the traps that did not contain yellow flies (Figure 4) from the aerial releases were to be found towards the south west of the release area. This is consistent with meteorological information from the Parafield airport showing that winds on the first two release dates at the time of release were south-westerlies (240°T). During the first release the wind was quite strong at 33.5 km/h. It is estimated that if a non-flying fly falls at 1 – 2 m/s, it would take 2.5 – 5 minutes for it to fall 300 m (release altitude). A SW wind of 30 km/h (0.5 km/min) may result in the fly landing between 1.25 – 2.5km NE of the release point. An examination of the chart for the aerial releases showed that the group of traps without yellow flies extended about 2 km into the release area from the SW.

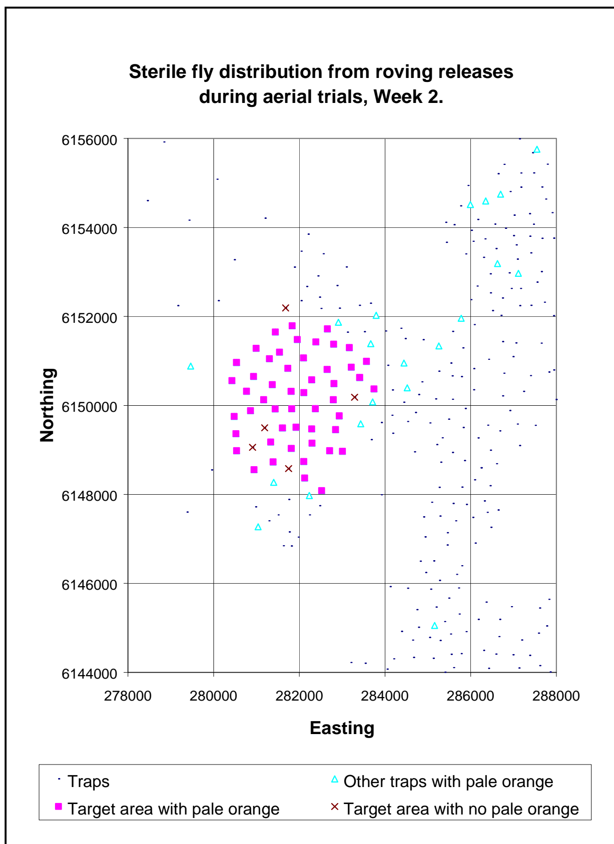
In the second week, 35% fewer flies were recaptured in the target area from the aerial method (2616 flies) compared with the roving release method (4051 flies). In that week, the traps that contained no flies from the aerial releases were spread throughout the target area (Figure 4). The weather conditions during that weeks releases were lighter winds from southeasterly (150°T, 27.7km/h) and northeasterly (60°T, 14.8km/h) directions.

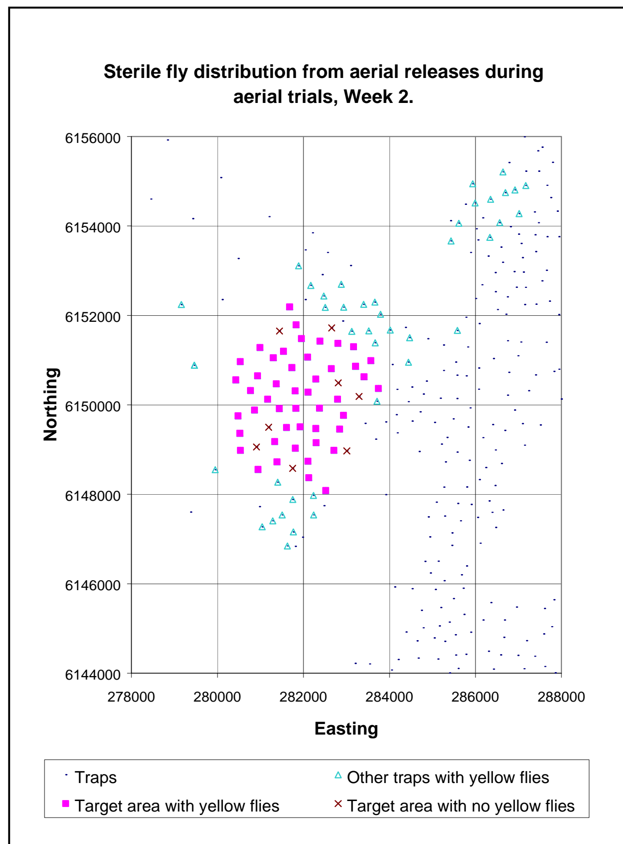
The results of this aerial trial provided a first estimation of the effect of wind on the distribution of the flies on the ground. Targeting the release area in future releases may be improved by taking this factor into account and adjusting the position of the flight pattern upwind accordingly.

Figure 4. Charts showing the relative locations of traps in the Salisbury area with catches of orange flies from roving releases (left) or with catches of yellow flies from aerial releases (right) in week 1 (upper) and week 2 (lower) of the aerial release trials.









These preliminary data for estimated losses due to aerial releases allowed us to calculate the relative costs and benefits of applying flies by air as compared to roving releases. Assuming that captures from aerial releases are 63% of captures from roving releases, for an SIT campaign based on 200,000 pupae per sqkm roving (approx. 140,000 sterile males per sqkm), 326,000 pupae (~228,000 sterile males) per sqkm would need to be applied by air to make up for losses due to the extra dispersal. For a 10-week release program, the relative costs of a single outbreak (7.5 sq km in area) indicate that roving releases are the most cost efficient. However, costs are more favourable for aerial releases as the size of the area to be treated increases. This is mainly because the labour costs involved in release increase dramatically for roving releases whereas larger areas can be covered in a small amount of time for aerial releases. Aerial releases may also be useful for country outbreaks. Adult flies would be delivered to the area and released by air, rather than shipping them as pupae and settling up a rearing facility in the area.

