**Cooperative Research Centre**
**for National Plant Biosecurity**

# Final Report

## CRC40024

## An integrated approach to the eradication of arthropod EPPs

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# 1. Executive Summary

For many reasons (economic, political and environmental) a singular approach to pest eradication will not be acceptable in the future and an integrated or systems approach will be required. The technologies used will be determined by the biology of the pest, the size of the outbreak, its location (urban or rural) and what eradication tools are available. As integrated pest management (IPM) has become the preferred approach to pest management so IPE will be developed as the preferred method of pest eradication.

As part of this project, eradication technologies currently used against the insect orders: Coleoptera, Diptera, Hemiptera, Lepidoptera were reviewed (Suckling *et al* 2008). Key eradication technologies include lure and kill, mating disruption, mass trapping, male annihilation, the sterile insect technique (SIT), use of bio-pesticides, use of broad-spectrum insecticides, tree/plant destruction, and mechanical/ physical methods.

The area-wide release of sterile insects to control and/or eradicate insect pests has been used against Lepidoptera for many years but is considered to be expensive. This limitation may be able to be overcome by properly taking into account inherited sterility, which lowers the over flooding ratio (number of insects needed for release) required to impact on the wild population.

In this project, we investigated the irradiation biology of the light brown apple moth (*Epiphyas postvittana*) (LBAM) as a model Tortricid species. Tortricid species which threaten Australia include summer fruit Tortrix, great brown twist moth , orange Tortrix, carnation Tortrix, omnivorous leaf-tier, South African carnation Tortrix, Manchurian fruit moth, tea Tortrix, grape berry moth, omnivorous leaf roller and false codling moth.

For successful eradication quality of released insects is paramount. If there is a negative effect of radiation on physical fitness, a greater number of irradiated moths must be released to compensate for their lower physical fitness. Alternatively, instead of releasing fully sterile male moths into the field, the release of partially sterile male moths (lower irradiation dose) may be feasible. At the optimum radiation dose the offspring resulting from successful pairings between irradiated males and wild females (F1) inherit the sterility thus are unable contribute to the next generation. As the optimal radiation dose for both full and inherited sterility varies between species the irradiation biology of the insect must be completed before committing to a dose.

The dose required to achieve 100% female sterility whilst inducing good levels of F1 sterility from irradiated male moths has been identified. Initial studies indicate at these doses sterile moths will have good competitiveness. Bottlenecks to successful mass rearing have been isolated.

SIT has a critical part to play in the final stages of Tortricid pest eradication but for it to be effective pest populations need to be first reduced to very low levels. Pheromones obviously have an important role to play in this and technologies such as attract and kill and mobile mating disruption show great promise. The integration of these technologies with SIT will be tested in phase 2 of this project.

## 2. Aims and objectives

To review past, present & potential eradication technologies and indentify those with most promise for integration into an IPE program.

To study the use of SIT in eradication of Lepidopteron pests using LBAM as the model species and further develop a sterile moth technique program for LBAM in collaboration between scientists from HortResearch, New Zealand, USDA ARS in Hawaii and the Department of Agriculture and Food, Western Australia.

Phase 2 of the project aims to investigate the integration of available eradication technologies against LBAM and use this as a template for the development of a generic integrated eradication strategy for Tortricid moth pests

## 3. Key findings

**Insect mass rearing methods**

An artificial diet has been widely used for LBAM, and recent improvements in rearing systems (Barrington unpublished data) have made major gains to support the development of mass rearing. The USDA APHIS teams in Phoenix, Arizona and Albany, California have recently found that LBAM can be reared on the diet used for mass rearing pink bollworm. However, methods for the separation of insects from diet, and the sexes from each other would be further enabling.

**Mass sorting of pupae by sex**

Current sex separation techniques for LBAM are labour intensive. LBAM do not pupate on top of artificial diets, and as with other leaf rollers create a large amount of webbing preventing their easy extraction. Extensive washing and soaking is required to remove excess diet and webbing. After removing pupae from the remaining diet, they are visually sorted by sex. Female pupae are generally larger than male pupae. However, there is some overlap in size between the smaller female pupae and larger male pupae thus the number of abdominal segments must be counted to accurately sex for pupae within this size range. Female pupae have one less segment than males. Nevertheless the size difference may provide a good basis for gross sex separation.