CONCLUSIONS

- Weekly distributional data were mapped in four categories onto digital maps for visual inspection of trap catch sizes. It is recommended that trap catches from future programs are plotted on the basis of whether or not they achieved the weekly target recapture rate (ie two categories). This target rate would be estimated using data from calibration trials, expected ambient temperatures for the period of the program, and other factors that may influence the recapture rate.
- The grid calibration trials suggested that the target recapture rate for any eradication program needs to be related to the ambient temperatures of the program. Programs conducted under warm weather conditions (20 – 28°C) could be guided by the current rule of thumb of aiming for an average of 100 flies per trap per week. It was estimated that on days where temperatures are between 17 – 20°C, the recapture rate may be expected to be only around 55 – 60% of that, and when temperatures are below 17°C, the recapture rate could be only 10 – 15% of the warm weather target. More data needs to be collected during hot weather (>28°C), to determine whether the observed relationship between temperature and recapture rate is maintained.
- The field longevity trial of a week-long pulse of 3.4 million yellow marked sterile flies, resulted in flies being caught up to five weeks after release. It was expected that if adult winter survival was good, these yellow flies would be recaptured early in the spring. This was not found to be the case, providing no evidence for the hypothesis that released medfly adults could over-winter in Salisbury.
- The first field trial looking at means of improving fly competitiveness indicated that protein-fed flies were recaptured in lower numbers than sugar fed flies. This trial triggered a series of laboratory cage experiments that will be described in detail in a later section.
- The results of the aerial trials provided a first estimation of the effect of wind on the distribution of the flies on the ground. Targeting the release area in future releases may be improved by taking this factor into account and adjusting the position of the flight pattern upwind accordingly. Based on these aerial release trials, the method would not be cost effective for ordinary outbreaks, but may be useful for outbreaks covering larger areas.

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EXPERIMENTAL WORK

CALLING BEHAVIOUR OF PROTEIN OR GINGER OIL TREATED FLIES

Introduction

Mediterranean fruit flies have a lek mating system (Shelly 2000) in which males form small aggregates within a tree canopy where they emit a sex pheromone attractive to females. This pheromone emission involves the eversion of a pheromone sac, an observable activity described as "calling". Females visiting a lek have the opportunity to inspect a number of the males before accepting a mate. Sterile male medfly used in SIT programs are typically poor at competing with wild males for mates (Shelly and Whittier 1996, Shelly and McInnis 2003, Kraaijeveld and Chapman 2004). One hypothesis contends that the high-density conditions under which mass-produced sterile flies are reared may be a cause of a decline in male courtship quality. Many production systems in SIT facilities now include a low-density reproductive stage to enable male competition/female selection processes to take place. An alternative hypothesis proposes that physical attributes such as the nutritional status or access to particular pheromone pre-cursors may be responsible for determining the quality of the pheromone that the males use to attract females. This has been addressed through investigations into the effects of diet and exposure to pheromone related substances (α-copaene-containing substances or trimedlure).

Studies examining the effect of diet have largely focussed on the presence of protein in the adult diet. In SIT programs, the larvae are reared on a high protein medium, but adult flies are typically supplied with only sugar and water in the few days prior to their release. Blay and Yuval (1997) found that a laboratory strain of medfly males, fed protein, copulated at a significantly higher rate than did protein-deprived males, but transferred significantly less sperm. Females mated to protein-fed males were less likely to re-mate the next day. Kaspi *et al* (2000) found that for wild flies emerging from infested guava, protein-fed males were more likely to emit pheromone in leks and, consequently, were more likely to copulate than protein-deprived males. Furthermore, protein-fed males tended to start calling earlier than their nutritionally deprived competitors. They hypothesized that, in lek mating systems where a considerable investment of time and energy is required by males, foraging successfully for nutritional resources prior to engaging in territorial or courtship behaviour would be essential for reproductive success. Shelly and Kennelly (2002) found that for mating trials involving wild flies, protein-fed males had a mating advantage over protein-deprived males. However, the addition of protein to the diet did not boost the mating success of sterile mass-reared males (Hawaiin bi-sex strain) in competition with wild or mass-reared males for wild females. They also found that the inclusion of protein in the male diet had no apparent effect on female remating tendency, copulation duration, or male longevity. We decided to see if feeding adult flies of the male-only Vienna 7 / Mix 99 strain with protein would have any significant effect on the calling rates of the flies in caged laboratory trials.

In parallel with work on protein, other studies have examined how α-copaene affects mating success. In laboratory trials, Nishida *et al* (2000) induced lek-like behavior in medfly using a plastic leaf models treated with (+)-α-copaene. Mating occurred exclusively on the artificial leaves treated with α-copaene. They reported that α-copaene was found as a minor component in the essential oils of various plant species, including its hosts such as orange, guava, and mango. Subsequently a number of studies have demonstrated positive effects of exposure to ginger root oil (known to

contain α-copaene) on the mating success of male medfly (Shelly 2001, Shelly and McInnis 2001, Shelly *et al* 2002, Barry *et al* 2003). All of these studies used wild flies or different strains of mass-reared medfly. We investigated the effect of ginger root oil exposure on the calling ability of our strain of sterile medfly.

Methods

Small batches of pupae were obtained from the WADA production facility. Three sub-samples were taken for pupal weight and flight-ability quality control measures.

Protein experiments

From November 2002 to July 2003, a number of different trial designs were tested, consisting of comparing calling of flies given either a diet of 1% or 2% protein hydrolysate in sugar agar, or access to 3g of a powdered yeast for *ad lib* consumption in addition to the sugar agar, with the calling of flies fed a pure sugar agar diet. All trials had the same statistical result. Only the powdered yeast trial is reported here (June 2003).

Two grams of pupae were placed in each of 10 rearing takeaway trays. All trays contained 5ml of sugar agar (from 250ml water, 1.21g agar, 44.65g sugar, 0.025g methylparaben), and five trays also contained 3g of powder yeast hydrolysate (protein-fed). The trays were maintained for 4 days (Days 0 – 3) at 25°C, 65%RH.

On the day before calling was recorded, the flies were immobilised by chilling (15mins in 4°C room), and 20 flies from each rearing container were transferred into two ventilated, clear plastic containers with 5ml sugar agar. The data for the 20 flies were pooled to produce a trial with five replicates per treatment.

Calling was recorded on the three subsequent days (Days 4, 5, and 6) at 9:30 am and 10:30am. Cages were observed in a random order at each recording session. Each cage was picked up and held for 45 seconds and the number of flies calling recorded after the first 15 seconds. Disturbance caused by lifting the cages appeared to be minimal. Calling flies that did cease calling as a result of the cages being moved were generally seen to resume calling by the end of the observation period.

Arc-sin transformed percentages were analysed with repeated-measures ANOVA (SPSS computer package, version 10.0). The data were normally distributed with homogeneous variances.

Ginger oil experiments

One trial design was conducted three times from August to October 2003. Two grams of pupae were placed in 5L cardboard rearing buckets with sugar agar diet and maintained at 25°C 60 – 70%RH for three days (Days 0 – 2). At 5pm on Day 3, either blank slips of filter paper (Whatman 541 quarters), or those containing 20µl of ginger root oil (GRO, Citrus and Allied Essences, Lake Success, NY) were inserted into each bucket. The buckets were maintained in separate incubators overnight (25°C 60 – 70%RH). At 9am the next day (Day 4), the flies were immobilised by chilling (15mins in 4°C room), and 20 flies from each bucket were transferred into two ventilated, clear plastic containers with 5ml sugar agar. The data for the 20 flies were pooled to produce a trial with five replicates per treatment.