**Mechanical based sex separation**

To remove the need for water and likelihood of damage from excessive stirring, a dry approach was taken based on the size difference of the pupae. Pupae of mixed sex were lightly shaken along a tilted wire rack. The distance between the wires of the rack were large enough to allow the smaller, likely male, pupae to fall through only into a collecting bucket below. The larger pupae were allowed to continue down the rack and fall off into the female collecting box at the end of the rack. A total of 13,234 pupae, 8,067 male and 5,167 female were separated this way. The male collection box contained 0.89 male and 0.11 female pupae compared with 0.96 female and 0.04 male pupae in the female box at the end of the trial. Some issues to overcome are the small sized females pupae slipping between the wires of the racks, landing in the male box and the assorted pupae hooking together

**Mass rearing in Western Australia**

Presence of moulds *Aspergillus niger*, *Aspergillus flavus* and a *Penicillium* species has been a major issue since the lab rearing of the moth started in 2007 despite the use of mould inhibitors in the diet. This was seriously impacting on pupae production and also represented a potential health hazard to those responsible for collecting the pupae. In an attempt to control the moulds from spreading in the media, the eggs after collection were bathed respectively in 0.1% sodium hypochlorite and 1% formalin, rinsed thoroughly with water before putting out to hatch, but very little improvement was noted. Based on work on controlling *Peculium roqueforti* in Drosophila colonies ( Holleley et al 2008) we increased the amount of Nipagin used in the preparation of 12 kg of the diet from 13.5gm to 28.5gm and added Calcium propionate (30 gm) as an additional preservative. Significant improvements were immediately noted, and moulds have now ceased to be a major issue and pupae production has increased

**Examination of irradiation biology**

Irradiation biology work has been carried out in laboratories in Western Australia and New Zealand. The irradiation biology of female and aspects of male LBAM is underway. The majority of male LBAM irradiation biology, including the effect on sexual and physical fitness has been assessed. Initial irradiation doses were 50, 100, 150, and 200 Gy while current radiation doses cover the previous doses and include 250 and 300 Gy. Sexual fitness assessments included cumulative mortality effects for the irradiated, F1 and F2 progeny. Male fitness was investigated in a wind tunnel, assessing the ability of male to fly upwind to a sex pheromone lure(Fig 1).

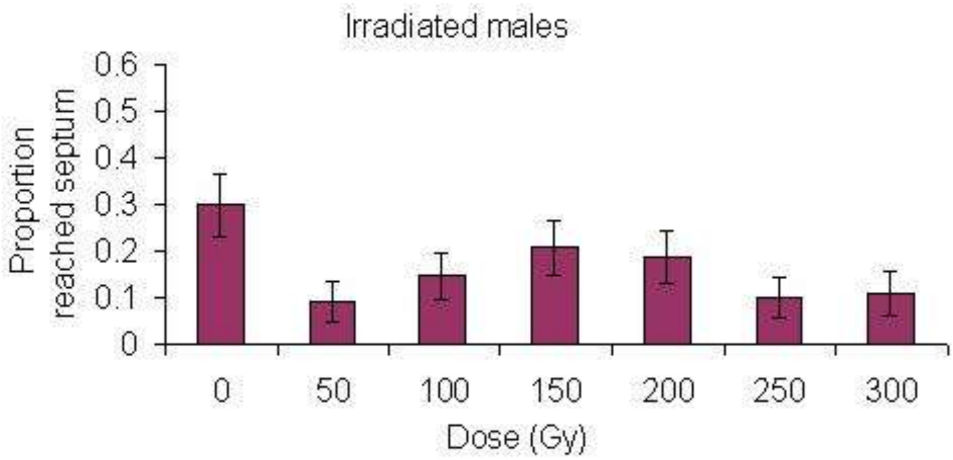
**Figure 1: The wind tunnel looking up wind with the moth release site in the foreground and the pheromone lure up wind.**

**Wind tunnel fitness**

The ability of males to reach females was examined under controlled conditions in a wind tunnel to investigate the role of radiation dose on the physical fitness of male LBAM. We predicted that with increasing dose of irradiation there would be a correlated decrease in fitness due to the burning of the wing muscles by the radiation. However, no fitness effect was perceived in the next generation as they were not subjected to radiation.