Calling was recorded on the two subsequent days (Days 5 and 6) at 8:30am, 10:30am and 12.30pm. Cages were observed in a random order at each recording session. Each cage was picked up and held for 45 seconds and the number of flies calling recorded during the last 30 seconds.

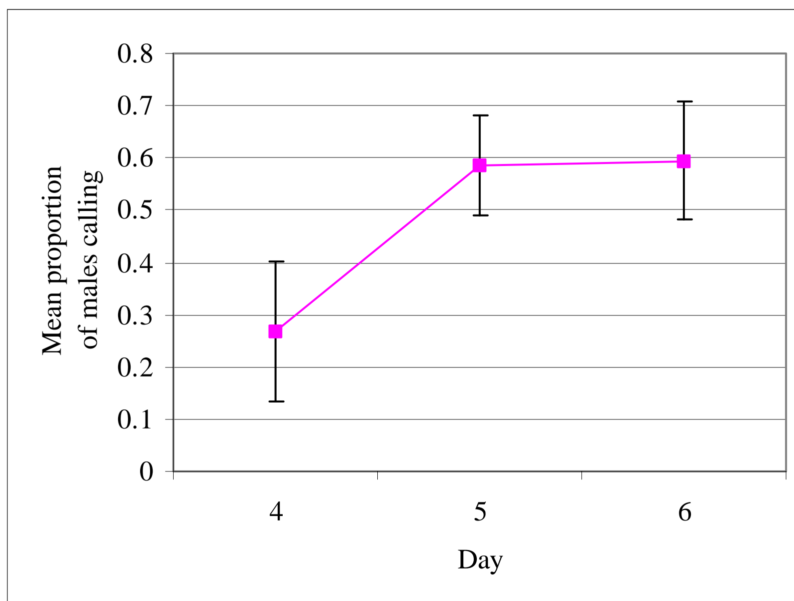
Arc-sin transformed percentages were analysed with repeated-measures ANOVA (SPSS computer package, version 10.0). The data were normally distributed with homogeneous variances.

Results

Protein experiments

The proportion of males calling was not affected by protein availability ($F_{[1,8]}$ = 3.191, p = 0.112). There was no effect of time of day ($F_{[1,8]}$ = 0.056, p = 0.853) or interaction effect of time of day and diet ($F_{[1,8]}$ = 0.775, p = 0.404). There was a significant effect of day ($F_{[2,16]}$ = 54.222, p = 0.000) with more flies calling on Days 5 and 6 than on Day 4 (Figure 5). There was no significant interaction between day and diet ($F_{[2,16]}$ = 54.222, p = 0.000) and there were no other significant interactions (day x time: $F_{[2,16]}$ = 0.595, p = 0.563; day x time x diet $F_{[2,16]}$ = 2.197, p = 0.144).

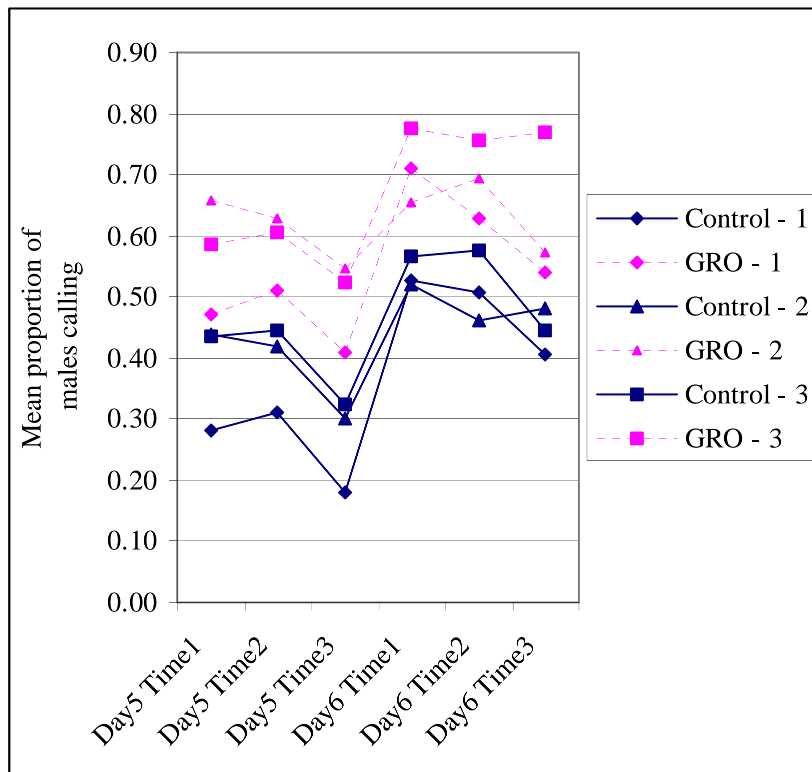
Figure 5. The proportion of all flies calling over three days (± 1 s.d).



Ginger oil experiments

The proportion of males calling during each observation period is illustrated in Figure 6.

Figure 6. The mean proportion of males calling at three times (8.30, 10.30, 12.30) over two days for untreated flies (Control) and flies exposed to ginger root oil (GRO) in three separate trials (designated by the symbols ♦, ▲, or ■).



The results of the repeated measures ANOVA indicated that a significantly higher proportion of GRO-treated flies called than non-treated flies in each trial (Table 9).

Table 9. Results of repeated measures ANOVA testing for the effect of GRO-treatment on calling frequency of sterile male Vienna7/Mix 99 medfly in laboratory cages.

Trial number	$F_{[1,8]}$ statistic	p
1	8.219	0.021
2	31.826	0.000
3	194.930	0.000

Each trial also showed an effect of day, suggesting that overall proportion calling on Day 5 was significantly less than the proportion calling on Day 6. Two of the three trials also showed an effect of time because the 12.30pm observation time had a lower proportion calling than the other two time periods (see Figure 6). There were no interaction effects.

Conclusions

Calling trials found no effect of protein availability during early emergence on the calling frequency of adult Vienna 7 / Mix 99 male medfly. There was an effect of day on calling frequency, with less calling on the first observation day (Day 4) relative to the next two. This could be an effect of "release", reflecting time needed for settling in to the new cage, or it could be an effect of age, with increasing maturity of the flies being reflected by increased calling percentages.

Ginger root oil was found to have a positive effect on calling. The results were consistent among three replicate trials. There was also a significant time effect which was probably not observed during the protein trials because the 12.30pm time-slot was not used for that experiment. There was also a significant effect of day. Like the protein experiment, there was less calling on the first observation day, in this case Day 5, relative to the next day. This would support the hypothesis that lower calling is related to a post-release adjustment period for the flies, rather than being a maturity effect.

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TRAPPING OF GINGER OIL TREATED FLIES

Introduction

Our laboratory experiments (previous section) supported the results of other studies that indicated that exposure to ginger root oil improved the mating competitiveness of sterile Mediterranean fruit fly (medfly) (Shelly and McInnis, 2001). Its incorporation into sterile release programs has been suggested as a means of improving the effectiveness of the sterile insect technique (Barry *et al*, 2003). As the South Australian medfly sterile release programs are monitored via Capilure-baited Lynfield traps on a 400 m suburban grid, the effect of exposure to ginger root oil on the recapture rate of released flies was investigated in orchard and field tent experiments.

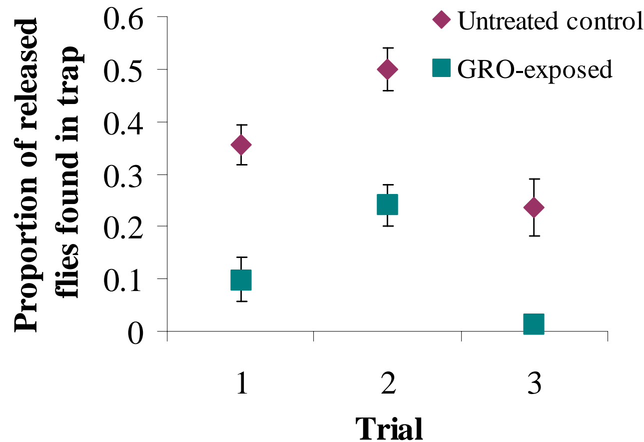
Methods

The medfly pupae (Vienna7 / Mix99) were reared, sterilised and dyed with two fluorescent colours (Fiesta) by the Western Australian Department of Agriculture, and then airfreighted to South Australia. Pupae in paper bags were placed with 40ml sugar-agar in 5L cardboard buckets with lids and maintained at 25°C, 60RH for 4 days. At 5pm on the 4th day, (1 – 2d post emergence) blank filter papers (controls) or those with 20µL of ginger root oil (Citrus and Allied Essences, Lake Success, NY) were inserted into the buckets, which were incubated separately overnight (25°C, 55RH). Dye colours were varied for each trial. Releases took place between 9 – 10am the following morning. The number of flies released was estimated using flight ability assessments for each pupal batch according to the manual, Product Quality Control and Shipping Procedures for Sterile Mass Reared Tephritid Fruit Flies (FAO/IAEA/USDA, 2003). A one-way ANOVA of trial and treatment was conducted for each experimental design.

Orchard experiments

In three orchard trials, flies from 7g pupae were released under five fruit trees each containing a Capilure-baited Lynfield trap. The traps were cleared after 3h and the flies counted. The proportion of flies found in the traps was significantly lower in the GRO treatment than the controls (Fig. 7. $F_{[1,26]} = 60.35$, $p = 0.000$).

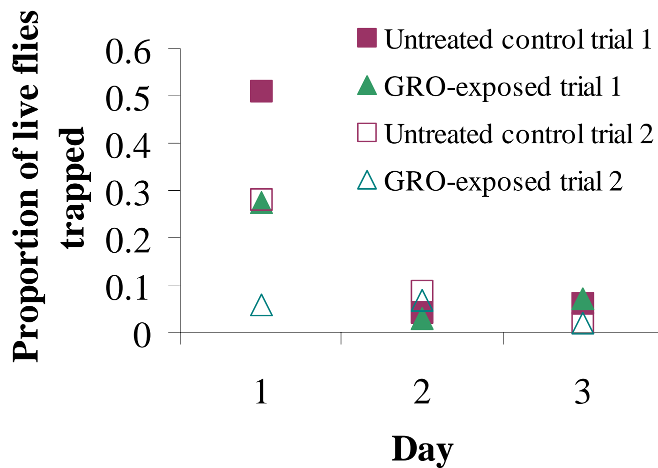
Figure 7. Orchard experiment results of Adelaide trials testing response to traps by ginger root oil exposed, and untreated sterile Mediterranean fruit fly.



Field tent experiments

In two trials, flies from 14g pupae were released into two field tents (6x4x2.3m) each containing 7 potted fruit trees. A trap was set for 1h on the first, second and third day after release. Dead flies were collected from the tent floor daily. Significantly fewer GRO-treated flies were trapped on day one (Fig. 8. $F_{[1,5]} = 46.60$, $p = 0.001$) and day two ($F_{[1,5]} = 6.99$, $p = 0.046$). On day three, there was no difference in recapture rate of GRO-treated and untreated flies ($F_{[1,5]} = 0.14$, $p = 0.720$).

Figure 8. Field tent experiment results of Adelaide trials testing response to traps by ginger root oil exposed, and untreated sterile Mediterranean fruit fly.



Conclusions

These results suggest that Capilure-baited Lynfield traps catch fewer sterile male medfly if they are treated with GRO prior to release. This result has implications for SIT programs should GRO be used in large scale eradication releases. If GRO treated flies are less attracted to traps, then monitoring of sterile fly density would need to take this into account to prevent concern that the sterile fly density was not high enough.

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SEXUAL COMPETITIVENESS OF GINGER ROOT OIL TREATED FLIES IN WESTERN AUSTRALIA

Introduction

The result of improved sexual competitiveness of sterile flies is that less flies need to be released to achieve eradication thereby reducing the cost of SIT. Recently USDA researchers in Hawaii reported that exposure of medfly to ginger root oil (GRO) significantly increased sexual competitiveness of sterile flies to a level where they out-competed wild males for mates. In field cage trials in Western Australia we investigated whether GRO was effective in increasing the competitiveness of
Vienna 7/ Mix 99 medfly. Promising initial trial results led us to continue research developing practical techniques for using GRO in the field and to large-scale field release trials.

Methods

Cage trials

In Perth, sterile male TSL medfly (Vienna 7/Mix 99) held in 5 litre paper buckets were exposed for 16 hours to 20 µ L of ginger root oil applied to strips of filter paper on glass slides. Flies that had been exposed to GRO were kept separate from unexposed TSL medfly at all times. Competitiveness (Fried) and mating compatibility tests were conducted in accordance with standard methods described in the manual, *Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies*. Green agar balls used as an oviposition medium in the tests were hung in a fruit tree within each cylindrical tent. Tents contained either GRO exposed or unexposed males that competed against wild males for wild females. The mating competitiveness of GRO treated versus non-treated males was compared by measuring the Relative Sterility Index (RSI), Competitiveness RSI (C-RSI) and Fried's Competitiveness Index (C-value).