We found that there was little direct effect of radiation on the physical fitness of LBAM up to a dose of 200Gy. However, during this work a strong, chlorine like contaminant odour developed in the wind tunnel requiring the replacement of the entire filtering system. We cannot rule out this affecting flight ability. As such this work is being further investigated and includes the 250 and 300 Gy doses. Thus far, there has been little effect of irradiation on the flight ability of the moths in the wind tunnel (Fig. 2)

**Fig 2: Results of the relationship of irradiation dose on male flight ability**




**Flightability studies in SA**

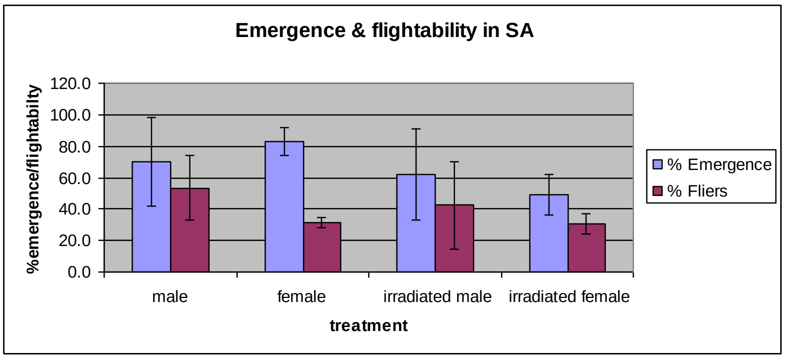
Tests to determine the number of adults that emerge from irradiated pupae and those with a basic capacity to fly have been used as standard quality control tests for Tephritid fruit flies for a number of years. The important statistics are percent emergence and percent fliers. Various types of containers have been used for the tests the standard now accepted for fruit flies  is a Plexiglas tube with a diameter of 9 cm painted black with a height of  10 cm.

The flight arenas tested at SARDI were (1) a standard fruit fly flight tube 100 (H) x 85 (D) mm tube constructed from 3 mm thickness Plexiglas (2) a tube 115 (H) x 150 (D) mm and (3) a tube 160 (H) x 150 (D) mm. The tubes were painted black on the inside surface so that light could only enter at the top. The base of each tube consisted of a petri dish lid covered with black paper cut to the lid dimensions on which LBAM pupae were placed. The tube plus lid base was placed in a ventilated cage (30 x 40 x 30 cm) at ambient room temperature (20oC) and natural light, and the number of fliers (i.e. moths that escaped from the flight arena) assessed daily.   Results showed little difference in emergence with all tubes giving good emergence but flightability was higher in the wide tube compared to the narrow fly tube and the height of the tube had little influence on flightability

Two lots of LBAM pupae reared by SARDI (Waite, SA) were flown to DAFWA, Perth, on 30 June and 7 July. Pupae were transported in a petri dish in a foam cooler with a cooler brick. Time for flight, dispatch & delivery was approximately 8 hrs. Upon arrival pupae were separated by sex and half of each sex was exposed to a gamma radiation dose of 200+/-10 Gy and half were left untreated.  Pupae were then flown back to the Waite, placed in 115 x 150 mm tubes in ventilated cages, and the percentage emergence and percentage fliers assessed.

There was large variation in the results and flightability of both non-irradiated and irradiated moths was low (Fig 3). This may be due to the long transit time from Adelaide to Perth and return. Based very limited data it appears that emergence will not be an issue for either males or females but there may be concerns with female flightability. Lower female flightability may be an artefact of the testing arena or it may be related to the biology of LBAM where females are heavier than males and tend to be sedentary.