Field trials

Sterile flies were released twice weekly from December 30, 2003 to March 29, 2004 at Katanning, an inland town some 300km south-east of Perth with a history of fruit fly problems. Prior to sterile male release the town was baited weekly from November to the end of December with an insecticide and protein mixture. For the trial the town was divided at the railway line into two areas, one which received flies treated with GRO (2 km^2) and the other untreated flies (3 km^2). Pupae were couriered to Katanning, then 60 ml of dyed pupae placed in 5l paper buckets for emergence with flightability averaging 82%. 0.1 mls of GRO was applied to a 10 cm^2 piece of filter paper and one piece placed in each bucket of pupae exposed to GRO. Flies were released from a vehicle driving at 20 km/hr with approximately 160m between release points giving a release rate of 250,000 flying males km^2. Results were compared by weekly trapping with Lynfield traps containing Capilure. There were 11 traps in the ginger

area and 15 in the untreated area arranged on a 400m grid pattern. Five traps were also placed in the adjacent town of Wagin (where sterile flies were not released) on March 19 and serviced weekly. Ripe samples of known medfly hosts (apple, apricot, feijoa, fig, Irish strawberry, cumquat, loquat, mandarin, nectarine, orange, peach, pear, plum, strawberry guava, tamarillo and tangerine) were collected across Katanning throughout the trial period and either dissected for evidence of larvae or held in containers over sand for emergence of flies.

Results

Cage trials showed an increase in sexual competitiveness of flies exposed to GRO. This was apparent in both the increased degree of sterility achieved and in the more frequent pairing between ginger treated males and wild females (Table 10).

Table 10: Competitiveness values (C), Relative Sterility Index (RSI) and Competitive Relative Sterility Index (C-RSI) for adult male Medfly exposed to Ginger Root Oil compared to untreated flies

Test	C		RSI		C-RSI	
	Ginger Root Oil	Untreated	Ginger Root Oil	Untreated	Ginger Root Oil	Untreated
1	1.57	0.26				
2	1.99	1.59	0.84	0.74	5.19	2.83
3	0.89	0.64	0.66	0.53	1.95	1.14
4			0.41	0.38	0.70	0.60
5	1.95	0.1	0.71	0.34	2.50	0.52
6			0.90	0.80	8.75	4.13
7	2.00	0.83	0.61	0.42	1.54	0.73
Mean	**1.68**	**0.684**	**0.69**	**0.54**	**3.44**	**1.66**

In the field trial, trap results demonstrated good control in both the GRO treated area and non-treated area whilst high numbers of flies were caught at Wagin (Table 11). Fewer flies were recaptured in the GRO treated area.

Fruit collection and dissection supported the trapping results. Of the 150 samples of fruit collected, medfly was found in only 1 sample of cumquats collected on the 29 April.

Conclusions

Cage trials demonstrated that exposure to GRO increased the competitiveness of sterile male medflies against wild flies. This was evidenced in both the Fried test which measures the degree of sterility induced in competitive mating and in the RSI and CRSI which measure propensity of sterile flies to mate with wild females. The positive results in these trials led to testing the use of GRO in the field.

In a laboratory situation emerged males can be exposed to GRO at an optimum age and for an optimum time. In the field where it is difficult to manipulate large numbers of emerged flies the only option was to add GRO at the pupal stage. It was postulated that sufficient GRO would still be present in the container once flies had emerged to have a significant effect on mating ability.

Table 11: Mean number of flies caught in Lynfield traps at Katanning and Wagin

Week	Katanning GRO treated	GRO wild	Untreated	Wild	Wagin Wild
Jan 1	267	0	299	0	
Jan 2	81.9	0	217.6	0	
Jan 3	183.9	0	307.9	0	
Jan 4	102.6	0	255.9	0	
Feb 1	115.4	0	311.6	0	
Feb 2	135.1	0	465.9	0	
Feb 3	221.8	0	467.4	0	
Feb 4	141.3	0	382.1	0	
Mar 1	205	0	347.7	0	
Mar 2	94.5	0	137.7	0	
Mar 3	498.1	0	699.5	0	
Mar 4	177.4	0	459.5	0	74.8
Apr 1	249.5	0	393.1	0	25.8
Apr 2	45.6	0	43.1	0.1	47.5
Apr 3	3.4	0	2.7	0.1	65.4
Apr 4	2.7	0	0.9	0.1	75.8
May 2	0.4	0.2	0.1	0	63.4
May 4	0.1	0	0	0.1	38.8

Good control was achieved by sterile fly release at Katanning in both the area receiving flies treated with GRO and the area receiving untreated flies. No wild flies were caught in traps or reared whilst sterile male release was being carried out. Therefore no positive effect can be assigned to release of flies treated with GRO. This may have been apparent if a lower rate of release of sterile flies was used.

On the other hand no negative effect was observed other than that smaller numbers of flies were caught in traps in the GRO area. It appears that flies exposed to GRO are less responsive to traps when compared with untreated flies. This result indicates that the technique used in the release buckets did expose the flies to GRO to some degree and caused a changed response to Capilure. Further research is needed to determine if the technique is exposing the flies sufficiently to GRO to improve their mating ability.

After releases were stopped small numbers of wild fly were caught in traps and reared from fruit. These flies may have come from a small population that was still surviving in the release area and increased in numbers once the suppression provided by sterile male release was lifted or the population may have derived from wild flies brought into the area from outside.

In the town of Wagin 30 km from Katanning both fly numbers caught in traps and fruit damage late in the season was high. In Katanning residents reported very little fruit damage and were very happy with the success of the sterile male release program and that cover sprays were not required.

SPINOSAD TESTING

Introduction

Alternative pesticides to malathion are being tested around the world for use against Mediterranean fruit fly (Peck and McQuate 2000, Burns *et al* 2001, Barry *et al* 2003). Spinosad has shown a lot of promise (Vargas *et al* 2001) and was chosen for testing. Its effectiveness against medfly was compared with malathion in a laboratory assay, and it was sprayed on trees to test for phytotoxicity in an orchard trial.

Methods

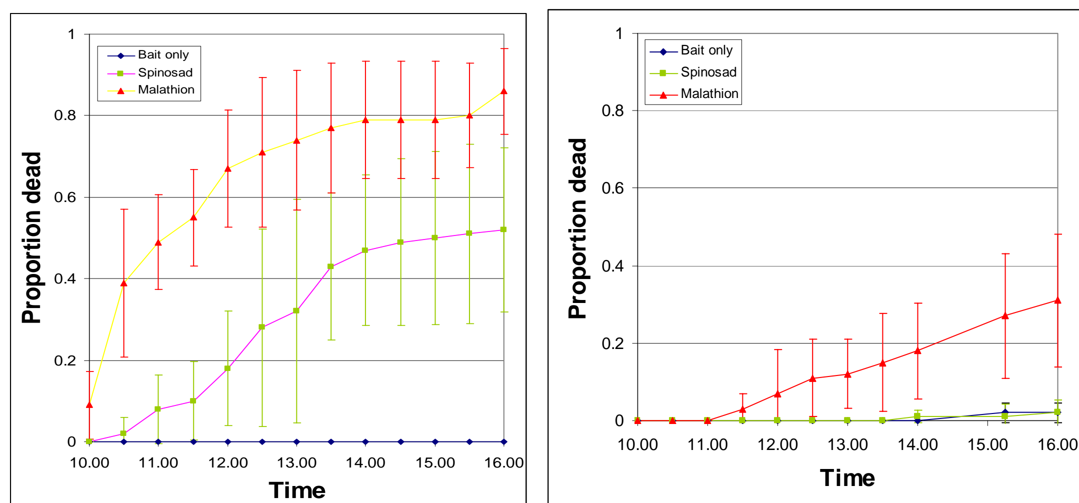
Laboratory assay.
Three *Citrus navelina* in Waite Orchard were sprayed with either Mauri bait only, the current bait spray (Mauri bait + malathion) or Spinosad bait spray (1:100 "Success":Mauri bait). Sterile male medfly pupae from Western Australia were reared in 5L cardboard tubs with sugar agar (15 g pupae per bucket) at 25°C, 60% RH in the Netley rearing facility. The assay was conducted twice; one day after spraying and eight days after spraying. Five leaves were cut from each treatment, transported to Netley, and each leaf hung in a mesh cage located on three tables outside under a roof. The flies were chilled and counted into batches of 20 flies that were then placed in each cage. The number of dead flies on the cage floor was counted half hourly between 10 am and 4 pm (except for an interruption during the second trial).

Orchard spray trial.
Eight apple trees (var. Pink Lady) were sprayed with Naturalure (GF120) at a dilution of 1: 6.5. The nozzle of the spray pack was adjusted to create droplets of about 4 - 6 mm (tested on newspaper on the ground), and the suspension was sprayed across an approximate area of 1m^2. This created a concentration of bait in the centre of the sprayed area. All trees were baited from the western side of the row. Four trees were sprayed with a single dose of 50ml of suspension. The remaining four trees were sprayed four times; with 100ml of suspension initially, then with another 50ml three times at three-day intervals. Within each of these treatments, the spray was directed over the outside of the foliage for two of the trees, and from within the canopy of the tree for the other two trees.

Results

Laboratory assay
The results (Figure 9) of the first trial suggested that the rate of mortality (slope of graph) for spinosad was less initially than the malathion treatment. In addition, the maximum mortality (at 4pm) for the spinosad treatment was only about 60% of the malathion treatment. The second trial using treated leaves that had weathered in the orchard for an additional 7 days showed that while malathion had a reduced, but still detectable effect, spinosad had lost activity as there was no difference in mortality between the spinosad-treated leaves and the bait-only leaves.

Figure 9. Results of laboratory assay testing spinosad, malathion and a bait only control one day (left) and eight days (right) after spraying in an orchard.



Orchard spray trial

The trees were examined at the end of the spray period. There were no signs of phytotoxicity in the leaves. Sooty mould was observed on all trees after the first week and tacky bait residue was seen on those trees that were treated repeatedly.

Conclusions

The limited work that was conducted on the potential for spinosad to replace malathion in baiting should be viewed within the context of the large amount of work done internationally. Our results supported other work which has shown that spinosad is slower acting in producing mortality in these flies, is not quite as effective as malathion and tends to last less long in the environment, which may necessitate more frequent applications of the bait. Our preliminary orchard phytotoxicity trial did not provide evidence that the spinosad in Mauri bait was phytotoxic to apples. More work needs to be done in this area. Specifically, if Naturalure becomes registered for use in Australia, laboratory trials comparing it with the bait currently in use would be essential to provide basic information on the expected baiting performance change should it be incorporated into eradication programs.

References

Barry, J. D., R. I. Vargas, N. W. Miller and J. G. Morse (2003). "Feeding and foraging of wild and sterile Mediterranean fruit flies (Diptera : Tephritidae) in the presence of spinosad bait." Journal of Economic Entomology **96**(5): 1405-1411.

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TECHNOLOGY TRANSFER

Catherine Smallridge attended the Sixth International Symposium on Fruit Flies of Economic Importance, Stellenbosch, South Africa, May 6 – 10, 2002. A poster entitled "Preventative sterile fly release for the management of a Mediterranean fruit fly outbreak in South Australia." was presented. Immediately after this conference, a workshop held by Dr Kevin Hoffman (CDFA) on the identification of sterile fruit flies through dissection techniques was attended (May 13 - 15, 2002). Guidelines on the identification of sterile flies were then prepared for the reference of SARDI entomology staff. Catherine ran a short workshop for SARDI entomologists Ken Henry, Greg Baker and Peter Taverner in order to pass on the skills required (Nov 2002). Vanessa Bosco and Gabriella Caon were later also trained in these methods of identification.

Vanessa Bosco attended the 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere, Florida, USA, May 16 – 21, 2004. A poster entitled "Ginger Root Oil: Effect on trap catches of sterile male Mediterranean fruit fly, *Ceratitis capitata*." was presented. In addition, collaborative research with colleagues from the University of Pavia, Italy, led to the presentation of another poster: Bonizzoni, M., Guglielmino, R., Gomulski, L.M., Smallridge, C.J., Malacrida, A.R. and Gasperi, G. (2004) "Medfly in Australia: a genetic perspective." These posters are available on the SARDI Entomology website: http://www.sardi.sa.gov.au/entomology/index.html. Vanessa, visited the Hawaii Fruit Fly Production Facility and fruit fly researchers in Hawaii, and inspected the fruit fly distribution facilities in California and Florida.