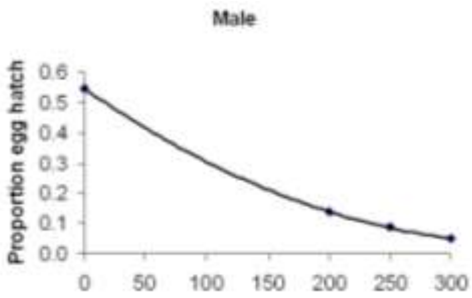
**Fig 3**: **Emergence and flightability of irradiated and untreated LBAM**




**Sterility**

Currently SIT programs release fully sterile males into the wild for population control. This method requires the release of large numbers of irradiated moths to counter the effect of radiation on physical fitness. By releasing semi-sterile moths, where the sterility is passed on to the offspring (inherited sterility) along the irradiated male line a greater return can be gained from releases as long as the F1 moths are as fit as wild moths. It appears that male sperm are more resistant to radiation than female eggs when irradiated. However, subsequent offspring from irradiated female moths do not appear to have inherited sterility. The larvae that hatch from the eggs of irradiated female moths appear to be perfectly healthy. As dose rate increased sterility level increased with full sterility reached for females at 250 Gy, while males did not reach full sterility at 300 Gy (Fig. 4). While there was successful adult F1 emergence at 200 Gy (Fig. 5), the male progeny were fully sterile at 200Gy (Fig. 5), although along the females line successful F2 emergence still occurred (Fig. 5). There appears to have been a radiation effect on the sex ratio of the surviving F1 pupae (Fig. 5) with a bias towards male production/survival with increasing radiation dose. These factors will be built into a model of the critical overflooding ratio.

**Fig 4: The effect of radiation on fertility of male and female irradiated LBAM.**



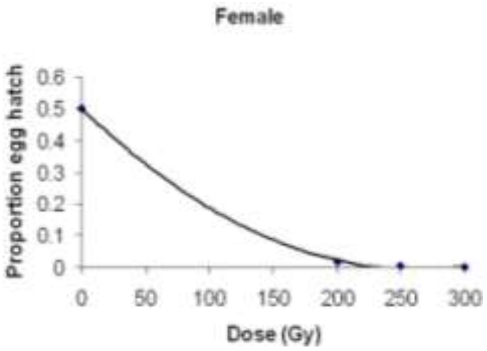
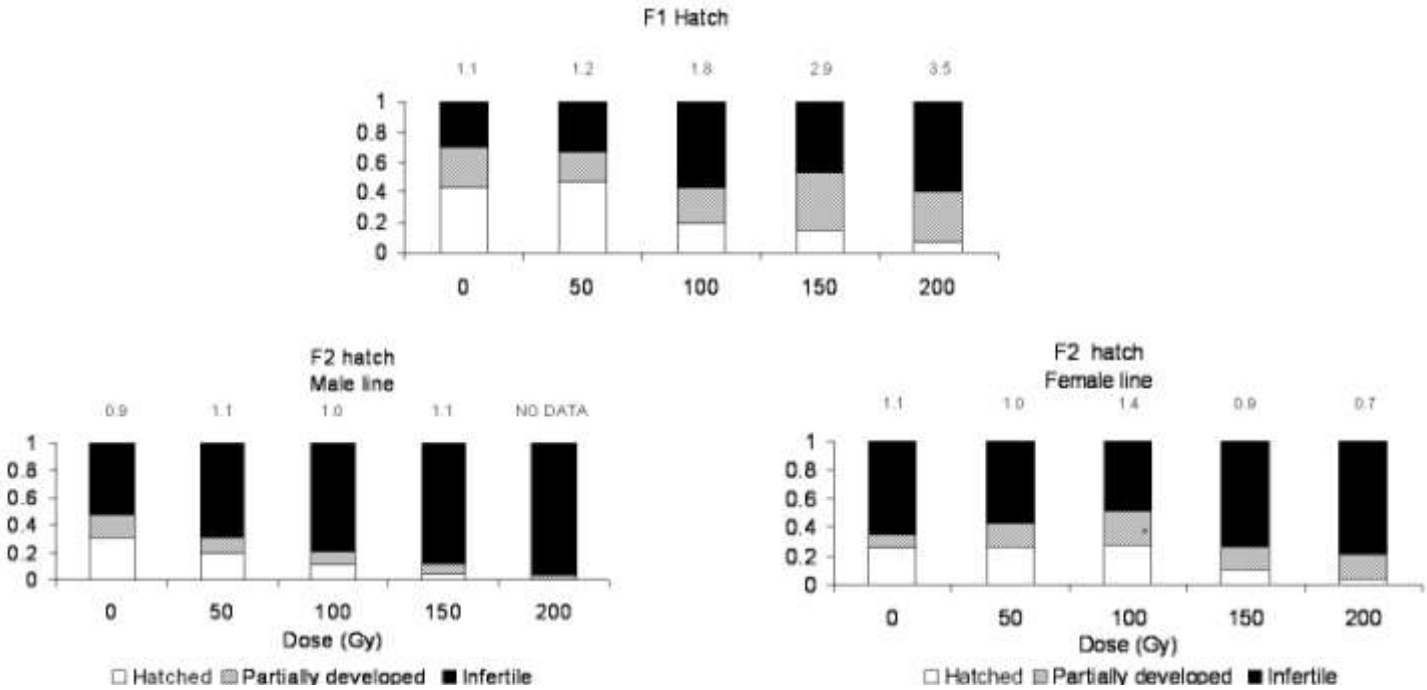