Visits by Dennis, Catherine and/or Vanessa to Western Australia for collaborative research took place in November 2001, December 2002, May 2003, and March 2004. A number of teleconferences were also arranged between the two departments. In 2002, Lib Colagiovanni from PIRSA's Plant Health Operations spent a week with the Western Australian Department of Agriculture passing on the benefits of his experience with roving releases, and assisting Western Australia to set up their own roving release trials.

Our research and operations were demonstrated to the Operations Manager of the South African Fruit Fly Facility during his visit in March 2004.

The Mediterranean fruit fly project was represented in a number of media articles:

- The Australian, Tuesday, August 19 2003. "Gingering up a wilting fruit fly's sex life."
- A Channel 10 news item in August 2003.
- PIRSA OpenGate, Sept 2003. "Fruit flies falling victim to aromas."
- Ecovoice, Sept/Oct 2003, Pg 30. "Viagra for sterile male fruit flies."
- SARDI Communicator Sept 2003 Vol. 12, Issue 5. "The sweet smell of success."

A limited number of controlled copies of the Sterile Insect Technique operations manual have been published.

Fact sheets for the public are available on fruit fly section of the PIRSA website: http://www.pir.sa.gov.au/dhtml/ss/section.php?sectID=1816&tempID=1

Recommendations

1. Plant Health Operations (PIRSA) public liaison staff report that the method has been well accepted by the general public. The reduced baiting period, and lower chemical insecticide use, as a result of SIT, is seen as a positive step in fruit fly management. **The use of the sterile insect technique for future Mediterranean fruit fly incursion eradication is recommended.**
2. The successful implementation of SIT depends on a reliable source of quality flies. Flies currently supplied by the Western Australian Department of Agriculture (WADA) have been demonstrated to be of a high quality. WADA are committed to research and development to improve sterile fly quality, either through pre-release treatments or strain changes. **A strong collaborative relationship has been developed between SARDI and WADA, and it is recommended that this be fostered and maintained.**
3. The sterile insect technique is a biological approach to fruit fly control, and as such is fraught with many sources of variation which, in different combinations, can determine the success or failure of any particular program. Each SIT program should be assessed by a trained biologist whilst the program is in progress. Before the program starts, this person should be consulted by Plant Health Operations to assist with preparations such as release routes, planned release rates, and calibration trial schedules. During the program, Plant Health Operations should supply this person with information on a weekly basis to allow the assessment of fly quality and distribution. This information will include quality control data, actual release rates, and recapture data, as well as other pertinent information such as host plant aggregations or baiting problems. The biologist will then be in a position to monitor the program and advise of changes to the program according to this assessment. **It is recommended that future SIT programs for medfly have a dedicated biologist/entomologist to provide ongoing technical appraisal and inputs into the program.**
4. The feasibility of eradicating medfly from Western Australia has been assessed in the past and considered to be not economic. Advances in SIT technology in recent years suggests the benefit/cost ratio of such a program has increased substantially. **It is recommended that the feasibility of an eradication program for medfly in WA be re-appraised.**

Appendix

Technical guidelines for the microscopic examination of Mediterranean fruit fly females for mating and ovarian maturity and males for signs of sterilisation

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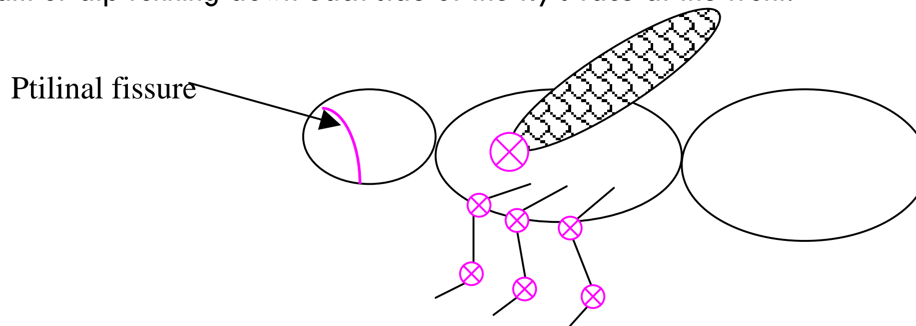
GUIDELINES FOR THE DETECTION OF FLUORESCENT DYE ON MEDFLY

1. The best place to look for dye is the ptilinum. Firstly, check for a glow that will come through the outer membrane of the ptilinum itself by using forceps to stand the fly vertically under the UV light of the microscope. If the glow is difficult to detect, there may be evidence of dye particles along the ptilinal fissure, which is a seam or dip running down each side of the fly's face at the front.



2. If the presence of dye seems uncertain, also check the "armpits" of the wings on the underside – cleaning in this location is difficult for the insect, and there are small plates surrounded in membranes that allow the wing to move, and that tend to trap dye. This locale is protected by the wing and least likely to have dye transferred there from other flies. The membranes between the leg segments, also tend to trap dye.

3. If the presence of dye is still not detected, check that the fly is a male. Males can be distinguished by the paddle-like hairs on their heads. If these have been knocked off (common in dried specimens), another feature is the presence of yellow hairs on the front pair of legs (and to a lesser extent the back pair) whereas females only have black hairs on these legs.

4. If it is a male, and there is no clear external dye, the ptilinum needs to be exposed. If this level of examination is required, the identifier should inform the operations leader, and the following process should be supervised by that person. The dehydrated fly is rehydrated by placing it in just-boiled water and allowing to soak for 15 minutes or more. The dissecting forceps and scalpel should be cleaned carefully and examined under the microscope for signs of dye, and the working surface under the microscope should also be cleaned and examined. There should be no possibility of external contamination of the fly to be examined. The fly is placed on its side under the UV microscope and pressure is applied to the upper-most facing eye with blunt forceps. The water in the head should push the ptilinal balloon out. If this does not happen (ie if the head is broken or pierced in any way, the pressure needed will be lost), then the front of the head can be pulled out with fine forceps to expose the inside of the ptilinum. If all else fails, place the fly in a dish of 70% alcohol (or water), and carefully cut the front of the fly's face off (the liquid should prevent accidental loss of the face-part). Examination of the back of the ptilinum should show some dye. In addition, the face-part can be laid front-up, and with forceps, the sides can be pulled apart laterally to expose the ptilinum.

Apart from these protected parts of the body, a fly must not identified as sterile on the basis of dye on any other parts of the body as contamination through dye transfer in the wild or in the trap occurs frequently.

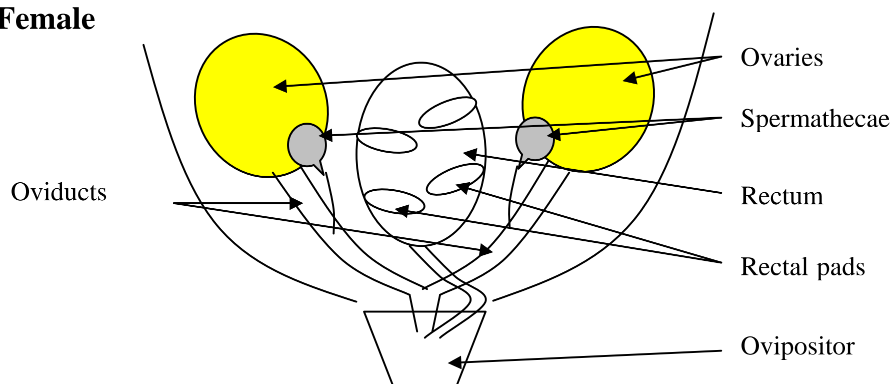
If there is no evidence of dye from the above investigations, the fly should be forwarded in alcohol to the Entomology Unit, Waite Precinct, SARDI, for further dissection by entomologists.

GUIDELINES FOR THE DETECTION OF SPERM IN THE SPERMATHECAE OF FEMALE MEDFLY AND OVARIAN MATURITY

Background

From the dorsal side of the fly, the ovaries of the female lie below and to each side of the rectum and the black/brown spermathecae will be seen lying on top of the ovaries. A small accessory glands lies under each ovary. Sperm can remain viable in spermathecae for months, and sperm is released with each egg which is fertilised in the lateral oviduct on the way out. It is important to check both spermathecae, since there is some evidence for asymmetrical storage and use of sperm.

In a fertile, mature female fly, the ovaries will be large, closely packed with eggs and fill up the area. Eggs may spill out when the tergites are removed, and some of the eggs will be large and shiny indicating they are covered by the chorion and ready to be laid. In a sterile or immature female fly, the ovaries are small. In immature flies they may appear to be bumpy with the growing eggs inside. In sterile females (unlikely to be captured with the use of the 'male-only' sterile strain) the ovaries will appear as translucent sacs, sometimes containing small cysts. In sterile females, the accessory glands sometimes enlarge as nutritive fluids accumulate due to the absence of egg release. The rectum in the centre is translucent and contains four rectal pads that function in retaining water from the faeces before they are passed out. Sometimes these pads may be mistaken for eggs if the rectum is damaged during dissection, however they are not as large as mature eggs and there are only four.



Dissection method for spermathecal exam

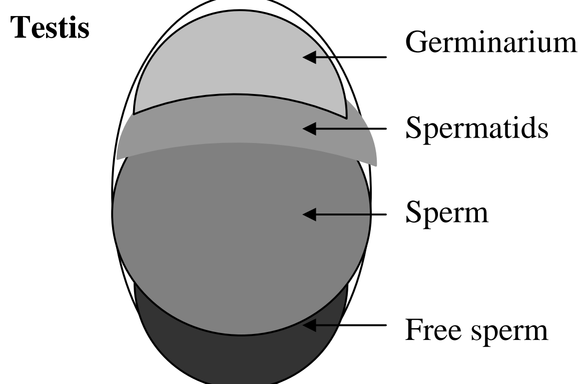
- If the fly is dry, place it in near boiling water for at least 15 minutes to allow it to soften and rehydrate.
- Lay the fly dorsal side up in a watchglass of alcohol under a dissecting microscope. The two spermathecae may be visible as two dark spots in the second or third to last segment of the fly.
- While holding the fly with one set of forceps, use another set to remove the outer body covering (tergite) of the section containing the spermathecae.
- With fine forceps, reach slightly under the dark spermatheca to grasp the fine tube that connects it to the lateral oviducts and ease the spermatheca out of the fly body. Place each one on a slide, and remove as much of the outer white tissue as possible without crushing the spermatheca.
- Draw a circle around the prepared tissue on the underside of the slide to show where they are. Allow to dry.

- Place a drop of 2% Aceto Orcein on the tissue and apply a coverslip.
- Wait for 5 – 10 minutes, then place the slide on a compound microscope under low magnification. Apply pressure to the coverslip while watching the preparation until the spermathecae are seen to crack open.
- Increase the magnification to look for signs of sperm issuing from the cracked spermathecae. They will look like a red “ball of yarn”.

GUIDELINES FOR THE EXAMINATION OF MALE MEDFLY FOR SIGNS OF STERILIZATION

Background

The testes of normal male medfly consist of germinarium in the top quarter (closely packed production cells), followed by small spots which are the forming spermatids, followed by formed spermatids which look like spots in the shape of a strawberry, followed by sperm bundles which look like bundles of hay, and finally, in older specimens (>2d) some free sperm looking like spaghetti.



There is no difference in the size of the testes of irradiated and non-irradiated flies. Irradiation has the effect of causing progressive pycnosis (nuclear collapse and cell death) of the germinarium and mutating the DNA of spermatids and sperm bundles. Sperm production starts before the flies emerge from the puparium, so at the time of irradiation, there are plenty of sperm bundles and spermatids which complete their formation process and become sperm. No further sperm is produced by the germinarium which lose their form and degrade. In older sterilized specimens, there will be no germinarium or spermatids evident, and the sperm bundles and free sperm take up the whole testes. In examining the testes of medfly, the absence of spermatids will be the first character to look for. In older flies, this will be accompanied by the pycnosis of the regularly organised germinarium into small irregularly spaced nuclei with gaps around them. In very old sterile flies, there will be no evidence of germinarium or spermatids. Very young sterile flies may look similar to young fertile flies since some spermatids may still be present in both.

The testes are yellow in fresh specimens and translucent-white in dried specimens, and they lie at the sides and in the second and third to last segments of the abdomen. In dried specimens, the sperm bundles sometimes look whiter than the rest of the tissue material, which can help in the location of the testes.

Dissection method

- If the fly is dry, place the abdomen in near-boiling water for at least 15 minutes to allow it to soften and rehydrate.
- Lay the fly dorsal side up in a drop of alcohol on a slide under a dissecting microscope.
- While holding the fly with one set of forceps, use another set to remove the outer body covering (tergite) of the entire abdominal section starting from the segments closest to the thorax.

- With fine forceps, carefully clear away the round white fat bodies that may be obscuring the other internal organs along the sides of the abdomen. Continue to carefully expose the one testis at a time and when clearly visible, grasp the vas deferens and carefully ease the testis onto the slide.
- Draw a circle around the prepared tissue on the underside of the slide to show where they are. Allow to dry.
- Place a drop of 2% Aceto Orcein on the tissue and apply a coverslip.
- Allow 5 – 15 minutes to stain, then examine the testes structure under the compound microscope.