**Fig 5: Hatch success of LBAM eggs, F1 and F2 of both male and female lines. The sex ratio of the subsequent pupae are noted across the top of the bars (males : one female).**



**Irradiation of pharate adults**

A trial was carried out in WA irradiating pupae les than one day from emergence at 0,150,200,250,300Gy. Pupae were selected where the fully formed wings of the adult could be seen through the pupal case. Although 0.05% of eggs hatched from females irradiated at 250 Gy none of the larvae survived to adulthood. Hatch from eggs of irradiated male pupae at this dose was 4.3% and 35% of the larvae from emerged as moths with a sex ratio were 7.8 ♂**:** 1♀. When these F1 males were crossed with normal females 8.4% of eggs hatched. Reduction in fertility compared to the control was 91% with parental males and 90% for the F1. Fecundity of untreated moths averaged 290 eggs/female and at 250 Gy untreated females crossed with irradiated males laid 281 eggs/female**.**

**Taxonomy**

The taxonomy of the forty or more members of the genus *Epiphyas* is unclear and is the subject of the CRCNPB PhD project of Bobbie Hitchcock at CSIRO, Canberra. Project members are collecting specimens of *Epiphyas* for taxonomic study with light trapping, pheromone trapping and larval collection. In WA light trapping has been carried out at Manjimup, Perth Hills and Swan Valley. An array of pheromone traps with varying ratios of (E)-11-tetradecenyl acetate to (E, E)-9,11-tetradecadienyl acetate have been placed in the southwest in an attempt to catch species, such as Western apple moth, E. pulla not attracted to the standard pheromone mix. Specimens are being sent to Ms Hitchcock for classical taxonomy and to Richard Newcomb of HortResearch, Auckland for DNA studies. It is hoped to extend the program to include study of pheromones utilizing HortResearch facilities at Lincoln, New Zealand. To date there appears to be little genetic variation in moth populations of LBAM in Australia with a population from WA the only one exhibiting any significant difference in mitochondrial DNA.

**Fig 6: Female *Epiphyas* from WA glasshouse colony**

**Mating competitiveness**

Mating competitiveness field cage trials were carried out in Perth in March 2007 in conjunction with Don McInnis of USDA/ARS, Hawaii. Techniques used previously with sterile fruit flies were modified for use with moths. Field cages (3m D x 2 m H) containing an artificial ornamental Ficus tree (1.8m high) were used. Recently emerged male and female adults and pupae <1 day from emergence were irradiated at either 200Gy+/-5% or 300Gy+/-5% and placed individually in 30ml containers for moth emergence. Irradiated or unirradiated moths were marked and just before dusk (when LBAM mate) they were placed into the cage. Type and duration of mating was recorded for approximately four hours using LED headlamps. Mating pairs were captured individually in tubes. Next morning moths were taken into the laboratory and released into one of five egging cages according to their treatment group. Percent egg hatch was recorded. The trial was replicated three times.

Mating competiveness was expressed as RSI (relative sterility index). RSI = sw / sw+ww. RSI values can vary from 0-1 where a value of 0.5 indicates equal competitiveness of sterile and wild males. Average values for 200Gy pupae and adults were 0.4 and for 300Gy pupae 0.3 and adults 0.4. This indicates good competiveness as for sterile fruit fly a value above 0.2 is considered acceptable. Mean egg hatch was 93% for unirradiated moths with egg hatch for irradiated moths and pupae between 8 and 20%. This trial needs to be repeated at a dose of 250Gy using moths bred from recently field collected larvae or eggs. However the initial results indicate that mating competiveness will not be an issue at a target dose of 250Gy.

**Critical over-flooding ratio**

The critical over flooding ratio is the ratio of irradiated to every wild moth needed to be release hold the wild population at zero growth. Increasing the number of moths released should have an impact on the natural population knocking it down to zero with prolonged releases. The ratio is based on fitness parameters, both sexual and physical Kean et al. (2008) and over flooding ratios can differ between radiation doses. Current parameters and values in the model are being redefined to determine the likely ratio needed to reduce a natural population of LBAM.

# 4. Implications for stakeholders

Incursions of exotic pests are a constant threat to horticultural industry sustainability. This project demonstrated the potential use of new technologies to eradicate these incursions. Further research is required to integrate these technologies into a sustainable, cost effective and reliable pest eradication package. The integration of these new eradication technologies will benefit all those involved in pest eradication including policy makers, effected parties, industry bodies, state and federal government and the wider community

The wider community will no longer accept eradication technologies that they perceive will be deleterious to themselves or the environment. Eradication programs will only go ahead if the technologies used are seen to be environmentally benign and without risk to public health

# 5. Recommendations

A dose rate of 250Gy will give complete female sterility. Progeny of males irradiated at this dose will be predominately male with inherited sterility. Competitiveness will not significantly impacted and an overflooding ratio of 20/1 should ensure eradication. Other measures such a mating disruption will be required to bring the target population down to a level where production of sufficient numbers of sterile moths to achieve the required overflooding ratio is economically feasible.

# 6. Abbreviations/glossary

| **ABBREVIATION** | **FULL TITLE** |
|---|---|
| LBAM | Light brown apple moth |
| SIT | Sterile insect technique |
| MD | Mating disruption |
| IPE | Integrated pest eradication |

# 7. Plain English website summary

Please complete table using plain English. This information will be published on CRCNPB's website for a public audience.

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|---|---|
| CRC project no: | CRC40024 |
| Project title: | An integrated approach to the eradication of arthropod EPP's |
| Project leader: | Bill Woods |
| Project team: | Max Suckling, Greg Baker, David Williams, Lloyd Stringer, Ian Lacey, Vanessa Mitchell, Alven Soopaya, Amandip Kaur |
| Research outcomes: | Eradication technologies reviewed, Irradiation biology and inherited sterility of LBAM documented, Competiveness of sterile moths tested , Integration of eradication technologies investigated |
| Research implications: | SIT has an important role as a key part of new age eradication technologies but integration with pheromones and other eradication tools needs further study |
| Research publications: | Integrated Pest Eradication for Perennial Horticulture: Current Technologies (2008) Suckling DM, Woods B, Williams DG, Baker G, Crisp P, Stephens AEA, Stringer LD, El-Sayed AM; CRCNPB Report 107pp |
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